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Inhibition of Prostaglandin Synthesis Antagonizes the Colchicine-Induced Reduction of Vasopressin-Stimulated Water Flow in the Toad Urinary Bladder

RONALD M. BURCH[1] AND PERRY V. HALUSHKA

Departments of Pharmacology and Medicine, Medical University of South Carolina, Charleston, South Carolina 29403

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SUMMARY

Inhibition of prostaglandin synthesis antagonizes the colchicine-induced reduction of vasopressin-stimulated water flow in the toad urinary bladder. Colchicine (20 μM), after preincubation with the hemibladders for 4 hr, inhibited vasopressin-stimulated osmotic water flow 49 ± 6% while vinblastine (20 μM) inhibited vasopressin-stimulated water flow 74 ± 5%. In addition, colchicine enhanced basal immunoreactive prostaglandin E (iPGE) biosynthesis from 0.10 ± 0.02 to 0.24 ± 0.01 pmole·min^{-1}·hemibladder^{-1} ($p < 0.001$; n = 24 pairs). Vasopressin (1 mU/ml) stimulated prostaglandin E biosynthesis to 0.29 ± 0.04 pmole·min^{-1}·hemibladder^{-1} in control hemibladders and to 0.39 ± 0.04 pmole·min^{-1}·hemibladder^{-1} ($p < 0.01$; n = 24 pairs) in colchicine-pretreated hemibladders. Similarly, 4-hr pretreatment with vinblastine (20 μM) significantly increased basal iPGE biosynthesis as well as the synthetic rate in the presence of vasopressin. Pretreatment of hemibladders with colchicine (20 μM) for only 1 hr did not significantly inhibit vasopressin-stimulated water flow nor significantly stimulate iPGE biosynthesis, whereas pretreatment for 2 hr did inhibit vasopressin-stimulated water flow and enhanced iPGE biosynthesis. Inhibition of colchicine-enhanced prostaglandin biosynthesis with indomethacin, meclofenamate, or naproxen completely reversed the inhibitory effect of colchicine on vasopressin-stimulated water flow. Pretreatment with meclofenamate decreased the vinblastine-induced reduction of water flow from 74 ± 5% to only 30 ± 9% ($p < 0.01$; n = 5). Colchicine also significantly ($p < 0.05$) enhanced the sensitivity of the toad bladder to exogenous prostaglandin E_1, lowering the ID_{50} for the inhibition of vasopressin-stimulated water flow by prostaglandin E_1 from 6.0 × 10^{-10} M to 2.2 × 10^{-10} M. However, cyclooxygenase inhibitors did not reverse the colchicine-induced inhibition of cyclic AMP-stimulated water flow. Thus, in addition to the other known actions of colchicine, its inhibitory effect on vasopressin-stimulated water flow in the toad urinary bladder may also be mediated, in part, by its ability to increase prostaglandin E biosynthesis and to enhance the inhibitory actions of PGE. Finally, we suggest that this unique combination of effects of colchicine may mediate some of its actions in other tissues.

INTRODUCTION

Vasopressin, exogenous cyclic AMP, and theophylline enhance the osmotic flow of water across the isolated toad urinary bladder (1, 2). Colchicine and other cytoskeleton-disrupting agents, such as vinblastine, antagonize the effects of these agents on the toad bladder (3-7). The isolated toad urinary bladder metabolizes arachidonic acid to PGE_2[2] (8); and vasopressin, but not cyclic AMP or theophylline, stimulates the synthesis of this metabolite (8). Exogenously added PGE is a potent antagonist of the effects of vasopressin and theophylline on water transport, whereas cyclic AMP-stimulated water flow is not affected by exogenous PGE (9). Colchicine has been reported to enhance prostaglandin synthesis in a number of tissues (10-14), as well as increasing tissue sensitivity to prostaglandins (15-19). However, in none of those studies was there a simultaneous assessment of the effect of colchicine on the rate of synthesis of PGE and the sensitivity of the tissues to exogenous PGE_1. The present study was performed to determine (a) the effect of colchicine and vinblastine on basal and vasopressin-stimulated metabolism of endogenous arachidonic acid to PGE, (b) the effects of inhibitors of prostaglandin synthesis on the actions of colchicine and vinblastine, (c)

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[2] The abbreviations used are: PGE, PGB, PGF, prostaglandins E, B, and F; iPGE, immunoreactive prostaglandin E.

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the effects of colchicine on the sensitivity of the isolated toad urinary bladder to exogenous PGE, and (d) the role of PGE in mediating the effects of colchicine on cyclic AMP- and theophylline-stimulated water flow.

METHODS

Materials. The following were generous gifts: PGE_2, Drs. J. Pike and U. Axen, Upjohn Company (Kalamazoo, Mich.); sodium meclofenamate, Parke Davis and Company (Detroit, Mich.); and naproxen, Syntex Corporation (Palo Alto, Calif.). The following were purchased from commercial sources: spectral grade solvents, Burdick-Jackson Company (Muskegon, Mich.); Pitressin, Parke Davis & Company (Detroit, Mich.); [5,6,8,11,12,14,15-^{3}H]-PGE_2 (150 Ci/mmole), New England Nuclear Corporation (Boston, Mass.); silicic acid (Biosil A 200–400 mesh), Biorad Laboratories (Richmond, Calif.); charcoal and dextran, Schwarz/Mann (Orangeburg, N. Y.); colchicine, vinblastine sulfate, indomethacin, cyclic AMP, and theophylline, Sigma Chemical Company (St. Louis, Mo.).

Osmotic water flow. Toads (*Bufo marinus*) of Mexican origin were obtained from W. M. Lemberger Company (Germantown, Wisc.) and were housed on moist pads. Toads were doubly pithed and hemibladders were mounted mucosal surface inward on glass tubes; one hemibladder from each toad served as a control; the other, as an experimental hemibladder. Osmotic water flow was measured by the method of Bentley (1). Water flow was normalized to mean hemibladder weight (200 mg for these experiments) and was expressed in $mg \cdot min^{-1} \cdot hemibladder^{-1}$. The serosal media were composed of 15 ml of a Ringer's solution containing 90 mM NaCl, 3.0 mM KCl, 25 mM $NaHCO_3$, 1.0 mM $MgSO_4$, 1.0 mM $CaCl_2$, and 6.0 mM glucose. The mucosal media consisted of 3 ml of the Ringer's solution diluted 1:5 with distilled water. A mixture of 95% O_2-5% CO_2 was continuously bubbled through the serosal media, and the pH was 7.4. The hemibladders were allowed to stabilize for 30 min, after which the media were replaced and colchicine or vinblastine was added to the experimental hemibladders. The experimental hemibladders were incubated for 4 hr with colchicine or vinblastine. Some hemibladders were also incubated with cyclooxygenase inhibitors for the final hour. All media were replaced every 30 min. Basal water flow was measured for 30 min, the media were replaced, and vasopressin (1 mU/ml), cyclic AMP (10 mM), or theophylline (10 mM) was added to the serosal media. Stimulated water flow was measured for 30 min starting immediately after the addition of the agent to the media. Colchicine, vinblastine, or cyclooxygenase inhibitors were present in media bathing the appropriate hemibladders during the stimulation period. Stimulated water flow was measured for 30 min, since vasopressin produces its peak effect after approximately 15 min, and then the response begins to decline (20). Thus, the peak response is well-contained within this measurement period. Furthermore, iPGE synthesis is stimulated to a peak level by vasopressin within 10 min and remains at a plateau until near the end of the 30-min period, thus approximating a linear synthetic rate over this time (20). In other studies, pairs of hemibladders were incubated for only 1 or 2 hr instead of 4 hr. In those incubated for 1 hr, colchicine (20 μM) was added to the experimental hemibladders. The media bathing both the control and experimental hemibladders were changed after 45 min and the new experimental media still contained colchicine. Basal water flow was measured for the next 30 min (i.e., from 45 to 75 min after the addition of colchicine) and the serosal media were collected for assay of iPGE. Vasopressin (1 mU/ml) was added, the new media bathing the experimental hemibladders still contained colchicine, and vasopressin-stimulated water flow was determined for 30 min (i.e., from 75 to 105 min after the addition of colchicine). The serosal media were collected for assay of iPGE. In those hemibladders incubated for 2 hr, basal water flow was measured from 90 to 120 min after the addition of colchicine followed immediately by the addition of vasopressin.

Radioimmunoassays for PGE. iPGE was quantitated after extraction of the serosal bathing media. To 4 ml of the serosal media was added [^{3}H]PGE_2 (1500 cpm) to account for recovery losses. The media were acidified to pH 3.5 with formic acid and extracted twice with 15 ml of ethyl acetate. The ethyl acetate was evaporated under a stream of nitrogen and the residue was redissolved and subjected to column chromatography on silicic acid as previously described (21). The PGE fraction was converted to PGB using 0.1 N methanolic KOH, then assayed using a previously described radioimmunoassay procedure (22). The term iPGE is used because the antibody cross-reacts with both the 1 and 2 series. The antibody does not significantly cross-react with PGD_2, $PGF_{2\alpha}$, 6-keto-$PGF_{s\alpha}$, 15-keto-PGB_2, or thromboxane B_2 (21).

Sensitivity of toad bladder to PGE_1. These experiments were performed in control hemibladders incubated for 3 hr in Ringer's solution and then pretreated with meclofenamate for 1 hr, or in experimental hemibladders pretreated with colchicine for 3 hr and colchicine plus meclofenamate for 1 additional hr. Hemibladders were pretreated with meclofenamate to reduce endogenous prostaglandin synthesis during the final hour. Vasopressin (1 mU/ml) was added and water flow was determined for 30 min. The vasopressin was washed out by changing the media three times at 10-min intervals. The mucosal media were replaced and the hemibladders were allowed to incubate at rest (still with meclofenamate or meclofenamate plus colchicine) for 30 min. PGE_1 vehicle or PGE_1 was added in concentrations from 10^{-11} M to 10^{-6} M and the bladders were incubated an additional 10 min. Vasopressin (1 mU/ml) was then added and water flow was determined again for 30 min. Water flow was expressed as the ratio of the PGE_1 vehicle or PGE_1 treatment period to the initial period with or without colchicine.

Statistical procedure. All data are presented as means ± standard error. All synthesis rates and water flow data were analyzed using Student's *t*-test for paired observations (23). Percentage inhibitions were determined by subtracting basal rates of water flow or synthesis from the rates in the presence of vasopressin, cyclic AMP, or theophylline for both control and experimental hemibladders, and the percentage reductions were calculated for each pair. These percentages were then averaged. The analysis of the effect of meclofenamate on the vinblastine inhibition of vasopressin-stimulated water flow

utilized Student's *t*-test for unpaired observations (23). The data in Fig. 1 were analyzed with an analysis of covariance adjusted for unequal treatment group sizes (23).

RESULTS

Effects of colchicine on iPGE synthesis and vasopressin-stimulated water flow. The effects of colchicine (20 μM) were tested after a 4-hr incubation, conditions which have been shown to maximally inhibit vasopressin-stimulated water flow (3). Arachidonic acid is converted to PGE_2 via the enzyme fatty acid cyclooxygenase. This enzyme catalyzes the transformation of arachidonic acid to endoperoxide intermediates which are then acted upon by other enzymes to yield PGE_2. In the control hemibladders, basal iPGE synthesis was 0.10 ± 0.02 pmole·min^{-1}·hemibladder^{-1} (Table 1), whereas in the paired hemibladders pretreated with colchicine for 4 hr, iPGE synthesis was significantly enhanced to 0.24 ± 0.01 pmole·min^{-1}·hemibladder^{-1} ($p < 0.001$, $n = 24$ pairs). Vasopressin significantly ($p < 0.001$) stimulated iPGE synthesis to 0.29 ± 0.04 pmole·min^{-1}·hemibladder^{-1}, whereas in the colchicine-pretreated hemibladders iPGE synthesis was stimulated to 0.39 ± 0.04 pmole·min^{-1}·hemibladder^{-1} ($p < 0.001$) (Table 1). Thus, the rate of iPGE synthesis in the presence of vasopressin was significantly greater in the colchicine-pretreated hemibladders than in the paired controls ($p < 0.001$; $n = 24$ pairs). Even though the absolute increment (vasopressin-basal) in synthesis appeared to be less in the colchicine-pretreated hemibladders (0.19 pmole·min^{-1}·hemibladder^{-1} in control hemibladders versus 0.15 pmole·min^{-1}·hemibladder^{-1} in the colchicine-pretreated hemibladders), they were not significantly different.

Colchicine had no effect on basal water flow (Table 2). Vasopressin (1 mU/ml) increased water flow in the control hemibladders from 1.8 ± 0.3 mg·min^{-1}·hemibladder^{-1} to 47.5 ± 6.4 mg·min^{-1}·hemibladder^{-1} (Table 2), whereas water flow in the paired colchicine-pretreated hemibladders was enhanced to only 24.6 ± 4.2 mg·min^{-1}·hemibladder^{-1} from a basal rate of 1.4 ± 0.3 mg·min^{-1}·hemibladder^{-1} ($n = 24$ pairs), an inhibition of 49 ± 6%, confirming previous observations (3, 4, 7).

TABLE 1

Effects of colchicine and vinblastine on basal and vasopressin-stimulated iPGE synthesis

Hemibladders were pretreated with colchicine for 4 hr. Basal and vasopressin-stimulated water flow were each determined for 30 min.

	iPGE	*n*
	pmoles·min^{-1}·hemibladder^{-1}	
Basal		
Control	0.10 ± 0.02	24
Colchicine (20 μM)	0.24 ± 0.01[a]	24
Control	0.33 ± 0.06	5
Vinblastine (20 μM)	0.49 ± 0.05[b]	5
Vasopressin-stimulated (1 mU/ml)		
Control	0.29 ± 0.04[b]	24
Colchicine (20 μM)	0.39 ± 0.04[a, c]	24
Control	1.04 ± 0.19[d]	5
Vinblastine (20 μM)	1.82 ± 0.34[a, d]	5

[a] $p < 0.001$, compared with control.
[b] $p < 0.05$, compared with control.
[c] $p < 0.001$, compared with basal.
[d] $p < 0.05$, compared with basal.

TABLE 2

Time course of inhibition of vasopressin-stimulated water flow and enhancement of iPGE synthesis by colchicine

Hemibladders were incubated with colchicine for 1 or 2 hr (see Methods). Basal and vasopressin periods were each 30 min; $n = 5$ for 1-hr preincubation, $n = 6$ for 2-hr preincubation. The 1-hr preincubation experimnent was performed in December 1980, and the 2-hr preincubation experiment was performed in March 1981. This seasonal variation in iPGE synthesis has been previously described (20).

Preincubation	iPGE		Water flow	
	Basal	Vasopressin (1 mU/ml)	Basal	Vasopressin (1 mU/ml)
	pmoles·min^{-1}·hemibladder^{-1}		*mg·min^{-1}·hemibladder^{-1}*	
Colchicine, 1 hr				
Control	0.23 ± 0.06	0.41 ± 0.06[a]	1.4 ± 0.7	33.6 ± 5.5
Colchicine	0.27 ± 0.06	0.47 ± 0.07[a]	1.5 ± 0.5	28.8 ± 4.3
Colchicine, 2 hr				
Control	0.067 ± 0.012	0.092 ± 0.017	0.7 ± 0.2	20.3 ± 1.2
Colchicine	0.095 ± 0.018[b]	0.18 ± 0.029[c]	0.8 ± 0.2	12.5 ± 0.5[d]

[a] $p < 0.05$, compared with basal.
[b] $p < 0.05$, compared with controls.
[c] $p < 0.02$, compared with basal.
[d] $p < 0.01$, compared with control.

Time course of the inhibition of vasopressin-stimulated water flow and enhancement of iPGE synthesis by colchicine. Because the effect of colchicine to reduce vasopressin-stimulated water flow is gradual in onset (2), we determined whether iPGE synthesis is enhanced by colchicine with the same time course. Paired hemibladders were incubated with either colchicine (20 μM) or the vehicle (Ringer's solution) for only 1 hr. In these hemibladders vasopressin stimulated water flow in the control and in the colchicine-pretreated hemibladders to a similar extent (Table 2). Furthermore, iPGE synthesis was no different in the control or colchicine-pretreated hemibladders both during the basal and vasopressin periods (Table 2). After incubation of the bladders for 2 hr with colchicine, vasopressin-stimulated water flow was inhibited 40 ± 4% ($p < 0.01$; $n = 6$ pairs) by colchicine (Table 2). In control hemibladders, iPGE synthesis in the presence of vasopressin was 0.092 ± 0.017 pmole·min^{-1}, hemibladder^{-1}, whereas in the colchicine-pretreated hemibladders, iPGE synthesis in the presence of vasopressin was 0.18 ± 0.029 pmole·min^{-1}, hemibladder ($p < 0.01$; $n = 6$ pairs) (Table 2).

Effects of vinblastine on iPGE synthesis and vasopressin-stimulated water flow. Other cytoskeleton-disrupting agents share colchicine's ability to inhibit vasopressin-stimulated water flow across the toad bladder (3, 4, 7). Thus, we determined whether the ability of colchicine to enhance iPGE synthesis in the toad bladder is shared by another of these agents, vinblastine. Hemibladders were incubated with vinblastine sulfate (20 μM)

for 4 hr, conditions under which vasopressin-stimulated water flow is maximally inhibited by this agent (7). After 4 hr of preincubation, water flow in control hemibladders was stimulated by vasopressin from 1.0 ± 0.4 mg·min^{-1}·hemibladder^{-1} to 28.9 ± 5.5 mg·min^{-1}·hemibladder^{-1}. In vinblastine-pretreated hemibladders, water flow was stimulated from 0.7 ± 0.1 mg·min^{-1}·hemibladder^{-1} to only 7.7 ± 2.6 mg·min^{-1}·hemibladder^{-1} ($p < 0.01$; $n = 5$ pairs, compared with vasopressin-stimulated water flow in controls). Basal iPGE synthesis was greater in the vinblastine-pretreated hemibladders than in the controls (0.49 ± 0.05 versus 0.33 ± 0.06 pmole·min^{-1}·hemibladder^{-1}; $p < 0.05$; $n = 5$ pairs) and in the presence of vasopressin, iPGE synthesis was greater in vinblastine-pretreated hemibladders than in the controls (1.82 ± 0.34 versus 1.04 ± 0.19 pmoles·min^{-1}·hemibladder^{-1}; $p < 0.05$; $n = 5$ pairs). Unlike the situation with colchicine, vasopressin increased iPGE synthesis to a greater absolute extent in the presence of vinblastine (1.33 pmoles·min^{-1}·hemibladder^{-1}) than in the controls (0.71 pmole·min^{-1}·hemibladder^{-1}) ($p < 0.05$; $n = 5$ pairs).

Effects of cyclooxygenase inhibitors on inhibition by colchicine and vinblastine of vasopressin-stimulated water flow. To test whether the colchicine-induced increase in arachidonic acid metabolism to PGE plays a role in its inhibition of vasopressin-stimulated water flow, we tested the effects of the fatty acid cyclooxygenase inhibitors indomethacin, meclofenamate, and naproxen on the colchicine-induced reduction of vasopressin-stimulated water flow. Since cyclooxygenase inhibitors have been shown to enhance vasopressin-stimulated water flow (24), both control and experimental hemibladders were incubated with the cyclooxygenase inhibitors prior to the addition of vasopressin (see Methods).

As previously indicated, colchicine had no effect on basal water flow (Table 3). Similarly, basal water flow in the presence of cyclooxygenase inhibitors was not different from basal water flow in the presence of cyclooxygenase inhibitors plus colchicine (Table 3). Although colchicine alone inhibited vasopressin-stimulated water flow 49%, all three of the cyclooxygenase inhibitors tested completely blocked the inhibitory effect of colchicine on water flow (Table 3). Since the concentration of indomethacin used in this study has been shown to inhibit cyclic nucleotide phosphodiesterase in the toad urinary bladder (25), the effect of theophylline (1 mM) on inhibition by colchicine of vasopressin-stimulated water flow was determined. This concentration of theophylline inhibits phosphodiesterase activity in the toad bladder to an extent similar to indomethacin (25). In hemibladders pretreated with theophylline for 1 hr, vasopressin-stimulated water flow was 53.8 ± 6.1 mg·min^{-1}·hemibladder^{-1}, whereas in the paired hemibladders pretreated with colchicine for the first 3 hr and colchicine plus theophylline for the final hour, vasopressin-stimulated water flow was 32.6 ± 7.2 mg·min^{-1}·hemibladder^{-1} ($p < 0.01$; $n = 6$ pairs, compared with vasopressin alone). Thus, the action of indomethacin to block the action of colchicine appears not to be related to an effect on phosphodiesterase. Meclofenamate does not inhibit phosphodiesterase in the concentration used in this study (22).

TABLE 3

Effects of colchicine and cyclooxygenase inhibitors on basal and vasopressin-stimulated water flow

Hemibladders were pretreated with colchicine for 4 hr, with cyclooxygenase inhibitors present for the final hour. Basal and vasopressin-stimulated water flow were each determined for 30 min.

	Water flow	n
	mg·min^{-1}·hemibladder^{-1}	
Basal		
Control	1.8 ± 0.3	24
Colchicine (20 μM)	1.4 ± 0.3	24
Cyclooxygenase inhibitors[a] + colchicine (20 μM)	1.6 ± 0.3	24
	1.5 ± 0.3	24
	1.5 ± 0.3	24
Vasopressin-stimulated (1 mU/ml)		
Control	47.5 ± 6.4	24
Colchicine (20 μM)	24.6 ± 4.2[b]	24
Indomethacin(50 μM)	49.0 ±7.5	7
Indomethacin + colchicine (20 μM)	46.6 ± 11.4	7
Meclofenamate (10 μM)	30.3 ± 7.6	6
Meclofenamate + Colchicine (20 μM)	29.6 ± 7.3	6
Naproxen (100 μM)	40.8 ± 7.0	8
Naproxen + colchicine (20 μM)	37.7 ± 6.8	8

[a] This group includes eight pairs each of hemibladdeers pretreated with indomethacin (50 μM), meclofenamate (10 μM), or naproxen (100 μM).

[b] $p < 0.001$, compared with control.

To confirm that indomethacin, meclofenamate, and naproxen had blocked arachidonic acid metabolism in these experiments, iPGE was measured in the serosal bathing media. During both the basal and vasopressin periods, iPGE was not detectable by radioimmunoassay, reflecting a synthesis rate of less than 0.005 pmole·min^{-1}·hemibladder^{-1}. Thus, there was greater than a 90% inhibition of PGE synthesis.

The cyclooxygenase inhibitor meclofenamate was also tested on vinblastine's inhibition of vasopressin-stimulated water flow. In an experiment performed simultaneously with that reported above for vinblastine's effect on vasopressin-stimulated water flow and iPGE synthesis, several pairs of hemibladders were preincubated in Ringer's solution for 3 hr or in Ringer's solution plus vinblastine (20 μM) for 3 hr. Meclofenamate (10 μM) was then added to the control hemibladders for 1 hr and meclofenamate (10 μM) plus vinblastine (20 μM) was added to the experimental hemibladder for 1 hr. In the hemibladders pretreated with meclofenamate alone, basal water flow was 1.2 ± 0.3 mg·min^{-1}·hemibladder^{-1} and was stimulated to 42.4 ± 6.7 mg·min^{-1}·hemibladder^{-1} by vasopressin. In the hemibladders pretreated with vinblastine plus meclofenamate, basal water flow was stimulated by vasopressin from 1.6 ± 0.4 mg·min^{-1}·hemibladder^{-1} to 30.1 ± 5.8 mg·min^{-1}·hemibladder^{-1} ($p < 0.05$ compared with vasopressin plus meclofenamate alone; $n = 5$ pairs), an inhibition of 30 ± 9%. Thus, meclofenamate significantly reduced the inhibition of

vasopressin-stimulated water flow by vinblastine from 74 ± 5% to 30 ± 9% ($p < 0.01$; $n = 10$, each group), but did not completely block the effect as it did with colchicine. iPGE synthesis in the presence of vasopressin plus meclofenamate was 0.062 ± 0.010 pmole·min^{-1}·hemibladder^{-1} in the control hemibladders; in the hemibladders pretreated with vinblastine plus meclofenamate, iPGE synthesis in the presence of vasopressin was 0.041 ± 0.005 pmole·min^{-1}·hemibladder^{-1} (difference not significant).

Since previous studies of the effects of colchicine on vasopressin-stimulated water flow in the toad bladder have used a supramaximal concentration of vasopressin (20 mU/ml) (3, 7), an additional experiment was performed using vasopressin at a concentration of 20 mU/ml. In control hemibladders, vasopressin (20 mU/ml)-stimulated water flow was 70.2 ± 6.4 mg·min^{-1}·hemibladder^{-1}, whereas in the presence of colchicine, vasopressin (20 mU/ml)-stimulated water flow was 38.6 ± 4.2 mg·min^{-1}·hemibladder^{-1} ($p < 0.01$, compared with vasopressin alone; $n = 8$ pairs). In similar experiments, vasopressin (20 mU/ml)-stimulated water flow in hemibladders pretreated with indomethacin was 75.5 ± 8.2 mg·min^{-1}·hemibladder^{-1}, whereas vasopressin (20 mU/ml)-stimulated water flow in hemibladders pretreated with indomethacin plus colchicine was 73.1 ± 8.6 mg·min^{-1}·hemibladder^{-1} ($n = 8$ pairs, difference not significant).

Effect of cyclooxygenase inhibitors on inhibition by colchicine of cyclic AMP- and theophylline-stimulated water flow. Colchicine and other cytoskeleton-disrupting agents inhibit the water flow response to exogenous cyclic AMP as well as to vasopressin (3). However, cyclic AMP-stimulated water flow has been reported not to be affected by PGE_1 (9). Thus, we determined the effects of cyclooxygenase inhibitors on the inhibition of cyclic AMP-stimulated water flow by colchicine. Basal water flow was not significantly different in control hemibladders compared with paired hemibladders pretreated with colchicine (20 μM) for 4 hr ($n = 6$ pairs) (Table 4). Cyclic AMP (10 mM)-stimulated water flow was significantly inhibited (58.7 ± 7.3%; $p < 0.02$). In parallel experiments, control and colchicine-pretreated hemibladders were pretreated with meclofenamate (10 μM) during the 4th hr of colchicine pretreatment. Basal water flow was not significantly different in the control hemibladders compared with the colchicine-pretreated hemibladders. Cyclic AMP (10 mM)-stimulated water flow was still significantly ($p < 0.02$) inhibited by colchicine (45.6 ± 3.8%) in the presence of meclofenamate. Thus, in contrast to vasopressin-stimulated water flow, cyclooxygenase inhibitors did not affect colchicine's inhibition of cyclic AMP-stimulated water flow.

TABLE 4

Effects of colchicine and cyclooxygenase inhibitors on basal and stimulated water flow

Hemibladders were pretreated with colchicine for 4 hr. Basal and stimulated water flow were each determined for 30 min.

	Water flow		n
	Basal	Stimulated	
	mg·min^{-1}·hemibladder		
Theophylline (10 mM)			
Control	1.1 ± 0.3	21.0 ± 4.6	6
Colchicine (20 μM)	0.8 ± 0.2	13.6 ± 5.0[a]	6
Meclofenamate (10 μM)	3.2 ± 1.1	38. 6 ± 3.8	
Meclofenamate + colchicine	3.8 ± 1.0	35.9 ± 4.1	
Cyclic AMP (10 mM)			
Control	1.1 ± 0.2	9.7 ± 0.8	6
Colchicine (20 μM)	0.9 ± 0.2	4.5 ± 0.9[b]	6
Meclofenamate (10 μM)	0.8 ± 0.3	10.7 ± 2.4	6
Meclofenamate + colchicine	0.8 ± 0.2	6.4 ± 1.7[b]	6

[a] $p < 0.001$ compared with control.
[b] $p < 0.02$ compared with control.

Theophylline stimulates water flow across the isolated toad bladder, presumably by inhibiting cyclic nucleotide phosphodiesterase to increase the intracellular accumulation of cyclic AMP generated by the basal activity of adenylate cyclase (2). Since the effect of colchicine on theophylline-stimulated water flow has not been previously reported, and because PGE_1 has been reported to inhibit theophylline-stimulated water flow (9), we determined the effects of colchicine on theophylline-stimulated water flow and the modification of the effect of colchicine by cyclooxygenase inhibitors. Hemibladders were preincubated for 4 hr with colchicine (20 μM) (Table 4). Basal water flows in control and colchicine-pretreated hemibladders were not significantly different. Theophylline (10 mM)-stimulated water flow was inhibited 45.2 ± 5.7% ($p < 0.01$; $n = 6$ pairs) by colchicine. In a parallel series of experiments, the control and colchicine-pretreated hemibladders were pretreated with meclofenamate (10 μM) during the 4th hr of colchicine pretreatment. Basal water flow was not significantly different in the control hemibladders as compared with the colchicine-pretreated hemibladders. In the presence of meclofenamate, theophylline stimulated-water flow in the control hemibladders was not significantly different from that in the colchicine-pretreated hemibladders (Table 4).

These experiments reveal that cyclooxygenase inhibitors block the inhibition by colchicine of vasopressin- and theophylline-stimulated water flow, but have no effect on inhibition by colchicine of cyclic AMP-stimulated water flow. These results are consistent with the established effect of PGE_1 to inhibit vasopressin- and theophylline-stimulated water flow while having no effect on cyclic AMP-stimulated water flow (8). To confirm that PGE_1 does not, in fact, affect cyclic AMP-stimulated water flow, the following experiments were performed.

Hemibladders were pretreated with meclofenamate (10 μM) for 1 hr to inhibit endogenous PGE synthesis. The experimental hemibladders were then pretreated with exogenous PGE_1 (10^{-8} or 10^{-7} M) for 30 min while basal water flow was being determined, and remained during a 30-min period during which cyclic AMP-stimulated water flow was being determined. In the control hemibladders, cyclic AMP (2 mM) stimulated ($p < 0.02$) water flow from 2.3 ± 0.4 to 7.3 ± 1.5 mg·min^{-1}·hemibladder^{-1} ($\Delta_{\text{cyclic AMP-basal}}$ = 4.6 ± 1.1 mg·min^{-1}·hemibladder^{-1}). In the hemibladders pretreated with PGE_1, cyclic AMP (2 mM) stimulated ($p < 0.02$) water flow from 2.8 ± 0.8 to 6.5 ± 1.1 mg·min^{-1}·hemibladder^{-1} ($\Delta_{\text{cyclic AMP-basal}}$ = 3.6 ± 1.0 mg·min^{-1}·hemibladder^{-1}). The Δ values were not significantly different ($n = 10$ pairs). Thus, PGE_1 (10^{-8}

or 10^{-7} M) did not affect cyclic AMP-stimulated water flow.

The possibility existed that, if basal PGE synthesis was increased, a greater effect of a cyclooxygenase inhibitor might be manifested. Therefore, in another series of experiments hemibladders were incubated in Ringer's solution for 11 hr, which results in enhanced basal PGE synthesis (26).[3] The paired experimental hemibladders were incubated for the first 10 hr in the presence of 1 μM meclofenamate, and for the final hour in 10 μM meclofenamate. In the control hemibladders, cyclic AMP (10 mM) stimulated water flow from 0.7 ± 0.2 mg·min^{-1}·hemibladder^{-1} to 9.6 ± 1.7 mg·min^{-1}·hemibladder^{-1} ($\Delta_{\text{cyclic AMP-basal}}$ = 8.9 ± 1.2 mg·min^{-1}·hemibladder^{-1}). In the hemibladders pretreated with meclofenamate, cyclic AMP (10 mM) stimulated water flow from 0.2 ± 0.3 to 6.7 ± 0.5 mg·min^{-1}·hemibladder^{-1} ($\Delta_{\text{cyclic AMP-basal}}$ = 6.5 ± 0.6 mg·min^{-1}·hemibladder^{-1}). The Δ values were not significantly different (n = 6 pairs). In the control hemibladders, iPGE synthesis during the cyclic AMP period was 0.62 ± 0.14 pmole·min^{-1}·hemibladder^{-1}. In the meclofenamate-pretreated hemibladders, iPGE accumulation was below detectable levels (0.012 pmole·min^{-1}·hemibladder^{-1}) in three hemibladders and ranged from 0.016 to 0.025 pmole·min^{-1}·hemibladder^{-1} in the remaining three hemibladders.

Effects of colchicine on the sensitivity of the toad bladder to PGE_1. The foregoing experiments demonstrate that colchicine enhances iPGE synthesis, and that inhibition of this synthesis blocks the effect of colchicine on vasopressin-stimulated water flow. Since PGE_1 and PGE_2 antagonize vasopressin-stimulated water flow, the effects described above could be explained by enhanced PGE synthesis alone. However, colchicine also enhances the sensitivity to prostaglandins in a number of tissues (15–19). Thus, the sensitivity of the toad bladder to PGE_1 was tested in the presence of colchicine.

In the presence of colchicine, PGE_1 was significantly ($p < 0.05$) more potent in its inhibitory effect on vasopressin-stimulated water flow (Fig. 1). The ID_{50} for PGE_1 in control hemibladders was 6.0×10^{-10} M, whereas in the presence of colchicine the ID_{50} for PGE_1 was 2.2×10^{-10} M ($p < 0.05$). To confirm that the increased sensitivity to PGE_1 in the colchicine-pretreated hemibladders was not due simply to the continued incubation with colchicine, four pairs of hemibladders were subjected to vasopressin for the second time without added PGE_1. In control hemibladders the second response to vasopressin was 98.5 ± 9.6% of the first response, whereas in the paired colchicine-pretreated hemibladders, the second response was 99.8 ± 12.0% of the first response (Fig. 1).

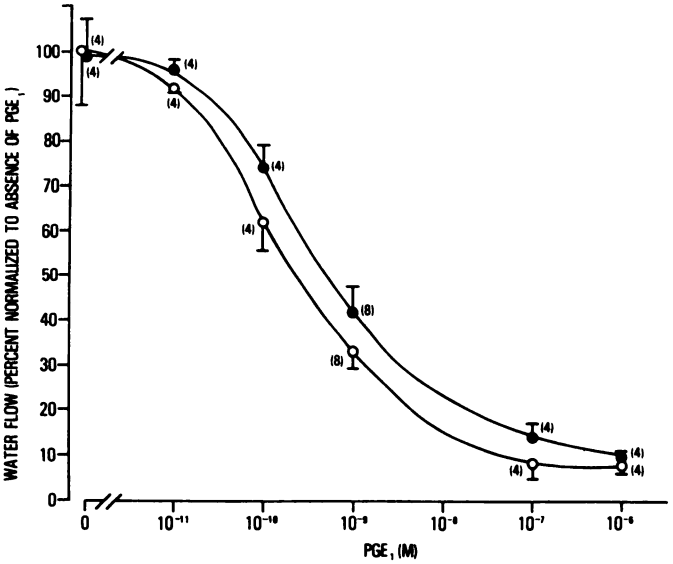

FIG. 1. *Effect of colchicine on the sensitivity of the toad bladder to PGE_1*

Control hemibladders were incubated with Ringer's solution for 3 hr and with Ringer's solution containing meclofenamate (20 μM) for 1 additional hr. Experimental hemibladders were incubated with colchicine (20 μM) for 3 hr and with colchicine (20 μM) plus meclofenamate (10 μM) for 1 additional hr. Vasopressin (1 mU/ml)-stimulated water flow was determined for 30 min, and the hemibladders were washed three times with Ringer's solution containing meclofenamate (10 μM) (controls) or with Ringer's solution containing colchicine (20 μM) plus meclofenamate (10 μM) (experimental hemibladders), and allowed to stabilize for 30 min. PGE_1 was added, and 10 min later vasopressin (1 mU/ml)-stimulated water flow was determined again. Water flow is expressed as the ratio of the second to first vasopressin period multiplied by 100. Hemibladders pretreated with colchicine were more sensitive to PGE_1; in controls (●) the ID_{50} concentration was 6.0×10^{-10} M, and in colchicine-pretreated hemibladders (○) the ID_{50} concentration was 2.2×10^{-10} M. Numbers in parentheses represent the number of hemibladders at each point.

DISCUSSION

Previous studies have shown that colchicine and other microtubule-disrupting agents inhibit enhancement of osmotic water flow by vasopressin (3–7). The time course for the onset of inhibition induced by colchicine is slow, consistent with binding of colchicine to microtubules, and the concentrations required to inhibit water flow are similar to those which are known to affect tubulin polymerization (7). Furthermore, the effect of colchicine has been correlated in the toad bladder to a reduction in the number of microtubules counted by transmission electron microscopy (4). Thus, this action of colchicine on microtubules has been implicated in mediating its effects on vasopressin-stimulated water flow.

Other studies have demonstrated that colchicine can enhance prostaglandin synthesis (11–14) and responsiveness to PGE_1 (15–19) in a variety of cultured mammalian cell lines. However, in none of these studies were the two phenomena assessed simultaneously. The effect of colchicine and vinblastine on vasopressin-stimulated water flow is slow in onset, is not seen during the 1st hr of incubation, and requires several hours to reach the maximal effect (7). Similarly, in this study it was observed that after 1 hr of incubation with colchicine, neither vasopressin-stimulated water flow nor iPGE synthesis was affected. However, by 2 hr colchicine had caused a significant reduction in vasopressin-stimulated water flow and a significant increase in basal iPGE synthesis. The increment in iPGE synthesis due to vasopressin was not greater in the presence of colchicine as compared with control bladders, but was greater than that found in

[3] P. V. Halushka and S. Kratz, unpublished data.

control bladders when vinblastine was present. However, the absolute amount of iPGE synthesized was always greater in the colchicine-pretreated hemibladders than in the controls, as was also the case with vinblastine. This finding suggests that it may be the total amount of PGE present, and not the incremental stimulation of PGE which is important in modulating vasopressin's action. When prostaglandin synthesis was inhibited by cyclooxygenase inhibitors, the inhibitory effect of colchicine on water flow was completely blocked and that of vinblastine was significantly reduced. The failure to block completely the effect of vinblastine by sodium meclofenamate may be due, in part, to the residual iPGE synthesis not eliminated by meclofenamate (0.041 pmole·min^{-1}·hemibladder^{-1} in the vinblastine plus meclofenamate-pretreated hemibladders versus less than 0.005 pmole·min^{-1}·hemibladder^{-1} in the colchicine plus meclofenamate-pretreated hemibladders). Additional effects of vinblastine, not mediated via PGE, may also account for some of the residual inhibition by vinblastine. The observation that cyclooxygenase inhibitors block the action of colchicine is not unique to the toad bladder. Rachmilewitz and Karmeli (27) have recently reported that colchicine enhances PGE synthesis in the jejunum and that inhibition of PGE synthesis completely blocks the effects of colchicine in that tissue.

The results of this study have suggested that enhanced PGE synthesis may be a mediator of the inhibition of vasopressin-stimulated water flow by colchicine. An observation difficult to reconcile with this notion is that cytoskeleton-disrupting agents inhibit cyclic AMP-stimulated water flow across the toad bladder (3, 7), but, PGE has been reported not to inhibit cyclic AMP-stimulated water flow (9). Consistent with the lack of inhibition by PGE_1 of cyclic AMP-stimulated water flow, it was found in the present study that meclofenamate, a cyclooxygenase inhibitor, does not block the inhibition of cyclic AMP-stimulated water flow induced by colchicine. Stimulation of water flow with theophylline has been reported to be inhibited by PGE_1 (9). In the present study, meclofenamate was found to block inhibition of theophylline-stimulated water flow by colchicine.

These observations are consistent with the notion that vasopressin and theophylline stimulate water permeability by a common mechanism sensitive to inhibition by PGE and colchicine, whereas cyclic AMP may stimulate water flow by a separate mechanism sensitive only to inhibition by colchicine. In support of this notion is the report by Cuthbert and Wong (28), who found that both vasopressin and theophylline stimulated release of calcium from the toad bladder, whereas cyclic AMP actually inhibited release of calcium. Furthermore, chlorpropamide, a sulfonylurea derivative, augmented vasopressin- and theophylline-stimulated water flow but inhibited cyclic AMP-stimulated water flow (29). Thus, the inhibition of cyclic AMP-stimulated water flow by colchicine is independent of the colchicine-induced increase in PGE synthesis.

Paradoxically, the sensitivity of the hemibladders to the inhibitory effects of exogenous PGE_1 on vasopressin-stimulated water flow was significantly enhanced in the presence of colchicine. The mechanism of this increased sensitivity remains unknown. However, this observation confirms that, in still another tissue, colchicine can enhance the pharmacological effects of PGE_1.

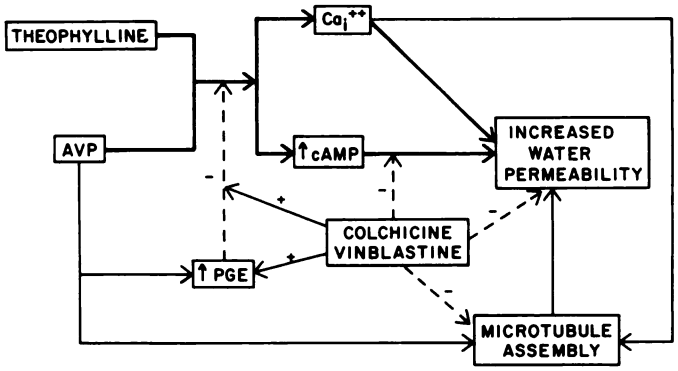

FIG. 2. *Proposed mechanism for the inhibition of stimulated water flow by microtubule inhibitors in the toad urinary bladder*

Vasopressin (*AVP*) and theophylline stimulate intracellular cyclic AMP accumulation and alter ^{45}Ca fluxes, leading to enhanced water permeability. Vasopressin also enhances PGE synthesis, which feeds back to inhibit the accumulation of cyclic AMP in response to the agents. Colchicine and vinblastine also enhance PGE synthesis and the sensitivity of the toad bladder to PGE by as yet unknown mechanisms. Stimulation of PGE synthesis and its effects appear to mediate the inhibition of vasopressin- and theophylline-stimulated water flow by colchicine and vinblastine. Colchicine and vinblastine also act independently of PGE to inhibit the enhancement in water permeability to cyclic AMP via either inhibition of microtubule assembly or other undefined mechanisms.

In conclusion, colchicine and vinblastine stimulate PGE synthesis in the toad urinary bladder, and prostaglandin synthesis inhibitors completely block or significantly reduce, respectively, their effects on vasopressin- or theophylline-stimulated water flow, whereas colchicine inhibition of cyclic AMP-stimulated water flow is not affected. The mechanism by which these cytoskeleton disrupting agents enhance PGE synthesis remains unknown. Figure 2 illustrates a tentative model for the mechanism of inhibition of vasopressin-stimulated water flow by colchicine. Although the model accounts for all of the experimental findings, additional experiments are clearly required to explore the complicated relationships among the various effectors. The results of this study suggest that enhanced PGE synthesis and enhanced sensitivity to the inhibitory effect of PGE may play a role in the effects of colchicine on vasopressin- and theophylline- but not cyclic AMP-stimulated water flow. Thus, this unique combination of actions of colchicine may mediate its effects in other tissues. However, additional mechanisms must also be considered by which colchicine and other cytoskeleton-disrupting agents exert their effects on water flow.

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Send reprint requests to: Dr. Perry V. Halushka, Department of Pharmacology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, S. C. 29425.