Thromboxane and Stable Prostaglandin Endoperoxide Analogs Stimulate Water Permeability in the Toad Urinary Bladder

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A B S T R A C T The effects of thromboxane B_2 and the stable prostaglandin endoperoxide analogs (15Z)-hydroxy-9α-11α-(epoxymethano)prosta-5Z,13E-dienoic acid (U44069) and (15Z)-hydroxy-11α,9α-(epoxymethano)prosta-5Z,13E-dienoic acid (U46619) were tested on water flow across the toad urinary bladder. In the presence of indomethacin or meclofenamic acid, inhibitors of prostaglandin and thromboxane A_2 synthesis, thromboxane B_2 stimulated water flow in a dose-dependent manner. U44069 (1 μM) stimulated water flow from 3.6 ± 0.8 to 12.4 ± 1.2 mg/min per 10 cm^2 hemibladder surface area, while U46619 (1 μM) stimulated water flow from 2.8 ± 1.0 to 21.8 ± 2.0 mg/min per 10 cm^2. The prostaglandin endoperoxide/thromboxane A_2 antagonist *trans*-13-azaprostanoic acid, an inhibitor of vasopressin-stimulated water flow, inhibited thromboxane B_2- and U46619-stimulated water flow in a dose-dependent manner. The inactive *cis*-13-azaprostanoic acid did not inhibit vasopressin-stimulated water flow in untreated hemibladders and had no effect on U46619-stimulated water flow in indomethacin or meclofenamic acid pretreated hemibladders. U46619 (1 μM) enhanced vasopressin-stimulated water flow in indomethacin pretreated hemibladders, producing a significant parallel shift ($P < 0.001$) in the dose-response relationship to submaximal concentrations of vasopressin (0.1–0.6 mU/ml), while not affecting water flow stimulated by supramaximal concentrations of vasopressin (10 mU/ml). *trans*-13-Azaprostanoic acid abolished the potentiating effects of U46619 on vasopressin-stimulated water flow. These results show that thromboxane A_2-like compounds stimulate water flow in the toad urinary bladder.

INTRODUCTION

Previous studies in the isolated toad urinary bladder have shown that vasopressin stimulates the synthesis of thromboxane A_2 (TXA$_2$)[1] (1), a labile (t$_{1/2}$ = 37 s), biologically active metabolite of arachidonic acid (2). Imidazole and 7-(1-imidazolyl)-heptanoic acid, inhibitors of thromboxane synthetase (3, 4), inhibit vasopressin-stimulated water flow and TXA$_2$ synthesis in the toad bladder (5). An antagonist of the effects of prostaglandin endoperoxides or TXA$_2$, *trans*-13-azaprostanoic acid (6), also inhibits vasopressin-stimulated water flow (5). Based on these observations it has been hypothesized that TXA$_2$ acts as a possible mediator of vasopressin-stimulated water flow (5). The extreme lability of TXA$_2$ precludes direct testing of its effects on water flow. Thus, stable compounds that may mimic its action must be sought to test the hypothesis. The stable prostaglandin endoperoxide analogs (15Z)-hydroxy-9α-11α-(epoxymethano)prosta-5Z,13E-dienoic acid (U44069) and (15Z)-hydroxy-11α-9α-(epoxymethano)prosta-5Z,13E-dienoic acid (U46619) possess biological activity that mimic the actions of TXA$_2$ and/or the prostaglandin endoperoxides in platelets and aortic strips (7). Recently, TXB$_2$, the stable metabolite of TXA$_2$, has been shown to exhibit actions similar to TXA$_2$ in the cardiovascular system (8). Thus, the present study was de-

[1] *Abbreviations used in this paper*: iTXB$_2$, immunoreactive TXB; PGE, prostaglandin E; TXA$_2$, B$_2$, thromboxane A$_2$ and B$_2$; U44069, (15Z)-hydroxy-9α,11α-(epoxymethano)prosta-5Z,13E-dienoic acid; U46619, (15Z)-hydroxy-11α,9α-(epoxymethano)prosta-5Z,13E-dienoic acid.

signed to determine the effects of TXB_2, U44069, and U46619 on basal and vasopressin-stimulated water flow in the isolated toad urinary bladder.

METHODS

Toads, *Bufo marinus*, of Dominican Republic or Mexican origin were obtained from National Reagents, Bridgeport, Conn., or W. M. Lemberger Co., Germantown, Wisc., respectively, and housed on moist pads. The following were generous gifts: 13-aza-prostanoic acid, Dr. G. LeBreton and Dr. D. Venton, University of Illinois, Chicago, Ill., and Drs. R. Broersma, P. Anzeveno, and B. Cregge, Dow Chemical Co., Indianapolis, Ind.; PGE_1, TXB_2, U44069, and U46619, Dr. J. Pike and Dr. U. Axen, Upjohn Co., Kalamazoo, Mich.; indomethacin, Merck & Co., Rahway, N. J.; and sodium meclofenamate, Parke Davis & Co., Detroit, Mich. The following were purchased from commercial sources: spectral grade solvents, Burdick & Jackson Laboratories, Inc., Muskegon, Mich.; Pitressin, Parke Davis & Co.; [5,6,8,11,12,14,15-^{3}H]TXB_2 (60–70 Ci/mmol), New England Nuclear, Boston, Mass.; silicic acid (Bio-sil A 200–400 mesh), Bio-Rad Laboratories, Richmond, Calif.; charcoal and dextran, Schwarz/Mann Div. Becton, Dickinson & Co., Orangeburg, N. Y.; Macherey-Nagel Sil G-25 thin-layer chromatography plates, Brinkmann Instruments, Inc., Westbury, N. Y.

Indomethacin, 36 mg, was dissolved in 10 ml 0.1 M phosphate buffer, pH 8 and diluted into 2 liters Ringer's solution of the following composition: 90 mM NaCl, 3.0 mM KCl, 25 mM $NaHCO_3$, 3.9 mM $MgSO_4$, 0.5 mM KH_2PO_4, 1.0 mM $CaCl_2$, and 6.0 mM glucose, pH 7.4. Sodium meclofenamate was dissolved directly into Ringer's solution. U44069, U46619, and TXB_2 were dissolved in ethanol to make stock solutions (10 mM), which were stored at $-20°C$. Aliquots of 10 μl were added to 100 ml Ringer's solution. Control media contained the ethanol vehicle. 13-Azaprostanoic acid (3.2–19 mg) was wetted with 25 μl of ethanol and dissolved in 10 ml of Ringer's solution at pH 9. This solution was diluted into 200 ml of Ringer's solution at pH 7.4. Control media contained the ethanol and pH 9 Ringer's vehicle.

Osmotic water flow. Toads were doubly pithed before removal of hemibladders. Hemibladders were mounted as sacs using the method of Bentley (9). The serosal medium was 15 ml Ringer's solution constantly gassed with 95% O_2-5% CO_2, and pH was 7.4. The mucosal medium was 3 ml Ringer's solution diluted 1:5 with distilled water. Mounted hemibladders were allowed to stabilize for 2 h, during which time the serosal media were replaced every 30 min. The control and experimental hemibladders were obtained from the same toad. Water flow was measured as weight loss per minute per hemibladder and expressed as milligrams/ minute per 10 cm^2 surface area. The mean surface area for the hemibladders used in these experiments was 10.4 ± 2.8 cm^2, $n = 38$. Surface area was calculated assuming the hemibladder sacs were spheres where volume = $(4/3)\pi r^3$ and surface area = $4\pi r^2$, where r is radius in centimeters. The volume was determined by submerging a hemibladder in a beaker of Ringer's solution containing the experimental agent, up to the level of the thread used to secure it to the glass tube. The hemibladder was then filled with the usual mucosal solution of diluted Ringer's to the point where the bottom of the water meniscus was level with the thread on the glass tube. At this point the pressure inside the hemibladder equalled the pressure outside the hemibladder so no distension occurred and the hemibladder appeared spherical. The mucosal solution was drawn up into a graduated

syringe and volume determined to within 0.1 ml. The procedure was then carried out a second time. The coefficient of variation of the differences between the two determinations was 1.4% ($n = 16$). After the experiment was completed, the volume of hemibladders treated with U44069, U46619, or TXB_2 was determined, then hemibladders were washed three times with Ringer's solution at 10-min intervals to remove the drug, and the procedure repeated.

Since U46619, U44069, and TXB_2 contracted the hemibladders, water flow was normalized to hemibladder surface area. Because water is assumed to move through pores in the apical membrane (10), a highly folded contracted bladder may impede water flow leading to an underestimate of water flow when compared with a hemibladder of equal weight that is not contracted. Determination of surface area, on the other hand, serves as an index of the amount of epithelium available for unimpeded flow (11). To test the hypothesis that hemibladder surface is a valid normalization parameter for water flow, one member of each of eight pairs of hemibladders was filled with 3 ml of diluted Ringer's solution while the other was fully distended by adding mucosal medium over a 1-h period, until no more could be accommodated, usually 20–30 ml. The distended hemibladders were emptied and 3 ml diluted Ringer's solution was added. These hemibladders remained distended after emptying. The hemibladders were allowed to stabilize 30 min, then vasopressin (50 mU/ml) was added and water flow was determined for 30 min. The weights of the hemibladders were not significantly different between the two groups (149 ± 8 mg in the undistended hemibladders and 133 ± 7 mg in the distended hemibladders, $n = 8$, each group). At the end of the vasopressin treatment period, the measured surface areas of the distended hemibladders were significantly greater than their paired controls (10.0 ± 3.0 cm^2 in the undistended hemibladders and 16.1 ± 3.0 cm^2 in the distended hemibladders, $P < 0.01$, $n = 8$, pairs). Water flows normalized to weight were significantly greater in the distended hemibladders than in their paired undistended controls (0.28 ± 0.04 mg/min per mg hemibladder in undistended hemibladders and 0.50 ± 0.07 mg/min per mg hemibladder in the distended hemibladders, $P < 0.01$, $n = 8$ pairs). However, when water flow was normalized to surface area there was no difference between the two groups (3.6 ± 0.2 mg/min per cm^2 hemibladder in undistended hemibladders and 3.7 ± 0.2 mg/min per cm^2 hemibladder in the distended hemibladders). Similar observations have been reported by Kachadorian and Levine (11). Although the data demonstrate that normalization of osmotic water flow to surface area is preferable in situations where surface areas may be different, for several representative experiments, we have normalized water flow to mean hemibladder wet weight in addition to the normalization to surface area. The mean hemibladder wet weight for these experiments was 164 ± 12 mg ($n = 38$). Since weight was not changed by the agents used in these studies, this further confirms that absolute water flow was increased by each experimental agent.

Measurement of immunoreactive TXB_2 ($iTXB_2$). Serosal media (10 ml) were collected and acidified to pH 3.5 with formic acid and [^{3}H]TXB_2 (1,500 cpm) was added to account for recovery losses. The media were extracted twice with 30 ml of ethyl acetate and the ethyl acetate was evaporated under a stream of nitrogen. Because U44069 and U46619 were found to cross react with the TXB_2 antibody, the dried extracts were redissolved in methanol and subjected to silica gel thin-layer chromatography using the solvent system chloroform:methanol:acetic acid:water (90:8:1:0.8). The retardation factor (R_f) for the analogs was 0.71 and for TXB_2 0.30. The zone corresponding to TXB_2 was scraped and TXB_2

was eluted from the silica gel using 5% acetic acid in methanol (vol/vol) (12) and the solvent was evaporated under a stream of nitrogen. The residue was redissolved in 50 μl methanol and 1 ml chloroform and subjected to column chromatography on silicic acid as previously described (1) and assayed for iTXB$_2$ with a previously described radioimmunoassay method (1). The TXB$_2$ antibody was provided by Dr. J. B. Smith, Cardeza Foundation, Philadelphia, Pa. The antibody does not significantly cross-react with prostaglandins 6-keto-PGF$_{1\alpha}$, PGD$_2$, PGF$_{2\alpha}$, PGE$_2$, or PGE$_1$ (1). Indomethacin (50 μM) reduced iTXB$_2$ synthesis to less than the limit of detection of the radioimmunoassay (0.009 pmol/min per hemibladder).

Effects of U46619 on vasopressin-stimulated water flow. Hemibladders were pretreated with indomethacin (50 μM) for the final hour of the stabilization period. U46619 (1.0 μM) was added to the experimental hemibladders and varying concentrations of vasopressin (0.1, 0.2, 0.3, 0.6, or 2.0 mU/ml) were added to both the control and experimental hemibladders, and osmotic water flow was measured for 30 min. The serosal bathing media were changed three times at 10-min intervals. Then the hemibladders were allowed to stabilize for 30 min. A supramaximal dose of vasopressin (10 mU/ml) was added to both the control and U46619-treated hemibladders and water flow was measured for 30 min. Water flow stimulated by the submaximal doses of vasopressin was expressed as a fraction of water flow stimulated by 10 mU/ml vasopressin.

Statistical methods. All data are presented as mean±SEM. Tests for significance were performed with Student's *t* test for paired observations comparing the two hemibladders from a single toad (13). The slopes of the lines in Fig. 3 were determined by linear regression analysis of the 0.2 to 2.0 mU/ml vasopressin concentrations. To determine the significance of the differences between the control and U46619-treated hemibladders at vasopressin concentrations of 0.2 to 2.0 mU/ml, an analysis of covariance was performed using the randomized complete-block design (13).

RESULTS

Effects of U44069 and U46619 on iTXB$_2$ synthesis. U44069, in addition to acting as a prostaglandin endoperoxide/TXA$_2$ agonist, has been reported to be a thromboxane synthetase inhibitor (14). At a concentration of 1 μM, U44069 had no effect on basal iTXB$_2$ synthesis (0.075±0.014 and 0.066±0.012 pmol/min per hemibladder, control and U44069, respectively, n = 6). However, U44069 significantly inhibited vasopressin (5 mU/ml)-stimulated iTXB$_2$ synthesis from 0.121±0.020 to 0.088±0.021 pmol/min per hemibladder (P < 0.01, n = 6) (Table I). U46619 had no effect on either basal or vasopressin-stimulated iTXB$_2$ synthesis (Table I).

Effects of TXB$_2$, U44069, and U46619 on basal water flow. In hemibladders not pretreated with indomethacin or meclofenamic acid, U46619 (1 μM) caused a significant increase in basal water flow, from 2.8±0.5 to 5.7±0.9 mg/min per 10 cm^2 (P < 0.05, n = 5), whereas U44069 tended to increase water flow (3.0±0.6 to 4.4±1.2 mg/min per 10 cm^2, P > 0.05, n = 5). We have found that the water flow responses are more uniform among hemibladders in the presence

of cyclooxygenase inhibitors (unpublished observations). Therefore, to reduce the contributions of variable amounts of endogenous PGE$_2$ and TXA$_2$ to the water flow response, hemibladders were pretreated with indomethacin (50 μM). Both U44069 and U46619 stimulated basal water flow in indomethacin pretreated hemibladders (Fig. 1). U44069 (1 μM) significantly stimulated basal water flow from 4.2±0.8 to 12.4±1.2 mg/min per 10 cm^2 (P < 0.05, n = 5), or from 4.2±0.8 to 9.1±1.1 mg/min per 150 mg hemibladder wet wt (P < 0.05, n = 5) (Methods). U46619 (1 μM) was more effective than U44069, increasing water flow from 2.8±1.0 to 21.8±2.2 mg/min per 10 cm^2 (P < 0.01, n = 5), or from 2.6±1.0 to 17.1±1.9 mg/min per 150 mg
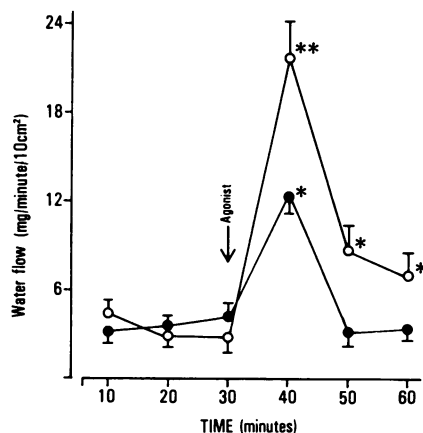

FIGURE 1 Stimulation of water flow in response to the endoperoxide/TXA$_2$ agonists U44069 and U46619. Hemibladders were pretreated with indomethacin (50 μM) for 1 h. Basal water flow was measured for 30 min, then U44069 (●) or U46619 (○) (1 μM) was added to the serosal bathing medium and water flow was measured for 30 min. Each point represents the mean±SEM for five hemibladders. * P < 0.05, ** P < 0.01, compared with basal.

hemibladder ($P < 0.01$, $n = 5$). A lower concentration of U46619 (0.1 μM) stimulated basal water flow from 2.7±1.1 to 13.4±1.0 mg/min per 10 cm^2 ($P < 0.02$, $n = 5$), or from 2.5±1.0 to 10.1±0.9 mg/min per 150 mg hemibladder ($P < 0.05$, $n = 5$) (data not shown). TXB$_2$ stimulated basal water flow in a dose-dependent manner (Fig. 2). The highest concentration of TXB$_2$ used, 50 μM, stimulated basal water flow from 2.1±0.3 to 15.9±0.8 mg/min per 10 cm^2 ($P < 0.02$, $n = 4$), or from 2.1±0.3 to 14.3±0.7 mg/min per 150 mg hemibladder ($P < 0.05$, $n = 4$). To determine that the stimulation of basal water flow was not due to some nonspecific effect of prostanoic acid derivatives, we tested the effects of PGE$_1$ on basal water flow. PGE$_1$ (2.5 μM) caused no change in basal water flow (2.1±0.8 to 2.3±0.6 mg/min per 10 cm^2, $n = 5$), confirming previous observations (15).

Effects of trans-13-azaprostanoic acid on TXB$_2$- and U46619-stimulated water flow. In the toad bladder *trans*-13-azaprostanoic acid has no effect on basal water flow, or on vasopressin-stimulated water flow in the presence of inhibitors of fatty acid cyclooxygenase (5). However, *trans*-13-azaprostanoic acid inhibits vasopressin-stimulated water flow in untreated hemibladders (5). Thus, the antagonistic effects of *trans*-13-azaprostanoic acid on water flow stimulated by TXB$_2$ and U46619 were assessed. *trans*-13-Azaprostanoic acid (300 μM) inhibited TXB$_2$ (25 μM)-stimulated water flow 79±9%, ($P < 0.02$, $n = 5$). *trans*-13-Azaprostanoic acid also antagonized the effects of U46619 in a dose-dependent fashion (Fig. 3). At a concentration of 300 μM, *trans*-13-azaprostanoic acid completely inhibited the water flow response to 0.1 μM U46619 (Fig. 3). Since indomethacin inhibits cyclic nucleotide phosphodiesterase as well as fatty acid cyclooxygenase
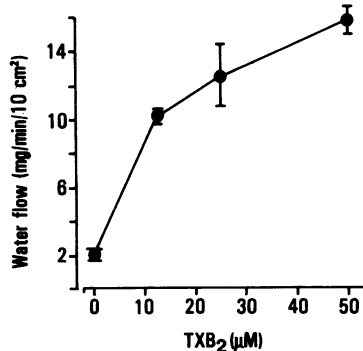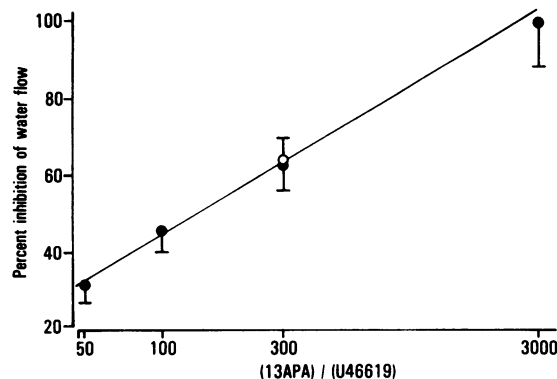

FIGURE 3 Inhibition of U46619-stimulated water flow with *trans*-13-azaprostanoic acid. The abscissa represents the molar concentration ratio of *trans*-13-azaprostanoic acid (13APA) to U46619. ●, Represent hemibladders pretreated with indomethacin (50 μM) and ○ represents hemibladders pretreated with meclofenamic acid (1 μM) for 1 h. The hemibladders were then preincubated for 10 min with *trans*-13-azaprostanoic acid before the addition of U46619. Each point represents the mean±SEM for five hemibladders.

(16), we also determined the effect of *trans*-13-azaprostanoic acid on U46619-stimulated water flow in the presence of another inhibitor of fatty acid cyclooxygenase, meclofenamic acid, which does not inhibit cyclic nucleotide phosphodiesterase (17). At a molar concentration ratio of 300:1, *trans*-13-azaprostanoic acid inhibited U46619-stimulated water flow 65±6% ($P < 0.02$, $n = 5$) in meclofenamic acid (1 μM)-pretreated hemibladders while U46619-stimulated water flow was inhibited 64±6% in indomethacin (50 μM)-pretreated hemibladders (Fig. 3). The absolute levels of TXB$_2$- and U46619-stimulated water flow were significantly ($P < 0.05$) greater in the indomethacin pretreated hemibladders than in the meclofenamic acid pretreated hemibladders. Since this concentration of indomethacin has previously been shown to inhibit cyclic nucleotide phosphodiesterase (16), the increased water flow in the presence of indomethacin compared with meclofenamate suggests that these agonists could be activating adenylate cyclase.

Effects of cis-13-azaprostanoic acid on U46619- and vasopressin-stimulated water flow. In the platelet the *cis* isomer of 13-aza-prostanoic acid is inactive as an antagonist of TXA$_2$ or the prostaglandin endoperoxide/TXA$_2$-like agonist U46619 (6). Thus, we tested the effects of *cis*-13-azaprostanoic acid on U46619-stimulated water flow. At a concentration of 300 μM, *cis*-13-azaprostanoic acid had no effect on water flow stimulated by 0.1 μM 46619. In control hemibladders U46619 stimulated water flow from 2.4±0.5 to 14.8±1.3 mg/min per 10 cm^2, and in the *cis*-13-azaprostanoic acid pretreated hemibladders, water flow was stimulated from 1.7±0.6 to 13.6±3.2 mg/min per 10 cm^2.

FIGURE 2 Stimulation of water flow in response to TXB$_2$. Hemibladders were pretreated with indomethacin (50 μM) for 1 h. Basal water flow was measured for 30 min ($n = 10$). TXB$_2$ was added to the serosal bathing medium and water flow was measured for three consecutive 10-min periods. Each point represents the mean±SEM for three or four hemibladders during the first 10-min period. Each point is significant at $P < 0.05$ or $P < 0.02$ compared with basal.

Having established that cis-13-azaprostanoic acid was inactive as an antagonist of U46619-stimulated water flow, we tested its effects on vasopressin-stimulated water flow. At a concentration of 300 μM, cis-13-azaprostanoic acid had no effect on water flow stimulated by 1.0 mU/ml vasopressin. In the control hemibladders water flow was stimulated from 1.4±0.4 to 42.5±4.0 mg/min per 10 cm^2 ($P < 0.02, n = 6$), and in the cis-13-azaprostanoic acid pretreated hemibladders, water flow was stimulated from 1.5±0.5 to 40.3±3.2 mg/min per 10 cm^2 ($P < 0.02, n = 6$). The difference between the two vasopressin-stimulated groups was not significant.

Effects of U46619 on vasopressin-stimulated water flow. Because TXA$_2$ may be a positive modulator of vasopressin-stimulated water flow, the effects of the prostaglandin endoperoxide/TXA$_2$ agonist U46619 was determined on vasopressin-stimulated water flow in indomethacin pretreated hemibladders. Vasopressin was added to the hemibladders and, at the same time, U46619 (1.0 μM) was added to one hemibladder of each pair. Water flow was determined for 30 min. Vasopressin increased water flow in a dose-dependent manner from 0.1 to 2.0 mU/ml (Fig. 4). U46619 significantly increased water flow at each concentration of vasopressin ($P < 0.001$); however, by paired t analysis the effect was not significant at the 2.0 mU/ml concentration (Fig. 3). The dose-response curve was significantly shifted to the left ($P < 0.001$) in a parallel fashion (slopes of 0.64 and 0.62, control and U46619, respectively) suggesting that vasopressin and U46619 are acting on the same sequence of events to increase water flow. In hemibladders pretreated with 300 μM trans-13-azaprostanoic acid ($n = 6$), U46619 did not significantly enhance vasopressin-stimulated water flow (data not shown).

DISCUSSION

Arachidonic acid metabolites have been shown to modulate vasopressin stimulation of water permeability in the toad urinary bladder. PGE$_2$ acts as a negative modulator of vasopressin-stimulated water flow (18), while TXA$_2$ appears to act as a positive modulator or mediator (1, 5). Since TXA$_2$ is a short-lived intermediate of arachidonic acid metabolism, direct demonstration of its effects in the toad bladder is technically difficult. Although TXB$_2$ is generally considered to be an inactive product of hydrolysis of TXA$_2$, at higher concentrations it has been shown to exhibit actions similar to TXA$_2$ (8). Thus, in the present studies, the effects of TXB$_2$ and the stable endoperoxide/TXA$_2$-like agonists U44069 and U46619 were assessed on the water flow response of the toad bladder. U46619 stimulated water flow in bladders that were not pretreated with cyclooxygenase inhibitors,
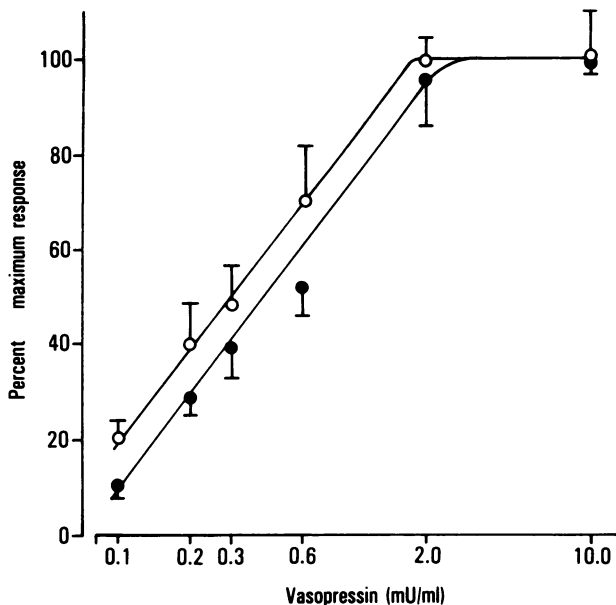

FIGURE 4 Effects of U46619 on vasopressin-stimulated water flow. Hemibladders were pretreated with indomethacin (50 μM) for 1 h. Vasopressin (●) or vasopressin plus U46619 (1 μM) (○) was added to the serosal baths and water flow was measured for 30 min. After thorough washing to return water flow to basal levels and a 30-min recovery period, a supramaximal dose of vasopressin (10 mU/ml) or vasopressin plus U46619 (1 μM) was added and water flow was determined for another 30 min. Water flow is expressed as a percentage of this maximum response (64.3±4.6 mg/min per 10 cm^2, $n = 72$). Each point represents the mean±SEM for six hemibladders. U46619 significantly ($P < 0.001$) increased vasopressin-stimulated water flow and significantly shifted the dose-response curve to the left ($P < 0.001$) in a parallel fashion (slopes were 0.64 for the control hemibladders and 0.62 for the U46619-treated hemibladders).

whereas U44069 did not. The failure of U44069 to stimulate water flow may be due to its simultaneous inhibition of TXA$_2$ synthesis, which would negate its agonistic properties. All three of these compounds stimulated basal water flow in indomethacin- and meclofenamic acid-pretreated hemibladders. The relative order of potency for the three compounds was similar to that which has previously been reported (7, 8). U46619 was twofold more potent than U44069, an observation that has been previously made in aortic strips (7). That the stimulation of the water flow response was not a nonspecific effect of prostanoic acid derivatives is borne out by the observation that PGE$_1$ did not stimulate water flow.

These compounds may be mimicking the action of either the prostaglandin endoperoxides or TXA$_2$. That the active arachidonic acid metabolite in the toad bladder is TXA$_2$ and not the endoperoxides is supported by the previous observations that the thromboxane synthetase inhibitors imidazole and 7-(1-

imidazolyl)-heptanoic acid inhibited vasopressin-stimulated water flow (1, 5), while the concentration of the endoperoxides would not have changed. The complete pharmacology of the agents used in these studies is not known. We have attempted to characterize some of their actions in the isolated toad bladder. U46619, U44069, and TXB₂, three compounds of different structure, but which in a variety of tissues have similar actions (7, 8), all exhibited similar actions in the toad bladder, at concentration ratios similar to those used in other tissues (7, 8). These actions have been assumed in the past to mimic TXA₂. However, definitive evidence supporting this notion is lacking and other actions of these compounds may exist. The effects of these compounds are antagonized by trans-13-azaprostanoic acid, but not by cis-13-azaprostanoic acid, properties consistent with specific receptor agonist activity (6). Furthermore, trans-13-azaprostanoic acid has no prostaglandin- or thromboxane-independent actions in the toad bladder (5). Finally, these agents were studied in the absence of endogenous arachidonic acid metabolism to avoid the influence of possible alterations in the cellular actions of endogenous prostaglandins. Thus, while alternative explanations may exist for the observations reported herein, the data are consistent with the hypothesis that TXA₂ may partially mediate the water flow response to vasopressin in toad bladder. Additional experiments are required to more rigorously test this concept.

U46619 enhanced vasopressin-stimulated water flow to submaximal concentrations of vasopressin, resulting in a parallel shift in the dose-response curve. The parallel shift supports the notion that vasopressin and U46619 are acting on the same pathway(s) leading to increased water flow. Since the response to vasopressin is enhanced, the step affected by the agonist may be rate-limiting at low doses. However, this step is not rate-limiting at high concentrations of vasopressin since the agonist did not enhance water flow to supramaximal doses of vasopressin. In contrast to these observations, in a preliminary study, Ludens and Taylor (19) found that U44069 and U46619 inhibited vasopressin-stimulated water flow in hemibladders not pretreated with cyclooxygenase inhibitors. Their findings may be reconciled with ours in part, since U44069 inhibited vasopressin-stimulated iTXB₂ synthesis (vide supra), which may account for the decrease in the vasopressin-stimulated water flow response. However, TXB₂ and U46619 also cause a marked sustained contraction of the bladders that reduced their surface areas ~5 and 25%, respectively. Therefore, simply weighing the empty hemibladder at the end of the experiment leads to a substantial overestimate of transporting surface in the agonist-treated hemibladders, with concomitant underestimation of water flow. Indeed, in preliminary experiments us-

ing the conditions of Ludens and Taylor (19), without correcting for the decrease in surface area, we also obtained a reduction in vasopressin-stimulated water flow in the presence of U44069 and U46619, compared with control hemibladders. However, in these experiments, TXB₂, U44069, and U46619 all increased the absolute magnitude of basal water flow.

trans-13-Azaprostanoic acid has previously been found to antagonize vasopressin-, but not cAMP-stimulated water flow (5). Further, the antagonistic effect of trans-13-azaprostanoic acid on vasopressin-stimulated water flow is dependent on intact arachidonic acid metabolism (5). In indomethacin- or meclofenamic acid-pretreated hemibladders, the trans isomer of 13-azaprostanoic acid antagonized the increase in water flow due to U46619 or TXB₂. In contrast, the cis isomer of 13-azaprostanoic acid had no effect on either vasopressin- or U46619-stimulated water flow. Thus, these studies support the concept that trans-13-azaprostanoic acid specifically antagonizes a site of action common to both vasopressin and U46619.

The significance of TXA₂ as a mediator or modulator of vasopressin-stimulated water flow is presently unknown. The previous observations that TXA₂ synthesis inhibition or TXA₂ antagonism resulted in approximately a 30% inhibition of vasopressin-stimulated water flow while PGE synthesis was not altered (5), and the present observations suggest that TXA₂ plays a role in the peptide's stimulation of water flow in the toad urinary bladder. Fatty acid cyclooxygenase inhibitors such as indomethacin inhibit the syntheses of both PGE₂ and TXA₂ in the toad urinary bladder (5, 18) and augment vasopressin-stimulated water flow in both the toad bladder (18) and in the mammalian kidney (20). In the toad bladder, the synthetic rate of PGE₂ is greater than that of TXA₂ (1, 5) and it appears to be more potent than TXA₂ (21); thus the net result of complete inhibition of the fatty acid cyclooxygenase would be expected to be augmentation of vasopressin-stimulated water flow. The studies with fatty acid cyclooxygenase inhibitors also point out that TXA₂ synthesis is not an absolute requirement for vasopressin-stimulated water flow in the toad urinary bladder. In these studies U46619 and TXB₂ were used in micromolar concentrations whereas the synthetic rate of TXA₂ yielded serosal concentrations in the picomolar range. Thus, the applicability of these observations in the toad urinary bladder to the mechanism of action of vasopressin in the mammalian kidney remains unknown.

ACKNOWLEDGMENTS

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