

Inhibition of Vasopressin-Stimulated Prostaglandin E Biosynthesis by Chlorpropamide in the Toad Urinary Bladder

MECHANISM OF ENHANCEMENT OF VASOPRESSIN-STIMULATED WATER FLOW

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ABSTRACT Chlorpropamide is known to enhance the water permeability response of the toad urinary bladder to vasopressin and to theophylline. In other studies, we have shown that prostaglandin E synthesis by the toad bladder inhibits the water permeability response to arginine vasopressin and to theophylline. In this study, the effect of chlorpropamide on vasopressin-, theophylline-, and cyclic AMP-stimulated water flow and on prostaglandin E biosynthesis was investigated in the toad urinary bladder in vitro. Chlorpropamide inhibited prostaglandin E biosynthesis during vasopressin-, theophylline- and cyclic AMP-stimulated water flow. Tolbutamide and glyburide, two other sulfonylurea compounds, also enhanced vasopressin-stimulated water flow and inhibited vasopressin-stimulated prostaglandin E biosynthesis. We conclude that the mechanism of enhancement on vasopressin-stimulated water flow by the sulfonylureas is the inhibition of prostaglandin E biosynthesis.

INTRODUCTION

Arginine vasopressin stimulates water flow along an osmotic gradient in the toad urinary bladder and certain other epithelial membranes (1). Vasopressin stimulates adenylate cyclase activity. This results in

an accumulation of cyclic AMP, which elicits an increase in water permeability (1). Exogenous cyclic AMP and theophylline, a cyclic nucleotide phosphodiesterase inhibitor, mimic arginine vasopressin (AVP)¹ in stimulating water permeability (2). Prostaglandin E (PGE) inhibits the accumulation of cyclic AMP (3, 4) thus inhibiting the water permeability response to AVP and to theophylline (5). We have recently shown that vasopressin stimulates PGE biosynthesis in the toad bladder (6). Vasopressin stimulates acylhydrolase (phospholipase) activity and increases the rate of arachidonic acid release from endogenous lipid stores which results in increased PGE biosynthesis. Vasopressin-stimulated PGE biosynthesis inhibits the vasopressin stimulation of adenylate cyclase and thereby decreases the water permeability response to vasopressin. The inhibition of endogenous PGE biosynthesis with mepacrine (a phospholipase inhibitor) or with non-steroidal anti-inflammatory agents such as naproxen (which inhibit the addition of oxygen to arachidonic acid) results in an augmented water flow response to vasopressin and theophylline (6).

Chlorpropamide, a sulfonylurea widely used in the treatment of diabetes mellitus, is effective in the treatment of pituitary diabetes insipidus (7, 8). It has been suggested that chlorpropamide decreases urine volume in patients with diabetes insipidus by increasing

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¹Abbreviations used in this paper: AVP, arginine vasopressin; PG, prostaglandin (used variously according to the identification of a given prostaglandin, i.e., PGE, PGE₁, PGE₂, and PGF_{2α}).

the release of antidiuretic hormone from the posterior pituitary (9) and (or) by enhancing the peripheral action of vasopressin in the kidney (10–13). In the toad urinary bladder in vitro, chlorpropamide enhances the antidiuretic effect of vasopressin and theophylline but inhibits cyclic AMP-stimulated water flow (11–13). The purpose of this investigation was to evaluate the role of endogenous prostaglandin E biosynthesis in the mechanism of action of chlorpropamide and other sulfonylureas.

METHODS

Toads, *Bufo marinus*, were obtained from National Reagents, Bridgeport, Conn. The urinary bladders were removed from doubly-pithed toads, and the hemibladders were mounted as sacs on bungs. Water flow was measured gravimetrically as previously described (6). Control and experimental paired hemibladders were selected randomly. Agents were added to the serosal solution. Naproxen was added 180 min before the basal period of water flow measurement. Prostaglandin E₂ was added 30 min before the basal period. Freshly prepared serosal solutions containing the appropriate agents were added immediately before the basal and test periods in all experiments. The prostaglandin E content of the serosal solution at the end of the basal and test periods was determined by radioimmunoassay as previously described (14). We have been unable to identify unequivocally as either PGE₁ or PGE₂ the actual prostaglandin produced by the toad urinary bladder. Although we feel that it is most likely PGE₂, we refer to it in the paper merely as prostaglandin E for the sake of accuracy. In those experiments in which we gave arachidonic acid, the specific precursor of PGE₂, we refer to the product as prostaglandin E₂. Vasopressin, cyclic AMP, and theophylline were used at concentrations that result in submaximal water flow in all experiments. Chlorpropamide, tolbutamide, or glyburide was added to the experimental hemibladder only.

To determine the site of action of chlorpropamide in prostaglandin E biosynthesis, hemibladders were incubated with [³H]arachidonic acid, 62 Ci/mmol (New England Nuclear, Boston, Mass.) for 18 h. The serosal solution was changed to fresh Ringer's solution and the experimental hemibladders were treated with 3 mM chlorpropamide for 30 min before the basal period. The serosal solution, after the basal period and after administration of 6 mU/ml vasopressin was extracted with chloroform at pH 3.5, and the

lipids were separated by thin-layer chromatography as previously described (14).

All experiments were performed at room temperature. Statistical analysis was done with Student's *t* test for "paired" observations (15).

Agents used in this study were: Arginine vasopressin (ICN Pharmaceuticals Inc., Cleveland, Ohio), theophylline (ICN Nutritional Biochemicals Div., Cleveland, Ohio), and cyclic AMP (Sigma Chemical Co., St. Louis, Mo.). Sodium tolbutamide, glyburide, and prostaglandin E₂ were kindly provided by Dr. Gerald Zins of The Upjohn Company, Kalamazoo, Mich., chlorpropamide by the Pfizer Chemicals Div., Pfizer, Inc., New York, and sodium naproxen by Syntex Laboratories, Inc., Palo Alto, Calif.

RESULTS

The effect of chlorpropamide on vasopressin-, theophylline-, and cyclic AMP-stimulated water flow and prostaglandin E biosynthesis (Table I). 3 mM chlorpropamide enhanced 1 mU/ml vasopressin- and 5 mM theophylline-stimulated water flow, but inhibited 15 mM cyclic AMP-stimulated water flow. This pattern of chlorpropamide action has been previously reported by Lozada et al. (13), and by Mendoza (11). Chlorpropamide inhibited PGE biosynthesis during vasopressin-, theophylline-, and cyclic AMP-stimulated water flow.

Chlorpropamide enhanced theophylline-stimulated water flow from 16.1 to 33.2 mg/min per hemibladder, and inhibited PGE biosynthesis from 0.6 to 0.4 pmol/min/hemibladder. To test whether such an apparently small difference in PGE biosynthesis results in such marked enhancement of water flow the following experiment was performed: endogenous PGE biosynthesis was completely inhibited in control and experimental hemibladders by incubation with 100 μ M naproxen for 3 h. Exogenous PGE₂ was added to the serosal solution of control hemibladder, 0.9 nM, and experimental hemibladder, 0.6 nM, the estimated mean PGE concentrations during the theophylline-chlorpropamide experiment. 10 mM theophylline-stimulated water flow was 1.8 \pm 0.6 and 11.6 \pm 2.2

TABLE I
The Effect of 3 mM Chlorpropamide on AVP-, Theophylline-, and Cyclic AMP-Stimulated Water Flow and PGE Biosynthesis

Agents added	n	Water flow*		PGE biosynthesis*	
		Control	Chlorpropamide treated	Control	Chlorpropamide treated
		mg/min per hemibladder		pmol/min per hemibladder	
AVP (1 mU/ml)	8	14.2 \pm 3.2	19.3 \pm 3.4†	5.0 \pm 0.2	2.2 \pm 0.1†
Theophylline (5 mM)	6	16.1 \pm 5.7	33.2 \pm 5.8†	0.6 \pm 0.1	0.4 \pm 0.1†
Cyclic AMP (15 mM)	6	39.7 \pm 4.7	24.5 \pm 7.7†	0.8 \pm 0.1	0.5 \pm 0.1†

* Each value represents the mean \pm SEM.

† *P* < 0.02.

TABLE II
The Effect of Naproxen and PGE₂ on the Effect of Chlorpropamide on Vasopressin- and Theophylline-Stimulated Water Flow

Agents added	Water flow	
	Control	Chlorpropamide treated
	mg/min per hemibladder	
AVP (0.25 mU/ml) + Naproxen (0.1 mM)	29.0±4.9	12.7±3.9*
AVP (3 mU/ml) + PGE ₂ (1 μM)	15.6±3.2	4.7±1.6*
Theophylline (3 mM) + Naproxen (0.1 mM)	20.4±2.1	9.1±1.1*
Theophylline (20 mM) + PGE ₂ (10 nM)	8.3±1.1	3.0±0.9*

PGE biosynthesis in naproxen-treated hemibladders was 0.01±0.01 pmol/min per hemibladder (mean±SEM, n = 24). * P < 0.02, n = 6.

mg/min hemibladder (n = 4, P < 0.005) in the control and experimental hemibladders, respectively. It is evident that theophylline-stimulated water flow is extremely sensitive to alterations in PGE₂ concentration. Because inhibition of PGE biosynthesis results in increased vasopressin- and theophylline-stimulated water flow (6), we conclude that the enhancement of vasopressin- and theophylline-stimulated water flow by chlorpropamide is secondary to the inhibition of PGE biosynthesis.

The effect of chlorpropamide on vasopressin- and theophylline-stimulated water flow in the presence of prostaglandin E₂ and naproxen (Table II). The stimulation of water flow in the toad urinary bladder by cyclic AMP is independent of the prostaglandin system (6). The inhibition of cyclic AMP-stimulated water flow by chlorpropamide is, therefore, secondary to an effect independent of the inhibition of PGE biosynthesis. To investigate this second effect of

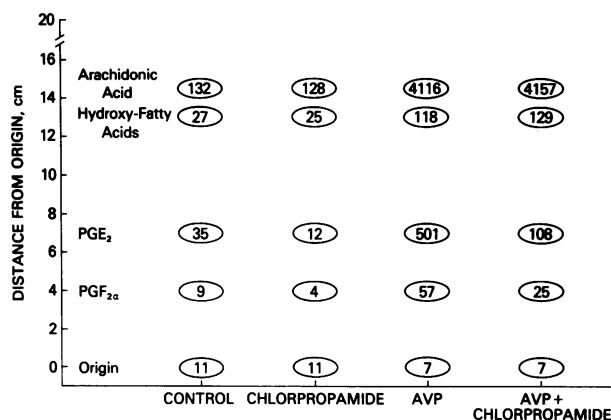


FIGURE 1 Thin-layer chromatogram of radioactive lipids released from toad urinary bladders in vitro after incubation with [³H]arachidonic acid. Chlorpropamide was added to the experimental hemibladder 30 min before the basal period. Each value represents the counts per minute of lipid released per minute per hemibladder and is the mean of six determinations. Arachidonic acid, PGE₂, and PGF_{2α} standards were used on each thin-layer chromatographic plate. The R_f values for arachidonic acid, PGE₂, and PGF_{2α} in this system were 0.70, 0.35, and 0.20, respectively. The hydroxy-fatty acids and the material at the origin were not identified.

chlorpropamide, vasopressin- and theophylline-stimulated water flow were measured after elimination of PGE biosynthesis with 0.1 mM naproxen or after the addition of 1 μM PGE₂. When PGE biosynthesis was completely inhibited by naproxen, chlorpropamide inhibited vasopressin- and theophylline-stimulated water flow. In the presence of exogenous PGE₂, chlorpropamide inhibited vasopressin- and theophylline-stimulated water flow.

The effect of tolbutamide and glyburide on vasopressin-stimulated water flow and prostaglandin E biosynthesis (Table III). Other sulfonyleureas were tested to see whether the effects of chlorpropamide are characteristic of this class of compounds. 3 mM

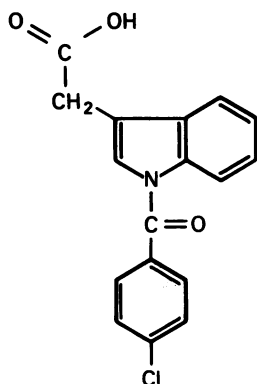
TABLE III
The Effect of Glyburide and Tolbutamide on AVP-Stimulated Water Flow and PGE Biosynthesis

Agents added	Water flow		PGE biosynthesis	
	Control	Experimental	Control	Experimental
	mg/min per hemibladder		pmol/min per hemibladder	
AVP (1 mU/ml)				
+ Glyburide (20 μM)	25.1±2.4	48.0±4.1*	4.5±0.2	1.3±0.1*
+ Tolbutamide (3 mM)	23.1±3.8	42.7±5.0*	4.2±0.2	1.3±0.1*
AVP (0.25 mU/ml + Naproxen (0.1 mM)				
+ Glyburide (20 μM)	36.7±5.2	16.2±3.6*	0.02±0.01	0.02±0.01
+ Tolbutamide (3 mM)	28.9±6.3	15.7±3.8*	0.01±0.01	0.01±0.01

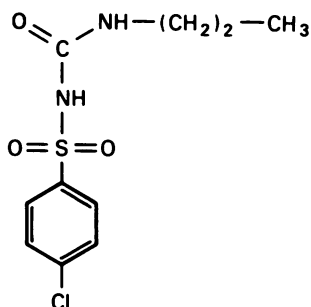
* P < 0.02, n = 6.

tolbutamide and 20 μ M glyburide increased 1 mU/ml vasopressin-stimulated water flow and decreased vasopressin-stimulated PGE biosynthesis. When PGE biosynthesis was inhibited by naproxen, tolbutamide and glyburide inhibited 0.25 mU/ml vasopressin-stimulated water flow. We interpret these results to mean that chlorpropamide, tolbutamide, and glyburide enhance vasopressin-stimulated water flow by inhibiting PGE biosynthesis. When PGE biosynthesis is inhibited by naproxen, the sulfonylureas inhibit vasopressin-stimulated water flow via a mechanism independent of the prostaglandin system.

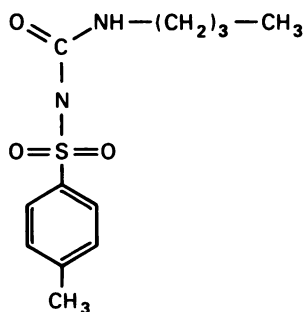
The step in prostaglandin biosynthesis affected by chlorpropamide (Fig. 1). Chlorpropamide had no effect on the rate of [3 H]arachidonic acid release from lipid stores during the basal period, but it decreased the rate of basal [3 H]PGE₂ release. Vasopressin markedly stimulated arachidonic acid release in both the control and chlorpropamide-treated hemibladders. The amount of [3 H]PGE₂ released after vasopressin stimulation, however, was diminished in the chlorpropamide-treated hemibladders. This pattern resembles that of hemibladders incubated with naproxen (6), an arachidonic acid oxygenase inhibitor.



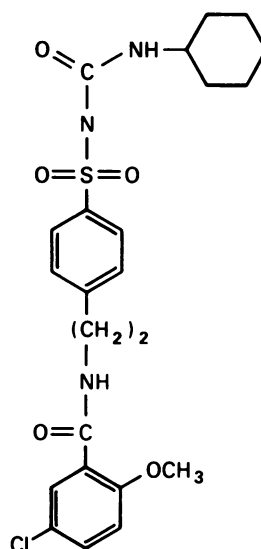
INDOMETHACIN



CHLORPROPAMIDE



TOLBUTAMIDE



GLYBURIDE

FIGURE 2 Chemical structures of indomethacin, chlorpropamide, tolbutamide, and glyburide.

DISCUSSION

Chlorpropamide has two effects on the water permeability of the toad bladder. First, the enhancement of vasopressin- and theophylline-stimulated water flow by chlorpropamide is due to the inhibition of PGE biosynthesis. The reduction in basal and vasopressin-stimulated PGE biosynthesis by chlorpropamide is responsible for the enhancement of theophylline- and vasopressin-stimulated water flow, respectively. It is interesting to note that chlorpropamide is particularly effective in enhancing the effect of low concentrations of vasopressin (16). The stimulation of PGE biosynthesis by vasopressin is most striking at low concentrations of vasopressin (6). It is thus not surprising that the enhancement of vasopressin-stimulated water flow, which is dependent upon the inhibition of PGE biosynthesis, is most marked at low concentrations of vasopressin. A second effect, the inhibition of cyclic AMP-stimulated water flow by chlorpropamide, is independent of the prostaglandin system. The mechanism of this effect is unknown, but it may represent a toxic action of chlorpropamide; this action is reflected in its inhibition of sodium transport by the bladder. Under the conditions used in the present study, chlorpropamide causes a 50% fall in the short circuit current of the toad bladder (17). When the influence of endogenous prostaglandin is eliminated by naproxen or by large amounts of exogenous PGE₂, this second effect is manifest as an inhibition of vasopressin- and theophylline-stimulated water flow.

Tolbutamide and glyburide also inhibit PGE biosynthesis and augment vasopressin-stimulated water flow. When PGE biosynthesis was inhibited by naproxen, vasopressin-stimulated water flow was inhibited by tolbutamide and glyburide as by chlorpropamide. These findings indicate that both the inhibition of PGE biosynthesis, and the other as yet unexplained effect of chlorpropamide, tolbutamide, and glyburide, are characteristics of the sulfonylurea class of organic compounds.

The synthesis of PGE₂ by the toad urinary bladder is dependent on a number of steps. Arachidonic acid, stored in cellular lipids (phospholipids, triglycerides, and cholesterol esters) is released by vasopressin via stimulation of an acylhydrolase (phospholipase). The release of arachidonic acid can be inhibited with mepacrine, a phospholipase inhibitor (18). Molecular oxygen is added to free arachidonic acid by an oxygenase to form a prostaglandin endoperoxide. The nonsteroidal anti-inflammatory agents, such as indomethacin and naproxen, inhibit this oxygenase (19). The prostaglandin endoperoxides are converted to PGE₂ via an isomerase and peroxidase. The sulfonylureas are structurally similar (Fig. 2) to indomethacin, and they

probably diminish PGE₂ biosynthesis by inhibiting the oxygenase.

The antidiuretic action of the sulfonylureas used in the treatment of diabetes insipidus, and the hyponatremia resulting from chlorpropamide therapy in patients with diabetes mellitus, are secondary to the enhancement of vasopressin action. This enhanced vasopressin action is probably due to the inhibition of vasopressin-stimulated renal PGE biosynthesis in vivo (20, 21) analogous to that described here. The inhibition of PGE biosynthesis with nonsteroidal anti-inflammatory agents has been shown to be effective in the treatment of diabetes insipidus (22).

It is interesting to consider the mechanism of action of sulfonylureas in the treatment of diabetes mellitus with respect to the inhibition of PGE biosynthesis. Robertson and Chen have shown that sodium salicylate, an oxygenase inhibitor, augments insulin release after a glucose load in the diabetic patient (23). It is possible that chlorpropamide inhibition of prostaglandin biosynthesis is related to the increased insulin release observed in chlorpropamide-treated patients with diabetes mellitus (24).

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