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Role of Prostaglandin E (PGE) in the Modulation of the Action of Vasopressin on Water Flow in the Urinary Bladder of the Toad and Mammalian Kidney

Jack Orloff and Randall Zusman

National Heart, Lung, and Blood Institute, Bethesda, Maryland

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Summary. PGE_1 and PGE_2 are known to interfere with the water permeability effect of vasopressin in toad bladder and kidney. It has been proposed that endogenous prostaglandin E (PGE), synthesized within cells of vasopressin-sensitive tissues, serves to modulate the permeability changes elicited by the neurohypophyseal hormone. Direct evidence in support of this hypothesis is as follows: vasopressin increases the biosynthesis of PGE_2 in renal interstitial cells and in isolated toad bladder. In the latter, inhibition of vasopressin-induced synthesis of PGE by a variety of inhibitors results in a greater water permeability response to vasopressin. It appears that vasopressin has two effects in toad bladder and kidney: (i) it activates adenylate cyclase thereby increasing the concentration of adenosine 3′,5′ monophosphate (cyclic AMP), the nucleotide responsible for the resultant increase in water permeability; and (ii) it activates a phospholipase that serves to release arachidonic acid, the precursor of PGE_2 from intracellular pools. The PGE derived from the arachidonic acid diminishes adenylate-cyclase activity, in consequence of which the response of the enzyme to vasopressin is modulated.

One of the most remarkable homeostatic mechanisms in man is that which regulates water balance, thereby maintaining plasma osmolality relatively constant despite wide variations in the intake of water and solute. The effector is antidiuretic hormone (arginine vasopressin in man) whose graded release from the posterior pituitary gland in response to deviations in plasma osmolality elicits appropriate changes in urine flow and urine osmolality.

Our laboratory's interest in this process spans several decades. In the early 1950's we attempted to unravel the renal effects of vasopressin in the intact animal with limited success (Orloff, Wagner & Davidson, 1958). It was not until the summer of 1957 when Professor Ussing spent several months in Bethesda that we became convinced of the utility of epithelial structures such as frog skin and toad bladder as model membranes for examination of hormone action on water and electrolyte transport. Within short order we demonstrated the role of cyclic AMP

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in the regulation of the water permeability of vasopressin-sensitive tissues (Orloff & Handler, 1962). It was obvious by now that graded release of vasopressin from the pituitary, the initiating step in the hypothalamicorenal conservation process that regulates water balance, was only part of a larger process involving many potential rate controlling sites or steps which serve to provide fine control of the volume and osmolality of the fluid compartments of the body. We shall discuss only one of these, the site of action and role of the naturally occurring fatty acid derivative, prostaglandin E, a putative intracellular modulator of the action of vasopressin.

In initial studies directed at elucidating the effect of PGE_1 on the water permeability response to vasopressin, two important observations were made: (i) the isolated collecting tubule of the rabbit was found to be exquisitely sensitive to vasopressin, such that concentrations of the hormone as low as 0.25 μU/ml elicited marked increases in water permeability, and (ii) exogenous PGE_1 (10^{-7} to 10^{-9} M) was noted to be capable of preventing full expression of the vasopressin effect on water permeability or reversing it (Orloff & Grantham, 1967; Grantham & Orloff, 1968). In view of the sensitivity to vasopressin of the collecting tubule, the time course of the vasopressin-stimulated water permeability effect, the gross control of vasopressin release via the osmoreceptor system, and the nonspecific mechanisms for vasopressin degradation, Grantham and Orloff (1968) suggested that prostaglandin E_1, which antagonizes vasopressin-stimulated water flow in the isolated collecting tubule of the rabbit, functions as an endogenous modulator of vasopressin action. It was their view that PGE synthesized within the collecting tubule cell could prevent "wide overshoots in permeability" which might otherwise occur in response to residual circulating vasopressin were there no intracellular agent capable of modulating the permeability effect of vasopressin.

No direct evidence in support of this thesis was available until the recent work of Zusman, Keiser, and Handler (1977). It is the purpose of this communication to review briefly the available information regarding the interaction of vasopressin and prostaglandin insofar as it is germane to the thesis under question.

The Role of Cyclic AMP in the Action of Vasopressin

It is now generally accepted (Orloff & Handler, 1962; Handler & Orloff, 1973) that vasopressin binds to a receptor on the surface of the

epithelial cells of toad bladder and distal nephron. As a consequence of this interaction the activity of adenylate-cyclase (a membrane bound enzyme system) is enhanced, thus increasing the conversion of ATP to cyclic AMP. The elevation in cyclic AMP concentration is responsible for an increase in the permeability of the membrane to water. The water permeability response of an epithelial membrane to vasopressin can be modified by alterations in hormone-receptor interaction, in the extent of activation of adenylate cyclase by vasopressin, or in the rate of the hydrolysis of cyclic AMP to an inactive nucleotide by cyclic nucleotide phosphodiesterase, an enzyme that is inhibited by methyl xanthines such as theophylline.

The Effect of Prostaglandin E on Vasopressin-Stimulated Water Permeability in the Toad Urinary Bladder

Orloff, Handler, and Bergstrom (1965) reported that prostaglandin E_1, at a concentration as low as 10^{-9} moles/liter, inhibited vasopressin (1 mU/ml)-stimulated water flow in the toad urinary bladder. They also noted that the fatty acid derivative inhibited theophylline-, but not cyclic AMP-, stimulated water flow in this tissue *in vitro* implying an inhibitory effect on the enzymatic generation of endogenous cyclic AMP. Lipson and Sharp (1971) confirmed the inhibition of vasopressin-stimulated water flow in the toad bladder by PGE_1, and demonstrated that large doses of vasopressin could overcome PGE_1-induced inhibition of vasopressin-stimulated water flow. Based on their findings that PGE_1 inhibited vasopressin- and theophylline-, but not cyclic AMP-, stimulated water flow, they concluded that PGE_1 inhibited both the basal and vasopressin-stimulated activity of adenylate cyclase. In considering the mechanism of inhibition of vasopressin-stimulated water flow by PGE_1, Lipson and Sharp (1971) proposed that PGE_1 either competitively inhibits the action of vasopressin on adenylate cyclase or that PGE_1 binds to a different membrane receptor and decreases the affinity of the vasopressin-receptor interaction. In order to assess the role of endogenous PGE in the regulation of vasopressin-stimulated water flow, Flores and Sharp (1972) studied the effect of indomethacin, an inhibitor of prostaglandin biosynthesis, in the toad bladder. Indomethacin enhanced the effects of vasopressin and of cyclic AMP on water flow; since indomethacin also inhibits phosphodiesterase activity, these effects could be explained on the basis of diminished cyclic AMP degradation. However,

when the effect of indomethacin on theophylline was investigated an enhancement of the water flow response was also observed. Since the concentration of theophylline (22 mM) utilized in their studies resulted in 98% inhibition of phosphodiesterase activity, Flores and Sharp (1972) concluded that the enhancement of vasopressin- and theophylline-stimulated water flow by indomethacin was at least in part due to the inhibition of endogenous prostaglandin E biosynthesis.

Ozer and Sharp (1972) studied the effect of PGE_1, PGE_2, and arachidonic acid, the latter the precursor of PGE_2, on vasopressin and theophylline-stimulated water flow in the toad bladder. They found that PGE_2 and arachidonic acid inhibit the water permeability response to vasopressin and to theophylline. They concluded that PGE_1, PGE_2, and arachidonic acid interact with adenylate cyclase to decrease basal and vasopressin-stimulated adenylate cyclase activity. Their conclusion concerning arachidonic acid is contrary to that of Zusman, Keiser and Handler (1977) and is discussed below.

Omachi, Robbie, Handler, and Orloff (1974) in our laboratory showed that, at a concentration which inhibits vasopressin-stimulated water flow, PGE_1 inhibits vasopressin-stimulated cyclic AMP accumulation in toad urinary bladder epithelium suggestive of an effect of the fatty acid on adenylate cyclase.

Evidence for Stimulation of Prostaglandin E Biosynthesis by Arginine Vasopressin

The synthesis of prostaglandins within the kidney occurs primarily in the medulla and papilla (Zins, 1975). Two types of cells have been shown to be sites of prostaglandin biosynthesis, the epithelial cells of the collecting duct, and the renomedullary interstitial cells (Janszen & Nugteren, 1971). Muirhead *et al.* (1972) isolated rabbit renomedullary interstitial cells and found that they synthesize significant amounts of prostaglandin E. Zusman and Keiser (1977*a*) have confirmed the findings of Muirhead and his co-workers and in addition have found that rabbit renomedullary interstitial cells in culture produce large quantities of prostaglandin E_2. Prostaglandin E_2 biosynthesis was enhanced by incubation in medium supplemented with arachidonic acid, the precursor of PGE_2, and markedly stimulated by arginine vasopressin. The stimulation of PGE_2 biosynthesis by the polypeptide was found to be secondary to an increase in the release of arachidonic acid from the cellular arachidonic

acid storage pool, primarily phospholipids. Vasopressin-stimulated prostaglandin E_2 biosynthesis could be inhibited by indomethacin, a non-steroidal anti-inflammatory agent which prevents the conversion of arachidonic acid to PGE_2, or by mepacrine, a phospholipase inhibitor which prevents hormone-stimulated arachidonic acid release (Zusman & Keiser, 1977*b*). The stimulation of PGE_2 biosynthesis is, therefore, dependent upon hormonal-activation of a cellular acylhydrolase with a resultant increase in free arachidonic acid which can then be converted to PGE_2.

The stimulation of prostaglandin E biosynthesis by vasopressin has also been demonstrated *in vivo* in the normal rat (Bell & Mya, 1977) and rabbit (Lifschitz & Stein, 1977), and in the rat congenitally deficient in vasopressin (Brattleboro rat) (Walker *et al.*, 1977).

Evidence for Vasopressin-Stimulated Prostaglandin E Biosynthesis as a Modulator of Vasopressin-Stimulated Water Flow

In order to study the role of endogenous PGE biosynthesis in the regulation of vasopressin-stimulated water flow in the toad urinary bladder, Zusman *et al.* (1977) have recently investigated PGE biosynthesis in this tissue. Although previous workers had suggested a role for endogenous PGE as a regulator of vasopressin action they did not address the question of whether vasopressin stimulated PGE biosynthesis by the toad bladder. Zusman *et al.* (1977) found that vasopressin stimulated PGE biosynthesis and water flow in the toad bladder. Although theophylline, and cyclic AMP mimic vasopressin in stimulating water flow in toad bladder, they had no effect on prostaglandin E biosynthesis by this tissue. Thus, whereas vasopressin-stimulated water flow is dependent upon adenylate cyclase activity and cyclic AMP accumulation, the stimulation of PGE biosynthesis by vasopressin appears to be independent of the adenylate cyclase-cyclic AMP system. Mepacrine, a phospholipase inhibitor, inhibited vasopressin-stimulated PGE biosynthesis and thereby enhanced water flow. When the blockage of arachidonic acid release induced by mepacrine was bypassed by addition of exogenous arachidonic acid, PGE biosynthesis increased significantly, and vasopressin-stimulated water flow was markedly diminished. The addition of indomethacin to bladders incubated with arachidonic acid inhibited PGE biosynthesis and enhanced vasopressin-stimulated water flow. Thus, contrary to the suggestion of Flores and Sharp (1972), arachidonic acid inhibits vasopressin-stimulated water flow because it increases PGE biosynthesis. Its effect

is not due to an inherent inhibitory property of the arachidonic acid. In the same studies, Zusman *et al.* (1977) found that mepacrine, naproxen, indomethacin, meclofenamic acid, and ibuprofen all inhibited prostaglandin E biosynthesis and enhanced vasopressin- and theophylline-stimulated water flow. Although indomethacin, mepacrine, naproxen, meclofenamic acid, and ibuprofen inhibited PGE biosynthesis, only indomethacin enhanced cyclic AMP-stimulated water flow. Thus the effect of indomethacin on cyclic AMP stimulated water flow as also noted by others (Flores & Sharp, 1972; Albert & Handler, 1974) is not due to the inhibition of PGE biosynthesis, but may be secondary to indomethacin-mediated inhibition of phosphodiesterase activity (Flores & Sharp, 1972).

In *in vivo* studies indomethacin enhances vasopressin-stimulated water reabsorption in the dog (Anderson *et al.*, 1975), in the rat (Lum *et al.*, 1977), and in man (Berl, Czaczkes & Kleeman, 1977). These studies suggest that endogenous PGE biosynthesis modulates the action of vasopressin in the mammalian kidney in a manner similar to that described in the toad bladder.

Summary and Conclusions

Vasopressin-stimulated water flow in epithelial membranes is due to the activation of adenylate cyclase and the resultant accumulation of cyclic AMP (Handler & Orloff, 1973). The marked sensitivity of the rabbit renal collecting tubule to vasopressin led Grantham and Orloff (1968) to conclude that an endogenous regulator of vasopressin action that would prevent continuous reabsorption of water in the presence of low concentrations of vasopressin must exist. They suggested that prostaglandin E_1 which inhibits vasopressin-stimulated water flow in the toad bladder and rabbit renal collecting duct might be such an intracellular regulatory substance. Since prostaglandin E_1 inhibited vasopressin- and theophylline-, but not cyclic AMP-stimulated water flow in toad bladder, it was concluded that PGE may inhibit adenylate cyclase activity. Omachi *et al.* (1974) confirmed that PGE_1 inhibited vasopressin-stimulated cyclic AMP accumulation in the toad bladder and Marumo and Edelman (1971) demonstrated that PGE_1 inhibited vasopressin-stimulated adenylate cyclase activity in a broken renal cell preparation. The stimulation of PGE biosynthesis by vasopressin was documented recently in cultured rabbit renomedullary interstitial cells (Zusman & Keiser, 1977*a*, 1977*b*), in toad urinary bladder *in vitro* (Zusman *et al.*, 1977),

and in the normal rat (Bell & Mya, 1977), Brattleboro rat (Walker *et al.*, 1977), and normal rabbit (Lifschitz and Stein, 1977) *in vivo*. Furthermore, the inhibition of prostaglandin biosynthesis has been shown to enhance the action of vasopressin in the toad bladder (Zusman *et al.*, 1977), in the rat (Lum *et al.*, 1977), in the dog (Anderson *et al.*, 1975), and in man (Berl *et al.*, 1977).

Within the mammalian kidney both the renomedullary interstitial cell and the epithelial cells of the collecting duct are capable of PGE biosynthesis. It is not known whether vasopressin-stimulated PGE biosynthesis in the renomedullary interstitial cell or in the collecting duct (or both) is responsible for the regulation of vasopressin-stimulated water permeability. The role of PGE as a regulator of renal vasopressin action is now well established. Agents which inhibit arachidonic acid release or the conversion of arachidonic acid to PGE_2 enhance vasopressin action on epithelial membranes sensitive to this hormone *in vivo* and *in vitro*. Although current data are consistent with the view that vasopressin has two independent effects, (i) activation of adenylate cyclase and the generation of cyclic AMP and (ii) activation of phospholipase and the generation of prostaglandin E_2, further study is required before this can be established as truth. It is still conceivable, particularly if one believes in the economy of nature, that activation of phospholipase is directly related to activation of the cyclic AMP system. That PGE is a naturally occurring modulator of the permeability effect of vasopressin appears to be established, however.

References

Albert, W.C., Handler, J.S. 1974. Effect of PGE_1, indomethacin, and polyphloretin phosphate on toad bladder response to ADH. *Am. J. Physiol.* **226**:1382

Anderson, R.S., Berl, T., McDonald, K.M., Schrier, R.S. 1975. Evidence for an *in vivo* antagonism between vasopressin and prostaglandin in the mammalian kidney. *J. Clin. Invest.* **56**:420

Bell, C., Mya, M.K.K. 1977. Release by vasopressin of E-type prostaglandins from the rat kidney. *Clin. Sci. Molec. Med.* **52**:103

Berl, T., Czaczkes, W., Kleeman, C.R. 1977. Effect of prostaglandin synthesis inhibition on the renal action of vasopressin: Studies in man and in the rat. *Am. J. Physiol.* (*in press*)

Flores, A.G.A., Sharp, G.W.G. 1972. Endogenous prostaglandins and osmotic water flow in the toad bladder. *Am. J. Physiol.* **223**:1392

Grantham, J.J., Orloff, J. 1968. Effect of prostaglandin E_1 on the permeability response of the isolated collecting tubule to vasopressin, adenosine 3′,5′-monophosphate and theophylline. *J. Clin. Invest.* **47**:1154

Handler, J.S., Orloff, J. 1973. The mechanism of action of antidiuretic hormone. *In:* Handbook of Physiology. Section 8, Renal Physiology. J. Orloff and R. Berliner, editors. p. 791. American Physiological Society, Washington, D.C.

Janszen, F.H.A., Nugteren, D.H. 1971. Histochemical localization of prostaglandin synthetase. *Histochemie* **27**:159

Lifschitz, M.D., Stein, J.H. 1977. Antidiuretic hormone stimulates renal prostaglandin E (PGE) synthesis in the rabbit. *Clin. Res.* **25**:440*A*

Lipson, L.C., Sharp, G.W.G. 1971. Effect of prostaglandin E_1 on sodium transport and osmotic water flow in the toad bladder. *Am. J. Physiol* **220**:1046

Lum, G.M., Aisenbrey, G.A., Dunn, M.J., Berl, T., Schrier, R.S., McDonald, K.M. 1977. *In vivo* effect of indomethacin to potentiate the renal medullary cyclic AMP response to vasopressin. *J. Clin. Invest.* **59**:8

Marumo, F., Edelman, I.S. 1971. Effects of Ca^{++} and prostaglandin E_1 on vasopressin activation of renal adenyl cyclase. *J. Clin. Invest.* **50**:1613

Muirhead, E.E., Germain, G., Leach, B.E., Pitcock, J.A., Stephenson, P., Brooks, B., Brosius, W.L., Daniels, E.G., Hinman, J.W. 1972. Production of renomedullary prostaglandins by renomedullary interstitial cells in tissue culture. *Circ. Res.* **30/31 (Suppl. II)**:II161

Omachi, R.S., Robbie, D.E., Handler, J.S., Orloff, J. 1974. Effect of ADH and other agents on cyclic AMP accumulation in toad bladder epithelium. *Am. J. Physiol.* **226**:1152

Orloff, J. Grantham, J.J. 1967. The effect of prostaglandin (PGE_1) on the permeability response of rabbit collecting tubules to vasopressin. *In:* Prostaglandins: Proceedings of the Second Nobel Symposium (1966). S. Bergstrom and B. Samuellson, editors. p. 143. Almquist & Wiksell, Stockholm

Orloff, J., Handler, J.S. 1962. The similarity of the effects of vasopressin, adenosine 3′,5′-phosphate (cyclic AMP) and theophylline on the toad bladder. *J. Clin. Invest.* **41**:702

Orloff, J., Handler, J. 1967. The role of adenosine 3′,5′-phosphate in the action of antidiuretic hormone. *Am. J. Med.* **42**:757

Orloff, J., Handler, J.S., Bergstrom, S. 1965. Effect of prostaglandin (PGE_1) on the permeability response of toad bladder to vasopressin, theophylline, and adenosine 3′,5′-monophosphate. *Nature* (London) **205**:397

Orloff, J., Wagner, H., Jr., Davidson, D. 1958. The effect of variations in solute excretion and vasopressin dosage on the excretion of water in the dog. *J. Clin. Invest.* **37**:458

Ozer, A., Sharp, G.W.G. 1972. Effect of prostaglandins and their inhibitors on osmotic water flow in the toad bladder. *Am. J. Physiol.* **222**:674

Walker, L., Whorton, R., France, R., Swigel, M., Frolich, J.C. 1977. Antidiuretic hormone increases renal prostaglandin E_2 production in rats (Brattleboro) with hereditary hypothalamic diabetes insipidus. *Fed. Proc.* **36**:402

Zins, G.R. 1975. Renal prostaglandins. *Am. J. Med.* **58**:14

Zusman, R.M., Keiser, H.R. 1977*a*. Prostaglandin biosynthesis by rabbit renomedullary interstitial cells in tissue culture: Stimulation by angiotensin II, bradykinin, and arginine vasopressin. *J. Clin. Invest.* (*in press*)

Zusman, R.M., Keiser, H.R. 1977*b*. Prostaglandin E_2 biosynthesis by rabbit renomedullary interstitial cells in tissue culture: Mechanism of stimulation by angiotensin II, bradykinin, and arginine vasopressin. *J. Biol. Chem.* **252**:2069

Zusman, R.M., Keiser, H.R., Handler, J.S. 1977. Vasopressin-stimulated water permeability in the toad urinary bladder: Role of endogenous prostaglandin E biosynthesis. *Fed. Proc.* **36**:631