Role of Inhibitory and Stimulative Effects of Prostaglandins on Vasopressin-stimulated Osmotic Water Flow in the Toad Bladder

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Abstract Vasopressin-prostaglandin (PG) interaction, especially the role of the inhibitory effects of PGE_2 on vasopressin action, was studied using toad urinary bladders. The PGH_2 , at 1×10^{-7} M, inhibited vasopressin-stimulated water flow (MARUMO, 1982); PGE_2 inhibited the water flow at 10^{-8} M, but PGD_2 , $\text{PGF}_{2\alpha}$, and PGI_2 did not do so even at 10^{-7} M. Thus, PGE_2 has a physiological effect in contrast to other PGs converted from PGH_2 . Indomethacin enhanced both the vasopressin- and cyclic AMP-stimulated water flow across the toad bladder. However, the half maximum activation dose for vasopressin was 2×10^{-10} M, but for cyclic AMP, as much as 3×10^{-8} M. The PGE_2 inhibited both vasopressin- and cyclic AMP-stimulated water flow. However, PGE_2 inhibited vasopressin action in a dose-dependent manner which was not noted as a PGE_2 effect on cyclic AMP action. The W-7, which is a specific inhibitor of calmodulin, suppressed cyclic AMP-stimulated water flow in a dose-dependent manner. Thus, PGE_2 may suppress vasopressin-stimulated water flow at a site of cyclic AMP generation under physiological conditions. Thromboxane B_2 (TXB_2) enhanced vasopressin-stimulated water flow but not cyclic AMP-stimulated one. Thus PGE_2 and TXB_2 may be concluded as negative or positive modulators of vasopressin action in the toad bladder on the step(s) as the site of cyclic AMP generation under physiological conditions.

Key words: vasopressin, prostaglandins, toad bladder, osmotic water flow, PGE_2 .

ORLOFF *et al.* (1965) were the first to report that prostaglandin E_1 (PGE_1) inhibits vasopressin- and theophylline-induced water flow across the toad bladder but not that induced by cyclic AMP. OMACHI *et al.* (1974) found PGE_1 diminished the accumulation of cyclic AMP in response to vasopressin in the toad bladder, and thus concluded that PGE_1 suppresses vasopressin-stimulated water flow by inhibiting adenylate cyclase activity. This is supported by other reports in which

mammalian tissues were used. MARUMO and EDELMAN (1971) previously reported that PGE_1 inhibits adenylate cyclase activity in the hamster kidney, and BECK *et al.* (1971) found the same in the rat kidney. However, SCHLONDORFF *et al.* (1981) observed that PGE_2 inhibited cyclic AMP-stimulated water flow when the bladders were pretreated with prostaglandin synthesis inhibitors, but had no effect on water flow (ORLOFF *et al.*, 1965; FLORES and SHARP, 1972; ALBERT and HANDLER, 1974). SCHLONDORFF *et al.* (1981) suggest that PGE_2 interferes with the stimulation of water flow at both "post cyclic AMP" and "pre cyclic AMP" sites, but they have not clarified at which of these two sites PGE_2 exerts its effects against vasopressin under physiological conditions.

Thromboxane B_2 (TXB_2) has been shown in several reports to enhance vasopressin-stimulated water flow (BURCH *et al.*, 1979; BISORDI *et al.*, 1980; BURCH and HALUSHKA, 1982). But the authors of those reports do not provide a clear indication as to whether the effects of TXB_2 are exerted at the site of or distal to cyclic AMP generation.

In the present study, we attempted 1) to examine the effects of prostaglandins on vasopressin-stimulated water flow across the toad bladder, 2) to determine whether the inhibitory effect of PGE_2 is exerted at the site of cyclic AMP generation or on a step after cyclic AMP generation under the physiological conditions, and 3) to determine whether the stimulatory effect of TXB_2 is exerted at the site or distal to cyclic AMP generation.

MATERIALS AND METHODS

Urinary bladders of the toad, *Bufo bufo japonicus*, reared on the ground surface for 1–4 weeks were used. After double pithing, the urinary bladders were immediately removed, placed in Ringer's solution, excised and halved. Each hemibladder was mounted in a glass chamber. To prevent mechanical distortion of the bladder membrane, both chamber orifices were covered with a nylon mesh as previously described (MARUMO and KIKAWADA, 1974). To measure the osmotic water flow volumetrically, Ringer's solution diluted by a fifth was introduced into the mucosal chamber. The area of either chamber orifice was 2.66 cm^2 . The composition of Ringer's solution was as follows (in mM): NaCl, 111; KCl, 3.5; CaCl_2 , 0.9; MgCl_2 , 1.5; NaH_2PO_4 , 1.9; Na_2HPO_4 , 8.1. The osmolality was 232 mOsm/l and the pH 7.4.

Prostaglandin E_1 (PGE_1), PGE_2 , PGD_2 , $\text{PGF}_{2\alpha}$, PGI_2 , and TXB_2 were purchased from Sigma (St. Louis). Adenosine 3',5'-monophosphate (cyclic AMP) was obtained from Yamasa Shoyu (Choshi, Japan), N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) from Shikagaku Kogyo (Tokyo), and vasopressin (Pitressin®) from Park-Davis (Detroit). Indomethacin and prostaglandins were dissolved in ethanol, cyclic AMP in Ringer's solution and adjusted to a pH of 7.4 with 0.1 N NaOH. The other drugs were dissolved in distilled water. All drugs were introduced into the chamber from the serosal side. Prostaglandins dissolved in

0.1 ml ethanol were applied into 15 ml Ringer's solution of the experimental chamber, and 0.1 ml ethanol to the control. When indomethacin or W-7 was used, pre-incubation with the drugs was performed for 30 min or 1 h, respectively, before the addition of vasopressin or cyclic AMP.

RESULTS

1. Effects of PGE_2 , PGD_2 , $PGF_{2\alpha}$, PGI_2 on vasopressin-stimulated osmotic water flow

As previously reported (MARUMO, 1982), PGH_2 inhibits the vasopressin-stimulated osmotic water flow of the toad bladder. Effects of products of PGH_2 , such as PGE_2 , PGD_2 , $PGF_{2\alpha}$, and PGI_2 on the water flow were examined. Table 1 shows the effects of prostaglandins on the 10 mU/ml vasopressin-stimulated water flow. The PGE_2 showed an inhibitory effect at 10^{-8} M, but $PGF_{2\alpha}$, PGI_2 , and PGD_2 failed to do so even at 10^{-7} M, although $PGF_{2\alpha}$ was effective at 10^{-6} and 10^{-5} M (Table 1). None of these prostaglandins by itself was found to have any effect on the osmotic water flow unless vasopressin was present.

2. Effects of PGE_2 on vasopressin- and cyclic AMP-stimulated water flow

Figure 1 shows the effects of indomethacin on vasopressin- and cyclic AMP-stimulated water flow in the toad bladder. Indomethacin was added to the serosal

Table 1. Effects of prostaglandins on vasopressin-stimulated water flow in the toad bladder.

Concentration (M)	PGE_1	PGE_2	$PGF_{2\alpha}$	PGI_2	PGD_2
10^{-5} Cont.			3.35 ± 1.16	3.31 ± 0.71	2.92 ± 0.52
Exp.			1.37 ± 0.44	3.36 ± 0.60	2.87 ± 1.11
			$n=6, p<0.01$	$n=6, p: \text{n.s.}$	$n=6, p: \text{n.s.}$
10^{-6} Cont.			2.51 ± 0.62	3.64 ± 1.27	3.54 ± 0.99
Exp.			1.66 ± 0.48	3.64 ± 0.77	3.17 ± 1.51
			$n=6, p<0.02$	$n=6, p: \text{n.s.}$	$n=6, p: \text{n.s.}$
10^{-7} Cont.		3.55 ± 0.70	2.62 ± 0.23	3.42 ± 0.75	3.10 ± 1.04
Exp.		1.98 ± 0.53	2.55 ± 0.83	3.45 ± 0.94	3.49 ± 1.11
		$n=6, p<0.0025$	$n=6, p: \text{n.s.}$	$n=6, p: \text{n.s.}$	$n=6, p: \text{n.s.}$
10^{-8} Cont.	3.30 ± 0.61	3.59 ± 0.60			
Exp.	2.24 ± 0.60	2.43 ± 0.95			
	$n=6, p<0.05$	$n=6, p<0.05$			
10^{-9} Cont.	3.24 ± 1.41	2.86 ± 0.88			
Exp.	2.84 ± 1.13	2.71 ± 0.52			
	$n=9, p: \text{n.s.}$	$n=9, p: \text{n.s.}$			

$\mu\text{l}/\text{min}$, mean $\pm$ S.D. Cont. indicates the control group, and Exp. that of the experimental. The concentration of vasopressin added was 10 mU/ml.

Fig. 1. Effects of indomethacin on vasopressin- and cyclic AMP-stimulated water flow in the toad bladder. Indomethacin was added to the serosal chamber 30 min prior to the addition of the above agents. The vertical line indicates the mean $\pm$ S.E.M., and the numbers of experiment are given in parentheses.

chamber 30 min before the addition of either vasopressin or cyclic AMP. Indomethacin significantly increased vasopressin-stimulated water flow at the doses higher than 10^{-9} M, and cyclic AMP-stimulated flow at the doses higher than 10^{-8} M. The figure shows a dissociation of indomethacin effects on vasopressin and cyclic AMP. The half maximum activation dose for vasopressin was 2×10^{-10} M, while that for cyclic AMP was 3×10^{-8} M. Indomethacin, at 10^{-4} M, continued to show its effect on the cyclic AMP-stimulated water flow, but this effect may be due to the inhibitory effect on phosphodiesterase activity instead of prostaglandin synthesis inhibition. FLORES and SHARP (1972) reported indomethacin to show an inhibitory effect on phosphodiesterase at high concentration, and the inhibitory effect of 2.8×10^{-6} M indomethacin on the enzyme activity to be equivalent to 5×10^{-5} M theophylline.

Figure 2 shows the effects of PGE_2 on vasopressin- and cyclic AMP-stimulated water flow. All drugs were added to the serosal chamber after 30 min pre-incubation with 1×10^{-6} M indomethacin. Figure 2 shows percent inhibition of vasopressin- or cyclic AMP-stimulated water flow with the addition of PGE_2 . PGE_2 inhibits the water flow in a dose-dependent manner as evident from the figure. On the other hand, PGE_2 inhibits cyclic AMP-stimulated water flow at both 10^{-8} and 10^{-7} M, but not at 10^{-9} and 10^{-6} M. However, the calmodulin inhibitors, which inhibit vasopressin action at steps following cyclic AMP generation (LEVINE *et al.*, 1981)

Fig. 2. Effects of PGE₂ on vasopressin- and cyclic AMP-stimulated water flow. Bladders were pre-incubated with 1×10^{-6} M indomethacin 30 min prior to the addition of the agents. The concentrations of vasopressin and cyclic AMP were 10 mU/ml and 5 mM, respectively.

such as W-7 (HIDAKA *et al.*, 1978) (Fig. 3) or trifluoperazine (data not shown), suppressed cyclic AMP-stimulated water flow in a dose-dependent manner. The PGE₂ showed no effect on cyclic AMP-stimulated water flow at 10^{-10} M.

3. Effects of thromboxane B₂ on vasopressin- and cyclic AMP-stimulated water flow

Thirty min following pre-incubation of bladders with 1×10^{-6} M indomethacin, TXB₂ with either vasopressin or cyclic AMP was introduced into the serosal chambers. In Fig. 4, TXB₂ significantly stimulates the 10 mU/ml vasopressin-stimulated water flow at a concentration of 1×10^{-7} M but not at 10^{-8} M. However, TXB₂ shows no effect on the 5 mM cyclic AMP-stimulated water flow either at 10^{-7} or 10^{-6} M in Fig. 5. TXB₂ alone, at 10^{-6} M, had no effect on the water flow.

DISCUSSION

A previous report (MARUMO, 1982) showed PGH₂ to inhibit vasopressin-stimulated water flow in the toad bladder, at a concentration of 1×10^{-7} M. Of the 4

Fig. 3. Effects of W-7 on cyclic AMP-stimulated water flow. W-7 was added to the serosal chamber 1 h before the addition of 5 mM cyclic AMP.

PGs converted from PGH_2 , only PGE_2 inhibited the water flow at 10^{-8} M, but PGD_2 , $\text{PGF}_{2\alpha}$, and PGI_2 were incapable of this even at 10^{-7} M. Concerning endogenous prostaglandins in the toad bladder, the synthesis of both PGE_2 and $\text{PGF}_{2\alpha}$ was stimulated by vasopressin (ZUSMAN *et al.*, 1977; BURCH *et al.*, 1979). That PGH_2 has an effect at 10^{-7} M indicates PGE_2 physiologically inhibits vasopressin action while $\text{PGF}_{2\alpha}$ has merely a pharmacological effect.

SCHLONDORFF *et al.* (1981) found PGE_2 , with or without vasopressin, to have an effect on water flow, cyclic AMP generation and cyclic AMP-dependent protein kinase activity in the toad bladder. They reported that 10^{-5} M PGE_2 increased cyclic AMP content and kinase ratio even more than vasopressin but had no effect on the rate of water flow, and that 5 mU/ml vasopressin and 10^{-5} M PGE_2 when added together, increased the water flow but had no effect on cyclic AMP content or the kinase ratio compared to that of PGE_2 when administered alone. ZUSMAN *et al.* (1977) were the first to report that vasopressin stimulates PGE_2 biosynthesis in the toad bladder, and that 1 mU/ml vasopressin increases PGE_2 synthesis from 0.5 ± 0.1 pmoles/min·hemibladder to 5.0 ± 0.4 . In a recent study, it was found that when PGE_2 biosynthesis in the toad bladder was at a basal level of 0.22 ± 0.01 pmoles/min·hemibladder, it was stimulated 5 times as much as by

Fig. 4. Effects of TXB₂ on vasopressin-stimulated water flow. TXB₂, at 1×10^{-7} M, was added to the experimental group with 10 mU/ml vasopressin, and vasopressin alone to the control.

vasopressin (BURCH *et al.*, 1980) and when at a basal level of 0.27 ± 0.05 pmoles/min·mg prot., it was stimulated by 5 mU/ml vasopressin to 0.53 ± 0.09 (BURCH and HALUSHKA, 1982). After removing the bladder from the toads, a preparatory period of no more than 2 h is necessary prior to the start of the experiment. During this period, the accumulation of PGE₂ in a hemibladder may be as much as 10^{-11} M without assuming any degradation. Compared to the endogenous generation of PGE₂, 10^{-5} M of PGE₂ used by SCHLONDORFF *et al.* (1981) to demonstrate the dissociation of cyclic AMP levels and kinase ratio from the hydro-osmotic response in the toad bladder, is extremely high, and may not be a physiological concentration. They found that low doses of PGE₂, such as 10^{-7} and 10^{-8} M, have no effect on 5 mM cyclic AMP-stimulated water flow, but could find no dose-dependent inhibitory effect at concentrations from 10^{-9} to 10^{-6} M. In the present study, PGE₂ significantly inhibited the cyclic AMP-stimulated water flow at 10^{-9} to 10^{-6} M, but no dose-dependency could be observed, showing agreement of our results with those of SCHLONDORFF *et al.* (1981) in regard to the effects of low doses of PGE₂ on cyclic AMP action.

Figure 1 shows different half maximum activation doses of indomethacin on vasopressin- and cyclic AMP-stimulated water flow and indicates endogenous

Fig. 5. Effects of TXB₂ on cyclic AMP-stimulated water flow. The solid line indicates the experimental group, and the dotted line the control. TXB₂, at 1×10^{-6} M, was added to the experimental group with 5 mM cyclic AMP, and cyclic AMP alone to the control.

PGE₂ to have a different mode of action on steps at the site of and following cyclic AMP generation. Our data show that PGE₂ inhibits both steps at the site of cyclic AMP generation and a step(s) distal to cyclic AMP, and that the action at the site of cyclic AMP generation has physiological significance while that at a step(s) distal to cyclic AMP may not be physiological. Recently, BURCH and HALUSHKA (1984) reported that vasopressin reduced the intra-cellular Ca²⁺ concentration while PGE₂ increased it. It has been well known that Ca²⁺ inhibits adenylate cyclase activity (MARUMO and EDELMAN, 1971; BIRNBAUMER, 1973) at relatively high concentration. Taken together, it is suggested that PGE₂ may inhibit vasopressin-stimulated

adenylate cyclase activity by increasing intracellular Ca^{2+} concentration.

The syntheses of thromboxanes are known to be stimulated by vasopressin (BURCH *et al.*, 1979; BISORDI *et al.*, 1980; BURCH and HALUSHKA, 1982). However, the authors of those reports do not provide a clear indication as to whether the effects of TXB_2 are exerted at the site of or distal to cyclic AMP generation. BURCH and HALUSHKA (1980) observed that TXB_2 alone stimulated the water flow in the toad bladder, though we were unable to do so. We found TXB_2 to enhance vasopressin-stimulated water flow but to have no effect on cyclic AMP-stimulated. At low concentrations, imidazole (BURCH *et al.*, 1980a,b) and OKY-1581 (MARUMO, 1985), both thromboxane synthesis inhibitors, reduced vasopressin-stimulated water flow, strongly indicating the possibility that TXB_2 stimulates vasopressin action at the site of cyclic AMP generation in the toad bladder. Thus, PGE_2 and TXB_2 were concluded to be negative or positive modulators of vasopressin-stimulated water flow at the step(s) of the site of cyclic AMP generation, under the physiological condition.

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