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 $1 \times 10^{-7}$ M, inhibited vasopressin-stimulated water flow (MARUMO, 1982); PGE$_2$ inhibited the water flow at $10^{-8}$ M, but PGD$_2$, 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 $2 \times 10^{-10}$ M, but for cyclic AMP, as much as $3 \times 10^{-8}$ 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

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mammalian tissues were used. MARUMO and EDelman (1971) previously reported that PGE₁ inhibits adenylate cyclase activity in the hamster kidney, and BECK et al. (1971) found the same in the rat kidney. However, SHLONDORFF et al. (1981) observed that PGE₂ 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 PGE₂ 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 PGE₂ exerts its effects against vasopressin under physiological conditions.

Thromboxane B₂ (TXB₂) 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 TXB₂ 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 PGE₂ 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 TXB₂ 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 present 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 cm². The composition of Ringer’s solution was as follows (in mM): NaCl, 111; KCl, 3.5; CaCl₂, 0.9; MgCl₂, 1.5; NaH₂PO₄, 1.9; Na₂HPO₄, 1.9; Na₃HPO₄, 8.1. The osmolality was 232 mOsm/l and the pH 7.4.

Prostaglandin E₁ (PGE₁), PGE₂, PGD₂, PGF₂α, PGI₂, and TXB₂ 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 PGE₂, PGD₂, PGF₂α, PGI₂ on vasopressin-stimulated osmotic water flow

As previously reported (MARUMO, 1982), PGH₂ inhibits the vasopressin-stimulated osmotic water flow of the toad bladder. Effects of products of PGH₂, such as PGE₂, PGD₂, PGF₂α, and PGI₂ on the water flow were examined. Table 1 shows the effects of prostaglandins on the 10 mU/ml vasopressin-stimulated water flow. The PGE₂ showed an inhibitory effect at 10⁻⁸ M, but PGF₂α, PGI₂, and PGD₂ failed to do so even at 10⁻⁷ M, although PGF₂α 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 PGE₂ 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>
<thead>
<tr>
<th>Concentration (M)</th>
<th>PGE₁</th>
<th>PGE₂</th>
<th>PGF₂α</th>
<th>PGI₂</th>
<th>PGD₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻⁵ Cont.</td>
<td>3.35 ± 1.16</td>
<td>3.31 ± 0.71</td>
<td>2.92 ± 0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp.</td>
<td>1.37 ± 0.44</td>
<td>3.36 ± 0.60</td>
<td>2.87 ± 1.11</td>
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<tr>
<td>10⁻⁶ Cont.</td>
<td>2.51 ± 0.62</td>
<td>3.64 ± 1.27</td>
<td>3.54 ± 0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp.</td>
<td>1.66 ± 0.48</td>
<td>3.64 ± 0.77</td>
<td>3.17 ± 1.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁻⁷ Cont.</td>
<td>3.55 ± 0.70</td>
<td>2.62 ± 0.23</td>
<td>3.42 ± 0.75</td>
<td>3.10 ± 1.04</td>
<td></td>
</tr>
<tr>
<td>Exp.</td>
<td>1.98 ± 0.53</td>
<td>2.55 ± 0.83</td>
<td>3.45 ± 0.94</td>
<td>3.49 ± 1.11</td>
<td></td>
</tr>
<tr>
<td>10⁻⁸ Cont.</td>
<td>3.30 ± 0.61</td>
<td>3.59 ± 0.60</td>
<td>n = 6, p &lt; 0.05</td>
<td></td>
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<tr>
<td>Exp.</td>
<td>2.24 ± 0.60</td>
<td>2.43 ± 0.95</td>
<td>n = 6, p &lt; 0.05</td>
<td></td>
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<tr>
<td>10⁻⁹ Cont.</td>
<td>3.24 ± 1.41</td>
<td>2.86 ± 0.88</td>
<td>n = 9, p: n.s.</td>
<td></td>
<td></td>
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<tr>
<td>Exp.</td>
<td>2.84 ± 1.13</td>
<td>2.71 ± 0.52</td>
<td>n = 9, p: n.s.</td>
<td></td>
<td></td>
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</tbody>
</table>

μl/min, mean ± S.D. Cont. indicates the control group, and Exp. that of the experimental. The concentration of vasopressin added was 10 mU/ml.

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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 $2 \times 10^{-10}$ M, while that for cyclic AMP was $3 \times 10^{-8}$ 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 $2.8 \times 10^{-6}$ M indomethacin on the enzyme activity to be equivalent to $5 \times 10^{-5}$ 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 $1 \times 10^{-6}$ 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)
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3. Effects of thromboxane $B_2$ on vasopressin- and cyclic AMP-stimulated water flow

Thirty min following pre-incubation of bladders with $1 \times 10^{-6}$ M indomethacin, TXB$_2$ with either vasopressin or cyclic AMP was introduced into the serosal chambers. In Fig. 4, TXB$_2$ significantly stimulates the 10 mU/ml vasopressin-stimulated water flow at a concentration of $1 \times 10^{-7}$ M but not at $10^{-8}$ M. However, TXB$_2$ shows no effect on the 5 mM cyclic AMP-stimulated water flow either at $10^{-7}$ or $10^{-6}$ M in Fig. 5. TXB$_2$ alone, at $10^{-6}$ M, had no effect on the water flow.

DISCUSSION

A previous report (MARUMO, 1982) showed PGH$_2$ to inhibit vasopressin-stimulated water flow in the toad bladder, at a concentration of $1 \times 10^{-7}$ M. Of the 4
PGs converted from PGH₂, only PGE₂ inhibited the water flow at 10⁻⁸ M, but PGD₂, PGF₂α, and PGI₂ were incapable of this even at 10⁻⁷ M. Concerning endogenous prostaglandins in the toad bladder, the synthesis of both PGE₂ and PGF₂α was stimulated by vasopressin (ZUSMAN et al., 1977; BURCH et al., 1979). That PGH₂ has an effect at 10⁻¹⁵ M indicates PGE₂ physiologically inhibits vasopressin action while PGF₂α has merely a pharmacological effect.

SCHLONDORFF et al. (1981) found PGE₂, 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 PGE₂ 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 PGE₂ when added together, increased the water flow but had no effect on cyclic AMP content or the kinase ratio compared to that of PGE₂ when administered alone. ZUSMAN et al. (1977) were the first to report that vasopressin stimulates PGE₂ biosynthesis in the toad bladder, and that 1 mU/ml vasopressin increases PGE₂ synthesis from 0.5 ± 0.1 pmol/min·hemibladder to 5.0 ± 0.4. In a recent study, it was found that when PGE₂ biosynthesis in the toad bladder was at a basal level of 0.22 ± 0.01 pmol/min·hemibladder, it was stimulated 5 times as much as by

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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.
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 Scholondorff 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 Scholondorff 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
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, BURGH and HALUSKA (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

\[ \text{Cumulative water flow} \]

\[ \text{µℓ} \]

\[ n = 6 \]

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 $1 \times 10^{-6}$ M, was added to the experimental group with 5 mM cyclic AMP, and cyclic AMP alone to the control.
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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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flow is decreased by thromboxane synthesis inhibition or antagonism. *Am. J. Physiol.*, 239: F160–F166.


