EFFECT OF PROSTAGLANDINS ON CYCLIC AMP CONCENTRATIONS IN TOAD BLADDER AND RAT KIDNEY

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1. Introduction

Prostaglandin PGE_1 increases sodium and water transport across the isolated toad bladder [1, 2]. It inhibits the water permeability response to both vasopressin and theophylline but does not affect the reponse to exogenous cyclic AMP [2, 3]. In renal collecting tubules, Grantham and Orloff [4] have reported a slight increase in water permeability following prostaglandin PGE_1 treatment, but an inhibition of the vasopressin-stimulated response. They proposed that both vasopressin and prostaglandin PGE_1 act on a common receptor. An alternative explanation by Orloff and Handler [5] suggests that two cell types exist in kidney tubules, which are separately stimulated or inhibited by prostaglandin PGE_1.

Measurements of the effects of vasopressin and prostaglandin PGE_1 on adenylate cyclase activity in toad bladder [1] and hamster kidney [6] have been somewhat inconclusive and the differential effects of prostaglandin PGE_1 on vasopressin-stimulated Na^+ and water transport have been considered to involve two adenylate cyclase systems in the toad bladder.

In the present work we have studied the dose response characteristic for vasopressin-stimulated cyclic AMP concentration in toad bladder and the effect of certain prostaglandins on the concentration of cyclic AMP stimulated by vasopressin and theophylline in rat kidney and toad bladder.

2. Materials and methods

Vasopressin (8-arginine) was obtained as pitressin (Parke Davis Co. Ltd.), the prostaglandins were generously supplied by Dr. J.E. Pike (Upjohn Co. Ltd.) and [^{3}H]cyclic AMP (specific activity 6.5 Ci/mmole) was obtained from the Radiochemical Centre, Amersham. Frog Ringer's solution contained Na^+ 113.5 mequiv./ℓ, K^+ 3.5 mequiv./ℓ, Cl^- 116.5 mequiv./ℓ, HCO_3^- 2.4 mequiv./ℓ and Ca^{2+} 0.89 mM. Robinson's kidney slice medium was prepared as described [7].

2.1. Vasopressin-stimulated cyclic AMP concentrations in toad bladder

All toads (*Bufo marinus*) were rapidly pithed and the half bladders promptly exsanguished, excised and weighed. They were incubated for 90 min at 22°, in aerated frog Ringer (10 ml) and then for a further 20 min as matched pairs in the presence of a given dose of pitressin or under control conditions. The tissue was homogenised in ice-cold 5.6% w/v trichloroacetic acid (4 ml) containing a trace of [^{3}H]cyclic AMP as an internal standard. The TCA was extracted into water-saturated diethyl ether (3 × 40 ml) and the aqueous layer collected and fractionated for cyclic AMP using a slightly modified method (Krishna et al. [8]). The cyclic AMP fraction was lyophilised and assayed in triplicate using the technique described by Brooker et al. [9]. The recovery of cyclic AMP was approx. 60%.

2.2. Time course experiments

Bladders excised and preincubated as previously described were incubated over a 20 min period in the presence of vasopressin (100 mU/ml) or vasopressin (2 mU/ml) plus theophylline (1 mM). The rate of increase in cyclic AMP concentration was measured by

removing bladder sections every 5 min during the incubation period for cyclic AMP assay.

2.3. Effect of prostaglandins

We have measured the effect of various concentrations of PGE_1 (10^{-9}–10^{-7} M) on the cyclic AMP concentration, stimulated during a 20 min incubation by 100 mU/ml vasopressin and 2 mU/ml vasopressin plus 1 mM theophylline in hypo- (10× dilution) and isotonic conditions.

A corresponding set of experiments has examined the effect of 1.4×10^{-7} M prostaglandin $PGF_{1\alpha}$ on the cyclic AMP levels stimulated in the presence of 1 mM theophylline by vasopressin (2 mU/ml and 100 mU/ml). In these experiments the bladder sections were excised, preincubated and cyclic AMP assayed as previously described. Each experiment has been repeated 2–3 times.

2.4. Rat kidney experiments

Male Wistar rats (150 g) were killed and the kidneys sliced using a Staddie Riggs microtome. The slices were incubated for 15 min at 30° in aerated Robinson's kidney slice medium (10 ml). The effect of prostaglandins on the vasopressin (2 mU/ml) plus theophylline (1 mM) stimulated cyclic AMP concentrations in kidney slices was studied by incubating rat kidney slices in the absence and presence of 10^{-7} M prostaglandins PGA_2, PGE_2 or $PGE_{2\alpha}$. In these experiments the cyclic AMP concentration was determined per mg wet weight and per mg protein, using Brown et al. [10] protein binding assay after extraction of cyclic AMP with perchloric acid.

3. Results

The vasopressin-stimulated cyclic AMP concentration in toad bladder (fig. 1) has a dose response characteristic of a single activation process. The dose required to give a half maximum response suggests a K_{ass} value for hormone-receptor binding of the order 10^8 ℓ/mole (450 U = 1 mg vasopressin) assuming that the increased concentration of cyclic AMP occurs as a direct effect of hormone interaction. The time course for vasopressin-stimulated cyclic AMP production in toad bladder shows

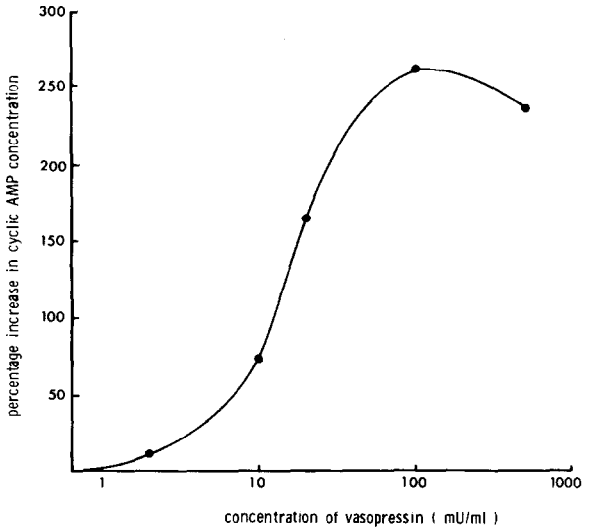

Fig. 1. Increased concentration of cyclic AMP in toad bladders, stimulated by various doses of vasopressin. Expressed as a percentage increase above the matched pair control. Each point represents the mean of three values. Mean value for the concentration of cyclic AMP in control tissue 2.13 pmole/mg wet wt.

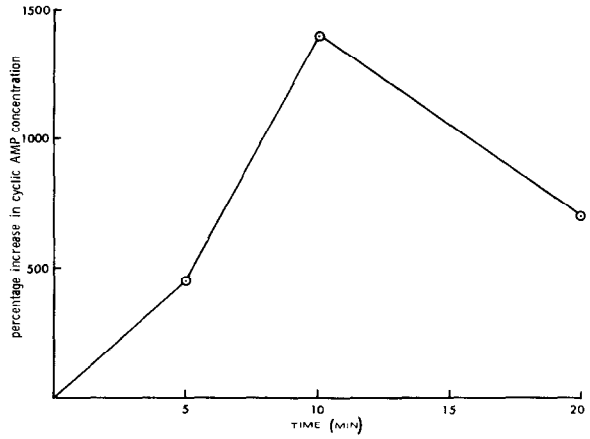

Fig. 2. Rate of increase of cyclic AMP concentration in toad bladder stimulated by 100 mU/ml vasopressin. Expressed as a percentage increase above the control (single expt.). Value for the concentration of cyclic AMP in control tissue 0.72 pmole/mg wet wt.

(fig. 2) a steady increase, reaching a maximum 10 min after addition of the hormone, following which cyclic AMP concentration in the tissue falls. In the presence of 1 mM theophylline the cyclic AMP concentration rises steadily over the 20 min period.

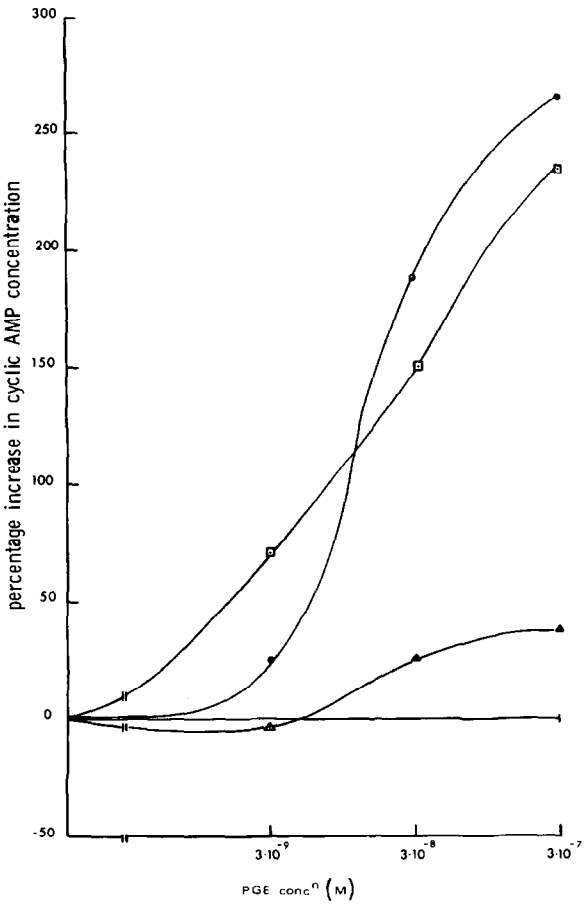

Fig. 3. Increased concentration of cyclic AMP in toad bladder stimulated by various doses of prostaglandin PGE_1 in isotonic conditions, in the presence of 100 mU/ml vasopressin (mean of 2 expts., △—△—△), or of 2 mU/ml vasopressin plus 10^{-3} M theophylline (mean of 2 expts., ○—○—○) and in hypotonic conditions 2 mU/ml vasopressin plus 10^{-3} M theophylline (mean of 3 expts., □—□—□). Mean value for the concentration of cyclic AMP in control tissues 1.48, 1.48, 1.39 pmoles/mg wet wt., respectively.

Studies of the effect of prostaglandin PGE_1 on the vasopressin plus theophylline stimulated cyclic AMP concentrations have shown (fig. 3) a potentiation effect at concentrations in the range 10^{-9}–10^{-7} M. This effect of prostaglandin PGE_1 is observed in both hypo- and isotonic conditions, a result contrary to that expected on the basis of its inhibitory effect on vasopressin and theophylline-stimulated water transport. In the presence of higher doses of vasopressin (100 mU/ml), the concentration of cyclic AMP in the tissue may be expected to be maximal and in these conditions prostaglandin PGE_1 has a more limited effect. A similar potentiation of the vasopressin plus theophylline stimulated cyclic AMP concentration has been observed, using prostaglandin $PGF_{1\alpha}$ (table 1), which again shows

Table 1
The effects of prostaglandin $PGF_{1\alpha}$ on toad bladder cyclic AMP levels in the presence of 1 mM theophylline.

	cyclic AMP (pmoles/mg wet wt.)
Vasopressin (2 mU/ml)	1.96
Vasopressin (2 mU/ml) + $PGF_{1\alpha}$ (10^{-7} M)	4.60
Vasopressin (100 mU/ml)	5.40
Vasopressin (100 mU/ml) + $PGF_{1\alpha}$ (10^{-7} M)	6.60

Table 2
Effect of prostaglandin A_2 on rat kidney cyclic AMP concentration.

	cyclic AMP	
	(pmoles/mg wet wt., mean ± S.E.M.)	(pmoles/mg protein, mean ± S.E.M.)
Control	1.60 ± 0.08	14.04 ± 1.31
Vasopressin (2 mU/ml)	2.59 ± 0.07	23.74 ± 0.92
Vasopressin + PGA_2	2.25 ± 0.09	20.17 ± 0.20
PGA_2 (1.4×10^{-7} M)	2.27 ± 0.10	21.09 ± 0.78

Table 3
Comparison of the effects of prostaglandins E_2, A_2 and $F_{2\alpha}$ on cyclic AMP concentrations in rat kidney in the presence of 10^{-3} M theophylline.

	cyclic AMP (pmoles/mg protein)		
	PGA_2	PGE_2	$PGF_{2\alpha}$
Control	14.0	17.6	17.6
Vasopressin (2 mU/ml)	23.7	30.4	30.4
Vasopressin + PG	20.2	27.2	24.6
PG (1.4×10^{-7} M)	21.1	38.2	37.3

The results are presented as the mean of 3–5 values.

a more limited effect in the presence of higher doses of vasopressin.

The effects of the renal prostaglandins PGA_2, PGE_2 and $PGF_{2\alpha}$ on cyclic AMP concentrations in rat kidney (tables 2 and 3) have shown that each has a stimulatory effect on basal cyclic AMP concentrations in the presence of 1 mM theophylline but an inhibitory effect on the vasopressin plus theophylline-stimulated concentrations.

4. Discussion

The dose response characteristic for vasopressin-stimulated cyclic AMP concentrations in toad bladder indicates a single adenylate cyclase sensitive to the hormone. The high level of hormone required to stimulate cyclic AMP maximally may not be related to the dose of hormone required for maximum stimulation of sodium transport [11], a finding that again raises the question of the role of increased cyclic AMP concentrations in the control of sodium permeability of the toad bladder.

In characterising the mechanism of action of octapeptide hormones on ion and water transport across the isolated toad bladder and frog skin [11, 12], a distinction has been made between cyclic AMP mediated effects that predominantly affect water permeability and a direct permeability effect of the hormone [13] that is believed to primarily affect Na^+ transport. There is a considerable amount of evidence supporting a separation of water and Na^+ transport effects of vasopressin [14–16] and it may be concluded that an important part of vasopressin action may not be related to the stimulated cyclic AMP concentration.

The dose response characteristics obtained for prostaglandin PGE_1 stimulation of cyclic AMP concentrations in the presence of vasopressin and theophylline have similar half maxima under different conditions. This result is taken to indicate a separate and specific high affinity binding site for the prostaglandin. The limited effect of prostaglandins PGE_1 and $PGF_{1\alpha}$ in the presence of high doses of hormone suggests an effect associated with a single adenylate cyclase that is maximally stimulated in the presence of hormone and prostaglandin.

The prostaglandin inhibition of vasopressin-stimulated water transport across the isolated toad bladder does not appear to involve a physiological effect on cyclic AMP concentrations. It was possible that previous studies on the effect of prostaglandins on toad bladder adenylate cyclase preparations might have failed to reveal any indirect effects of the prostaglandins. However, the present work indicates that the inhibitory effects of prostaglandin on the vasopressin-stimulated water transport is probably independent of any effect on cyclic AMP levels. A similar result has been found for 10 mM Ca^{2+} which inhibits the vasopressin water transport response but has no effect on cyclic AMP levels in toad bladder [17].

There is some evidence of an inhibitory effect of the prostaglandins PGE_2, $PGF_{2\alpha}$ and PGA_2 on renal cyclic AMP concentrations. This result agrees with an earlier report by Beck et al. [18], who obtained inhibitory effects of prostaglandin PGE_1 in renal medullary tissue, and suggests that a prostaglandin or similar substance may act in the kidney to regulate vasopressin action.

References

[1] L. Lipson, S. Hynie and G.W.G. Sharp, Ann. N.Y. Acad. Sci. 180 (1971) 261.
[2] J. Orloff, J.S. Handler and S. Bergstrom, Nature 205 (1965) 397.
[3] P. Eggera, I.L. Schwartz and R. Walter, J. Gen. Physiol. 56 (1970) 250.
[4] J.J. Grantham and J. Orloff, J. Clin. Invest. 47 (1968) 1154.
[5] J. Orloff and J.S. Handler, Am. J. Med. 42 (1967) 757.
[6] F. Marumo and I.S. Edelman, J.Clin. Invest. 50 (1971) 1613.
[7] J.R. Robinson, Biochem. J. 45 (1949) 68.
[8] G. Krishna, B. Weiss and B.B. Brodie, J. Pharmacol. Exp. Ther. 163 (1968) 379.
[9] G. Brooker, L.J. Thomas and M.M. Appleman, Biochemistry 7 (1968) 4177.
[10] B.L. Brown, J.D.M. Albano, R.P. Ekins, A.M. Sgherzi and W. Tampion, Biochem. J. 121 (1971) 561.
[11] D.W. Wright and R.S. Snart, Comp. Biochem. Physiol. 38B (1971) 269.
[12] R.S. Snart and T. Dalton, Experientia 28 (1972) 1028.
[13] R.S. Snart and N.N. Sanyal, Biochem. J. 108 (1968) 369.
[14] J. Bourguet and F. Morel, Biochem. Biophys. Acta 135 (1967) 693.
[15] M.J. Petersen and I.S. Edelman, J. Clin. Invest. 43 (1964) 583.
[16] R.S. Snart, Hormones 1 (1970) 233.
[17] P.Y.D. Wong, J.R. Bedwani and A.W. Cuthbert, Nature New Biology 238 (1972) 27.
[18] N.P. Beck, T. Kaneko, U. Zor, J.B. Field and B.B. Davis, J. Clin. Invest. 50 (1971) 2461.