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6-KETOPROSTAGLANDIN E_1 STIMULATION OF RAT AND RABBIT RENAL ADENYLATE CYCLASE-CYCLIC AMP SYSTEMS

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Summary

6-Ketoprostaglandin E_1 effects on rat and rabbit renal adenylate cyclase-cyclic AMP systems were examined. Adenylate cyclase activity was assessed in the $1000 \times g$ fractions prepared from different areas of kidney. 6-Ketoprostaglandin E_1 caused a dose-dependent increase in rat cortical and medullary adenylate cyclase activity with $8 \cdot 10^{-6}$ M being the lowest effective concentration. Combinations of maximal stimulatory concentrations of 6-ketoprostaglandin E_1 and prostaglandin I_2 caused stimulation similar to that seen with either agent alone. In contrast, the combination of either prostaglandin with parathyroid hormone (cortex) or antidiuretic hormone (medulla) resulted in enzyme activity significantly greater than with either agent alone. Similar results were observed in the rabbit. In addition, rabbit cortical and medullary slice cyclic AMP content was increased by 6-ketoprostaglandin E_1. Maximal stimulatory effects of 6-ketoprostaglandin E_1 on adenylate cyclase activity and cyclic AMP content were similar to prostaglandin I_2. Therefore, the similarity in physiologic actions of 6-ketoprostaglandin E_1 and prostaglandin I_2 may be due to the stimulation of adenylate cyclase by both agents. These prostaglandins and the polypeptide hormones appear to activate different renal adenylate cyclase-cyclic AMP systems.

Introduction

Prostaglandins have been generally considered to be local modulators of cellular function because the major products of prostaglandin synthesis either

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have a short half-life as prostaglandin I_2 [1], or they are inactivated during one passage through the lungs as prostaglandin E_2 and prostaglandin $F_{2\alpha}$ [2,3]. 6-Ketoprostaglandin E_1 is a stable product of prostaglandin I_2 metabolism [4]. The liver is a site of 6-ketoprostaglandin E_1 synthesis. Not only is 6-ketoprostaglandin E_1 stable in biological solution but it also escapes inactivation by the lung. Thus, Quilley et al. [5] had shown equivalent biological effects of 6-ketoprostaglandin E_1 when administered intravenously or intra-arterially. In addition, Baer et al. [6] have shown that a 10-fold greater dose of prostaglandin I_2 is required to obtain a similar hypotensive effect in the eviscerated rat compared to the control rat. This suggests there is significant hepatic conversion of prostaglandin I_2 to 6-ketoprostaglandin E_1 in vivo and that the 6-ketoprostaglandin E_1 generated exerts a significant biological effect in vivo. Thus, 6-ketoprostaglandin E_1 could be considered a candidate for a circulating substance with significant biological activity which would act like a hormone.

Prostaglandin I_2 and its metabolite 6-ketoprostaglandin E_1 reduce blood pressure and lower renal vascular resistance in the rat [5]. These are the only prostaglandins known to exert such an effect on the rat kidney. It has been proposed that certain renal effects of prostaglandins could be mediated by a cyclic-AMP-dependent mechanism [7]. Prostaglandin I_2 is an effective activator of renal adenylate cyclase, a finding consistent with such a proposal [8]. Because 6-ketoprostaglandin E_1 has physiologic effects similar to prostaglandin I_2 in kidney [5], blood vessels [5], and platelets [9], it was decided to evaluate the effect of 6-ketoprostaglandin E_1 on renal adenylate cyclase and to compare its effects with those of prostaglandin I_2, antidiuretic hormone and parathyroid hormone.

Materials and Methods

Cyclic [G-^{3}H]AMP (31 Ci/mmol) was obtained from New England Nuclear, Boston, MA. [α-^{32}P]ATP (10 Ci/mmol) and ACS scintillation fluid were obtained from Amersham Searle (Arlington Heights, IL). Synthetic arginine vasopressin (antidiuretic hormone, 100 I.U./ml), parathyroid hormone (180 U/mg), Dowex 50W-X4 (200—400 mesh), neutral alumina, ATP, GTP, and cyclic AMP were obtained from Sigma Chemical Company, St. Louis, MO. The phosphodiesterase inhibitor (±)-4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (RO 20-1724) was a gift from Hoffman-LaRoche, Nutley, NJ. Cyclic AMP binding protein was prepared as described by Gilman [10]. 6-Ketoprostaglandin E_1 and prostaglandin I_2 were gifts from Dr. John Pike (The Upjohn Company, Kalamazoo, MI). 6-Ketoprostaglandin E_1 was dissolved in absolute ethanol, 7 mg/ml, and stored at −20°C. Gas mixtures were obtained from Liquid Carbonic (St. Louis, MO). All other chemicals were purchased in the highest available grade from standard sources. Male Sprague-Dawley rats weighing 250—300 g and New Zealand white rabbits weighing 1.5—2 kg were obtained from Eldridge Laboratory Animals (Barnhart, MO).

Preparation and assay of renal membrane fractions. Rats were anesthetized with diethyl ether, and rabbits with sodium thiopental (20 mg/kg, intravenously) and the kidneys were immediately removed, bisected and sliced with a Stadie-Riggs microtome. Tissue slices (0.5 mm thick) from cortex, outer

medulla and inner medulla in rat or from cortex and medulla in rabbit were collected separately, minced and homogenized. Homogenization was accomplished with six strokes of a Teflon-glass homogenizer at 1400 rev./min in 10 mM Tris-HCl (pH 7.6)/1 mM EDTA/3 mM $MgSO_4$/0.25 M sucrose (0.1 g tissue/ml). The final $1000 \times g$ pellets were prepared as described previously [7]. The reaction mixture for assay of adenylate cyclase contained 2.0 mM ATP (Lot No. 117C-72301), 4—8 cpm/pmol [α-^{32}P]ATP, 1.3 mM cyclic AMP, 0.008 mM GTP, 5 mM $MgSO_4$, 20 mM caffeine, 20 mM creatine phosphate, 67 U/ml creatine phosphokinase, 0.4 mg/ml bovine serum albumin, 40 mM Tris-HCl, pH 7.6, and concentrations of test agents as indicated in the results. Cyclic [^{3}H]AMP was added at the end of the reaction to monitor recoveries. The cyclic [^{32}P]AMP product was isolated using a two-step column chromatographic procedure [11]. Using methods described previously [12], there was no measurable cyclic nucleotide phosphodiesterase activity with the standard reaction mixture for adenylate cyclase. Adenylate cyclase activity was expressed as pmol cyclic AMP produced/mg protein per min. Maximal stimulatory concentrations of prostaglandin I_2, antidiuretic hormone and parathyroid hormone determined in this study are similar to those previously reported for rat renal cortical, outer and inner medullary $1000 \times g$ fractions [8,13,14]. The final concentration of ethanol in the adenylate cyclase assay mixture was 4%. Immediately before use, prostaglandin I_2 was dissolved in 20 mM Tris-HCl, pH 9.4. Control (nonstimulated) adenylate cyclase activity was determined in the presence of the diluent controls for 6-ketoprostaglandin E_1 (ethanol) and prostaglandin I_2 (Tris-HCl). Protein concentrations were estimated by the method of Lowry et al. [15], using bovine serum albumin as a standard.

Slice preparation, incubation, and determination of cyclic AMP content. Tissue slices were dissected, weighed, and placed in plastic scintillation vials containing Krebs-Ringer bicarbonate buffer (pH 7.4), 1 mg/ml glucose and 1 mg/ml bovine serum albumin for 20 min. Slices were then transferred to corresponding media containing 1.0 mM dl-4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (RO 20-1724) for 30 min. Test agents were present during the last 10 min of the incubation. Slices were incubated at 37°C with a gas phase of 5% CO_2/95% O_2. These incubation conditions are optimal for production of cyclic AMP [12]. At the conclusion of the incubation, slices were extracted with 0.5 ml 50 mM sodium acetate buffer (pH 4.0) at 95°C. Cyclic AMP was assayed directly by the protein binding method [10] as modified by Mashiter et al. [16]. Duplicate determinations were made on each sample at more than one dilution. Cyclic AMP content of each slice is expressed as pmol cyclic AMP/mg wet weight.

Results represent typical experiments containing at least three samples. Data are expressed as mean ±S.E.; statistical differences were evaluated by Student's *t*-test for unpaired values ($P < 0.05$).

Results

6-Ketoprostaglandin E_1 caused a dose-dependent stimulation of adenylate cyclase in rat renal cortex, outer medulla and inner medulla (Fig. 1). 6-Ketoprostaglandin E_1 caused the greatest relative increase compared to control in the cortex (3.3-fold) and the largest absolute increase over control in the inner

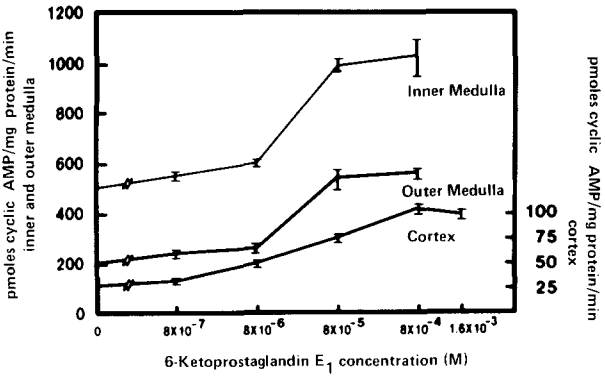

Fig. 1. Effects of 6-ketoprostaglandin E_1 on rat cortical, outer, and inner medullary adenylate cyclase activity. Results are expressed as mean ±S.E., $n = 3$ or more.

medulla (553 pmol/mg per min). In all three areas of the kidney, $8 \cdot 10^{-6}$ M 6-ketoprostaglandin E_1 was the lowest effective dose studied which caused a statistically significant increased in cyclase activity compared to control. Maximum stimulation was at $8 \cdot 10^{-5}$ M 6-ketoprostaglandin E_1 in the renal outer and inner medulla, with $8 \cdot 10^{-4}$ M in the cortex.

Combinations of 6-ketoprostaglandin E_1 with prostaglandin I_2 and renal active polypeptide hormones were evaluated in the rat (Table I). As previously shown [8], prostaglandin I_2 stimulates adenylate cyclase in each area of the kidney. In the cortex and medulla, prostaglandin I_2 and 6-ketoprostaglandin E_1 stimulated adenylate cyclase to a similar extent. Combinations of maximal stimulatory concentrations of 6-ketoprostaglandin E_1 and prostaglandin I_2 were not significantly different from either prostaglandin alone. However, combinations of either 6-ketoprostaglandin E_1 or prostaglandin I_2 with parathyroid hormone or antidiuretic hormone were significantly different than

TABLE I

STIMULATION OF RAT KIDNEY ADENYLATE CYCLASE BY 6-KETOPROSTAGLANDIN E_1, PROSTAGLANDIN I_2, AND POLYPEPTIDE HORMONES

Prostaglandins were present at $8 \cdot 10^{-4}$ M, antidiuretic hormone at 10^{-7} M, and parathyroid hormone at 5 units/ml. Results represent mean ±S.E. with $N = 3$ or more. Values represent pmol cyclic AMP/mg protein per min.

Condition	Cortex	Outer medulla	Inner medulla
Control	28 ± 3	149 ± 11	568 ± 16
6-Ketoprostaglandin E_1	115 ± 2	419 ± 40	1121 ± 100
Prostaglandin I_2	93 ± 2	423 ± 50	1132 ± 58
6-Ketoprostaglandin E_1 + prostaglandin I_2	114 ± 7	455 ± 7	1116 ± 100
Parathyroid hormone	86 ± 4		
Parathyroid hormone + 6-ketoprostaglandin E_1	132 ± 6 *		
Parathyroid hormone + prostaglandin I_2	123 ± 3 *		
Antidiuretic hormone		690 ± 10	2026 ± 84
Antidiuretic hormone + 6-ketoprostaglandin E_1		1020 ± 27 *	2411 ± 95 *
Antidiuretic hormone + prostaglandin I_2		993 ± 33 *	2232 ± 5 *

* $P < 0.05$ compared to the corresponding value for either agent alone.

TABLE II

STIMULATION OF RABBIT KIDNEY ADENYLATE CYCLASE BY 6-KETOPROSTAGLANDIN E_1, PROSTAGLANDIN I_2, AND POLYPEPTIDE HORMONES

Prostaglandins were present at $8 \cdot 10^{-4}$ M, antidiuretic hormone at 10^{-6} M, and parathyroid hormone at 10 units/ml. Results represent mean ±S.E. with $N = 3$ or more. Values represent pmol cyclic AMP/mg protein per min.

Condition	Cortex	Medulla
Control	94 ± 2	101 ± 3
6-Ketoprostaglandin E_1	292 ± 20	184 ± 8
Prostaglandin I_2	249 ± 11	175 ± 4
6-Ketoprostaglandin E_1 + prostaglandin I_2	264 ± 10	170 ± 5
Parathyroid hormone	329 ± 12	
Parathyroid hormone + 6-ketoprostaglandin E_1	469 ± 20 *	
Parathyroid hormone + prostaglandin I_2	440 ± 22 *	
Antidiuretic hormone		205 ± 9
Antidiuretic hormone + 6-ketoprostaglandin E_1		262 ± 9 *
Antidiuretic hormone + prostaglandin I_2		275 ± 25 *

* $P < 0.05$ compared to corresponding value for each agent alone.

either agent alone. 6-Ketoprostaglandin E_1 was a more potent stimulator of cortical adenylate cyclase than parathyroid hormone while in the medulla antidiuretic hormone was more potent than 6-ketoprostaglandin E_1.

Effects of 6-ketoprostaglandin E_1 on rabbit renal adenylate cyclase were examined (Table II). 6-Ketoprostaglandin E_1 stimulated cortical adenylate cyclase 3.1-fold and medullary 1.8-fold. Combinations of maximal stimulatory concentrations of 6-ketoprostaglandin E_1 and prostaglandin I_2 were not significantly different than either agent alone. Combinations of either 6-ketoprostaglandin E_1 or prostaglandin I_2 with parathyroid hormone or antidiuretic hormone were significantly different than either agent alone. 6-Ketoprostaglandin E_1 stimulated adenylate cyclase activity to a similar extent as parathyroid hormone in the cortex and antidiuretic hormone in the medulla.

The effects of 6-ketoprostaglandin E_1 and prostaglandin I_2 on rabbit cortical and medullary slice cyclic AMP content were evaluated (Table III). Both prostaglandins caused a similar maximal increase in cyclic AMP content. In the cortex, slice cyclic AMP content was increased more than 6-fold and in the medulla more than 2-fold.

TABLE III

EFFECT OF 6-KETOPROSTAGLANDIN E_1 AND PROSTAGLANDIN I_2 ON THE CYCLIC AMP CONTENT OF RABBIT KIDNEY SLICES

Concentrations of 6-ketoprostaglandin E_1 and prostaglandin I_2 were $8 \cdot 10^{-6}$ M and $8 \cdot 10^{-4}$ M, respectively. Results represent mean ±S.E., $n = 4$ or more. Values represent pmol cyclic AMP/mg wet weight.

	Cortex	Medulla
Control	0.50 ± 0.06	3.17 ± 0.5
6-Ketoprostaglandin E_1	3.37 ± 0.8 *	7.39 ± 1.0 *
Prostaglandin I_2	4.82 ± 0.4 *	6.11 ± 0.6 *

* $P < 0.05$ compared to corresponding control value.

Discussion

These results demonstrate 6-ketoprostaglandin E_1 stimulation of the renal adenylate cyclase-cyclic AMP systems in both rabbit and rat. The conditions of these experiments indicate that these effects on cyclic AMP accumulation are due to an effect on adenylate cyclase. Plasma membrane incubations contained 20 mM caffeine and 1.3 mM unlabeled cyclic AMP. Phosphodiesterase activity was not detectable under these conditions. In the slice experiments, the potent phosphodiesterase inhibitor RO 20-1724 was used to prevent degradation of cyclic AMP [12]. Therefore, both preparations, isolated membranes and tissue slices, indicate that 6-ketoprostaglandin E_1 activates renal adenylate cyclase. However, a separate effect of 6-ketoprostaglandin E_1 on phosphodiesterase cannot be excluded.

The data in each tissue, cortex, outer and inner medulla, in both species, rat and rabbit, and with each technique, membranes and slices, indicate that 6-ketoprostaglandin E_1 and prostaglandin I_2 cause a similar maximal activation of adenylate cyclase. Because the effects of both prostaglandins were not additive in any of the circumstances tested, the data also indicate that 6-ketoprostanglandin E_1 and prostaglandin I_2 activate the same adenylate cyclase enzyme. Tissue receptors for prostaglandins have been demonstrated [17] and one interpretation of these data is that both 6-ketoprostaglandin E_1 and prostaglandin I_2 utilize the same receptor mechanism to activate renal adenylate cyclase. Previous studies have shown that 6-ketoprostaglandin $F_{1\alpha}$, a stable nonenzymatic metabolite of prostaglandin I_2, does not stimulate renal adenylate cyclase or increase cyclic AMP content [8]. In addition, 6-ketoprostaglandin $F_{1\alpha}$ does not alter prostaglandin I_2- or antidiuretic hormone-mediated increases in adenylate cyclase activity.

One postulated role for prostaglandins in the kidney is competition with polypeptide hormones. For example, prostaglandin E_1 appears to inhibit the ability of antidiuretic hormone to increase water permeability of the isolated perfused collecting duct [18]. It has been further suggested that those effects are mediated at the level of receptors for the polypeptide-sensitive adenylate cyclase [19,20]. Our experiments indicate that both 6-ketoprostaglandin E_1 and prostaglandin I_2 are additive with parathyroid hormone in cortex and with antidiuretic hormone in medulla with respect to their capacities to activate adenylate cyclase. This suggests that these two prostaglandins and the two polypeptide hormones activate different adenylate cyclase-cyclic AMP systems. Perhaps 6-ketoprostaglandin E_1 and prostaglandin I_2 act on renal vascular cyclase and parathyroid hormone and antidiuretic hormone act on renal epithelial cyclase.

Prostaglandin I_2 is synthesized in the kidney [21] and other tissues. Although it is not cleared by the lungs, prostaglandin I_2 is unstable and is rapidly converted nonenzymatically to 6-ketoprostaglandin $F_{1\alpha}$. The latter compound is considered an inactive metabolite of prostaglandin I_2 [22]. However, 6-ketoprostaglandin $F_{1\alpha}$ can be enzymatically converted by 9-hydroxyprostaglandin dehydrogenase to 6-ketoprostaglandin E_1 [4]. Therefore, prostaglandin I_2 can be converted to a stable metabolite that exhibits similar physiologic properties. The present study also demonstrates that both prostaglandin I_2

and 6-ketoprostaglandin E_1 stimulate the renal adenylate cyclase-cyclic AMP systems. This may explain the similar physiologic properties of prostaglandin I_2 and 6-ketoprostaglandin E_1.

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