Prostaglandin Biosynthesis by Rabbit Renomedullary Interstitial Cells in Tissue Culture

STIMULATION BY ANGIOTENSIN II, BRADYKININ, AND ARGININE VASOPRESSIN

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ABSTRACT Rabbit renomedullary interstitial cells were isolated and grown in tissue culture. These cells synthesize 0.8 ng of prostaglandin E_2 (PGE_2) per microgram cellular protein per hour in monolayer tissue culture; prostaglandins A_2 and $F_{2\alpha}$ (PGA_2 and $PGF_{2\alpha}$) biosynthesis was 10 and 5% of PGE_2 biosynthesis, respectively. Arachidonic acid markedly stimulated the production of PGE_2 and $PGF_{2\alpha}$, with conversion rates of 0.24 and 0.02%/h, respectively. Angiotensin II, hyperosmolality, bradykinin, and arginine vasopressin each stimulated PGE_2 biosynthesis; maximum stimulation was 20, 3.7, 3.6, and 3.2 times basal production, respectively. PGE_2 biosynthesis by the renomedullary interstitial cells was inhibited by isoproterenol, potassium, nonsteroidal anti-inflammatory agents (indomethacin, naproxen, ibuprofen, suprofen, meclofenamate, and acetylsalicylic acid), mepacrine (a phospholipase inhibitor), hydrocortisone, and cortisone.

The rabbit renomedullary interstitial cell in tissue culture is a model system for the study of hormonal regulation of PGE_2 biosynthesis.

INTRODUCTION

Investigation of the role of prostaglandins in renal physiology suggests that prostaglandins may mediate or modulate the actions of vasoactive peptides (1). McGiff et al. (2) have shown that infusions of angiotensin II result in the appearance of material similar to prostaglandin (PG)[1] E in renal venous blood; Fröhlich et al. (3) have demonstrated increases in urinary PGE_2 as measured by gas chromatography-mass spectroscopy after angiotensin II infusion. Furthermore, prostaglandin synthetase inhibition has been shown to potentiate the renal vasoconstriction observed after angiotensin II administration (4), suggesting that prostaglandins modulate the action of this potent polypeptide. Bradykinin is a potent renal vasodilator and natriuretic agent (1, 5); its actions are mediated by a bradykinin-stimulated increase in prostaglandin biosynthesis (6–9). PGE has been shown to antagonize the effect of antidiuretic hormone in the toad urinary bladder (10, 11), whereas indomethacin, a potent prostaglandin synthetase inhibitor, potentiates antidiuretic hormone in in vitro and in vivo studies (12–14).

The renal medullary interstitial cell is the major site of prostaglandin biosynthesis within the kidney (15). These highly specialized cells exist in high density in the renal medulla and papilla (16, 17), and contain large amounts of arachidonic acid, the precursor of PGE_2 biosynthesis (18).

To study the production of prostaglandins by renal interstitial cells independent of the many interlocking systems which characterize renal function, we have grown rabbit renal interstitial cells in tissue culture. The rabbit renomedullary interstitial cell in tissue culture is a model system for the study of the molecular events associated with hormonal stimulation of PGE_2 biosynthesis. The characterization of prostaglandin production by these cells and the effects of a number of vasoactive peptides, hormones, physiologic stimuli, and drugs on PGE_2 biosynthesis comprise this investigation.

METHODS

Isolation of rabbit renomedullary interstitial cells in tissue culture. Monolayer tissue cultures of rabbit renomedullary interstitial cells were obtained by minor modification of the technique of Muirhead et al. (19). Under pentobarbital

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[1] *Abbreviation used in this paper*: PG, prostaglandin.

anesthesia, male New Zealand white rabbits, 3 kg, underwent unilateral nephrectomy. The renal medulla was isolated by sharp dissection and was cut into 5 × 5 × 5-mm fragments. These fragments were homogenized for 15 s in 15 ml of RPMI-1640 tissue culture medium (Grand Island Biological Co., Grand Island, N. Y.), containing 25 mM Hepes buffer, 20% (vol/vol) fetal bovine serum (Grand Island Biological Co.), penicillin (2.5 mg/ml), and streptomycin (2.5 mg/ml) in a 20-ml capacity Waring blender. The crude homogenate was injected subcutaneously at four sites on the rabbit's abdomen. 28–35 days later, the firm, highly vascularized, lipid-laden, subcutaneous nodules were removed with aseptic technique. The nodules were minced into 1-mm^3 explants, and tissue cultures were initiated in 75-cm^2 plastic flasks (20–25 explants per flask) with the RPMI-tissue culture medium supplemented with Hepes buffer, fetal bovine serum, penicillin, and streptomycin as described above (15 ml/flask). Cultures were incubated at 37°C in a 95% air-5% CO_2 atmosphere, and the tissue culture medium was changed every 48–72 h. Sheets of confluent fusiform cells developed from the cultured explants after 25–35 days. Confluent cell layers were dispersed with 0.25% trypsin in Dulbecco's phosphate-buffered saline containing 0.1% EDTA (National Institutes of Health Media Unit), centrifuged at 150 *g*, and dispersed in fresh medium for subculture. For experimental studies involving dose-response curves, trypsin-dispersed cells were subcultured in replicate, with Costar Cluster[24] 24-cup plates (Costar, Data Packaging Corp., Cambridge, Mass.). Tissue culture medium was changed at 24–48-h intervals.

Measurement of prostaglandin content of tissue culture medium. PGA_2, PGE_2, and $PGF_{2\alpha}$ were isolated and measured by radioimmunoassay as previously described (20). Tissue culture medium or buffered solutions were acidified to pH 3.5 and extracted with 15 vol of chloroform. Tritium-labeled prostaglandins (New England Nuclear, Boston, Mass.) were added to the samples for subsequent recovery correction. The chloroform was evaporated under a nitrogen stream. Prostaglandins of the A, E, and F series were separated by silicic acid column chromatography (CC-4 Special 200–325 mesh, Mallinckrodt, Inc., St. Louis, Mo.). Prostaglandins of the A and E series were converted to PGB (21) and rechromatographed by silicic acid column chromatography. Prostaglandins of the A and E series were measured as PGB with an antibody against B series prostaglandins and a standard curve derived from PGB_2 (22). Prostaglandins of the F series were measured with an antibody against F series prostaglandins, and a standard curve derived from $PGF_{2\alpha}$ (22).

Effect of cellular growth on PGA, PGE, and PGF biosynthesis by rabbit renal interstitial cells in tissue culture. Trypsin-dispersed cells from 75-cm^2 flasks were pooled and replicate 25-cm^2 flasks containing 5 ml of fresh tissue culture medium were inoculated. Tissue culture medium was changed at 24-h intervals. At each 24-h interval, two flasks were scraped to remove the cells from the plastic surface, and the cellular protein was determined by the method of Lowry et al. (23), with bovine serum albumin as the standard.

Characterization of the factors affecting prostaglandin biosynthesis by renal interstitial cells in tissue culture. Before the study of the effects of vasoactive substances and prostaglandin synthesis inhibitors on prostaglandin biosynthesis, the effects of osmolality, time of incubation, sodium, potassium, pH, and arachidonic acid on prostaglandin biosynthesis were determined as follows:

The interstitial cells were incubated in a buffered solution, pH 8.1, containing magnesium sulfate 1.2 mM, calcium chloride 1.3 mM, disodium hydrogen phosphate 0.4 mM, potassium chloride 3 mM, sodium chloride 22 mM, sodium bicarbonate 25 mM, glucose 10 mM, reduced glutathione 2 mM, and mannitol *q.s.* to a final osmolality of 300 mosmol/liter. Changes in the buffer composition were made as necessitated by the individual experiment as noted below. Cells were washed twice with saline and were incubated in the designated buffer for 1 h at 37°C in a 95% air-5% CO_2 atmosphere, and the fluid was assayed for prostaglandin content.

(*a*) Effect of sodium concentration: Potassium bicarbonate and dipotassium hydrogen phosphate replaced the sodium salts in the buffered solution. Sodium chloride was added to the buffer in a final concentration of 0–100 meq/liter. (*b*) Effect of potassium concentration: Potassium chloride was added to the buffer in a final concentration of 0–100 meq/liter. (*c*) Effect of buffer osmolality: Mannitol was added to the buffered solution to give a final osmolality of 125–1,250 mosmol/liter. (*d*) Effect of pH: The pH of the buffered solution was adjusted to pH 7.0–9.0 with hydrochloric acid or sodium hydroxide. (*e*) Effect of time of incubation: After incubation for 0, 10, 20, 30, 45, and 60 min, the buffer was removed and assayed for PGE_2. (*f*) Effect of arachidonic acid: Arachidonic acid (NuChek Prep, Elysian, Minn.) was solubilized in RPMI-1640 tissue culture medium supplemented with Hepes buffer, fetal bovine serum, penicillin, and streptomycin, as described above. Cells were incubated in arachidonic acid containing medium (10–1,000 μg/ml) for 1 h at 37°C in an atmosphere of 95% air-5% CO_2. The tissue culture fluid was then assayed for PGE_2 and $PGF_{2\alpha}$.

Characterization of the effect of vasoactive substances and prostaglandin synthesis inhibitors on PGE_2 biosynthesis by rabbit renal interstitial cells in tissue culture. In all subsequent experiments the following protocol was followed: the test substance was dissolved in the buffered solution described above. Replicate-plated renal interstitial cells 48–72 h old, in Costar Cluster[24] plates (Costar, Data Packaging Corp.), were incubated with the appropriate test solution for 1 h at 37°C in an atmosphere of 95% air-5% CO_2. The test solution was aspirated and assayed for PGE_2. The cells were scraped from the plastic surface, and cellular protein was determined by the method of Lowry et al. (23), with bovine serum albumin as a standard.

Substances evaluated as prostaglandin biosynthesis inhibitors were: indomethacin (Merck Sharp & Dohme, Division of Merck & Co., West Point, Pa.), naproxen (Syntex Laboratories, Inc., Palo Alto, Calif.), ibuprofen (The Upjohn Co., Kalamazoo, Mich.), suprofen (Janssen Pharmaceutica, Beerse, Belgium), meclofenamate (Parke, Davis & Co., Detroit, Mich.), acetylsalicylic acid, hydrocortisone, cortisone (Sigma Chemical Co., St. Louis, Mo.), and mepacrine (K and K Laboratories, Inc., Plainview, N. Y.).

The following substances also were evaluated: bradykinin (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.), angiotensin I, angiotensin II, epinephrine, dihydroxyphenylalanine (DOPA), serotonin, histamine, norepinephrine, dopamine (Calbiochem, San Diego, Calif.), isoproterenol, dibutyryl cyclic AMP (Sigma Chemical Co.), dibutyryl cyclic GMP (Boehringer Mannheim Biochemicals, Indianapolis, Ind.), fetal bovine serum (Grand Island Biological Co.), and arginine vasopressin (kindly provided by Dr. Joseph Handler).

Statistical analysis. Statistical comparisons were made by use of Student's *t* test (24).

RESULTS

After 25–35 days, a confluent monolayer of fusiform cells developed from explants of tissue from the iso-

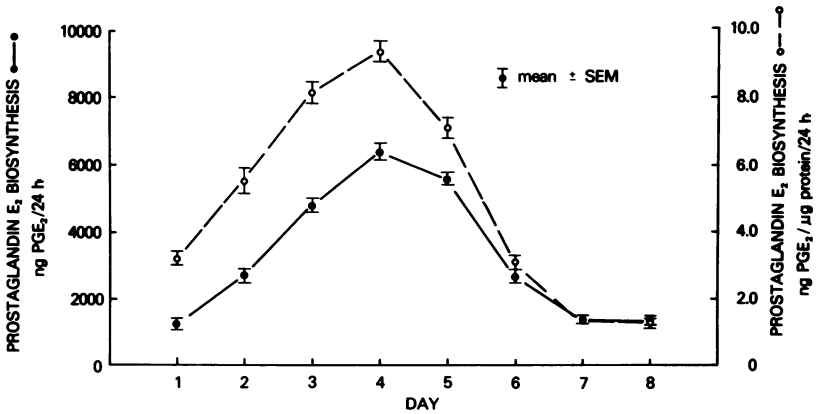

FIGURE 1 PGE_2 biosynthesis by rabbit renomedullary interstitial cells during cellular growth in tissue culture (n = 20).

lated subcutaneous renal interstitial cell nodules. These cells reached confluence within 5 days after trypsin dispersion and formed a dense monolayer over the plastic growth surface. Oil Red O staining of cells grown on a glass cover slip revealed lipid-containing vacuoles throughout the cellular cytoplasm. Electron microscope evaluation of the renal interstitial cell in tissue culture revealed numerous dense osmiophilic vesicles and a prominent Golgi apparatus. These light and electron microscopic properties are characteristic of the renomedullary interstitial cell *in situ* and in tissue culture.

To confirm the radioimmunoassay of prostaglandins in tissue culture fluid, gas chromatographic-mass spectroscopic analysis of the acidic lipid fraction was done. This evaluation confirmed the presence and predominance of PGE_2 in comparison to PGA_2 and $PGF_{2\alpha}$.[2] Negligible amounts of PGE_1, PGA_1, and $PGF_{1\alpha}$ were found, and the radioimmunoassay results are thus expressed as PGE_2, PGA_2, and $PGF_{2\alpha}$. When the renomedullary interstitial cells were assayed for intracellular PGE_2 content, no PGE_2 was found. Furthermore, gas chromatographic-mass spectroscopic analysis failed to identify any degradative metabolites of PGE_2. The PGE_2 content of the culture medium, therefore, represents the biosynthesis of PGE_2 by the interstitial cells in tissue culture.[2]

When tissue culture fluid from interstitial cells was sampled during the cells' growth phase before attaining confluence, PGE_2 biosynthesis (nanograms of PGE_2 produced/24 h) increased progressively over the first 96 h and subsequently fell as the culture attained monolayer confluence (Fig. 1). A similar pattern was observed when PGE_2 biosynthesis was expressed as nanograms of PGE_2 per micrograms of cellular protein/24 h (Fig. 1). Depletion of the biosynthetic precursor arachidonic acid in the culture medium was not the reason for a decrease in PGE_2 biosynthesis at cellular confluence, since tissue culture medium was replaced at 24-h intervals. PGA_2 biosynthesis was 10% of PGE_2 biosynthesis (Fig. 2). When tritium-labeled PGE_2 was incubated in a tissue culture flask containing renomedullary interstitial cells for 24 h, <2% of the tritium label appeared as PGA_2 by thin-layer chromatography. This indicates that the PGA_2 found in the culture did not arise from the nonenzymatic dehydration of PGE_2 during incubation, extraction, and/or chromatography. $PGF_{2\alpha}$ biosynthesis was highest on the 1st day of growth and subsequently fell to a stable 24-h production (Fig. 3). The ratio of $PGF_{2\alpha}/PGE_2$ biosynthesis was 0.05±0.002 (mean±SEM, n = 65).

PGE_2 biosynthesis was linear over a 60-min incubation period and insensitive to alterations in the sodium content of the buffer (Fig. 4). The optimum pH for PGE_2 biosynthesis was between 8.0 and 8.2, and all studies were done at pH 8.1. PGE_2 biosynthesis was strikingly diminished by the addition of potassium to the buffer in which the cells were incubated (Fig. 4). As little as 2 meq potassium/liter resulted in a 42% decrease in PGE_2 synthesis. From 2 to 100 meq/liter potassium, a linear suppression of PGE_2 biosynthesis was observed. At a potassium concentration of 100 meq/liter, PGE_2 biosynthesis was only 25.7±1.6% (mean±SEM, n = 8) of control. In order to approximate more closely the physiological milieu of the renal interstitial cell, all studies involving incubation of the cells in buffer were done at a potassium concentration of 3 meq/liter, although in doing so, a significant suppression of peak PGE_2 biosynthesis was accepted. The effect of total buffer osmolality was evaluated by the addition of varying amounts of mannitol to the buffered solution in which the cells were incubated (Fig. 5). PGE_2 biosynthesis was stable in the range of 250–375 mosmol/liter. At 125 mosmol/liter PGE_2 biosynthesis

[2] Cagen, L., and R. M. Zusman. Unpublished observations.

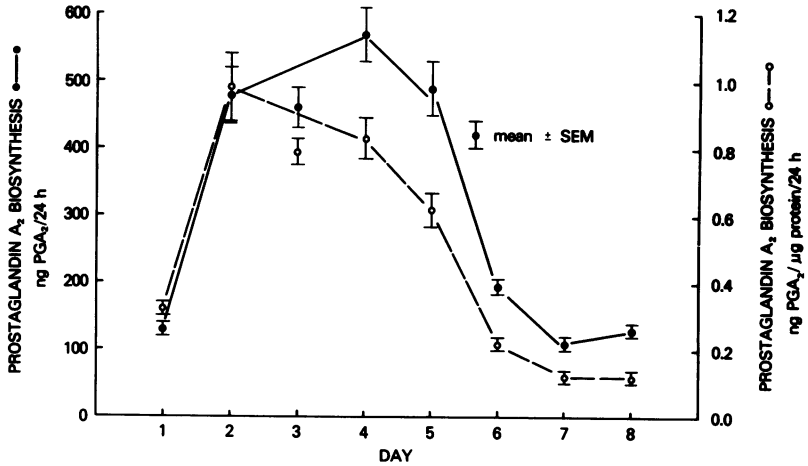

FIGURE 2 PGA_2 biosynthesis by rabbit renomedullary interstitial cells during cellular growth in tissue culture (n = 6).

was 142% of that observed at 250–375 mosmol/liter. With progressive increases in osmolality, PGE_2 biosynthesis was found to increase significantly. At a buffer osmolality of 1,250 mosmol/liter, PGE_2 biosynthesis was 3.5±0.1 ng PGE_2/µg protein per h (mean±SEM, n = 5), compared to 1.0±0.1 ng PGE_2/µg protein per h (mean±SEM, n = 10) at an osmolality of 250–375 mosmol/liter. To approximate the physiological milieu and to study the cells under conditions where there was potential for increased PGE_2 biosynthesis, all studies were done at an osmolality of 300 mosmol/liter.

Arachidonic acid is the biosynthetic precursor of PGE_2 and $PGF_{2\alpha}$ (25). The addition of arachidonic acid to the tissue culture medium in which the cells were incubated resulted in a striking stimulation of PGE_2 and $PGF_{2\alpha}$ biosynthesis (Figs. 6 and 7). PGE_2 biosynthesis increased from 1.0±0.2 ng PGE_2/µg protein per h (mean±SEM, n = 4), with no added arachidonic acid, to 68±4 ng PGE_2/µg protein per h (mean±SEM, n = 4), at an arachidonic acid concentration of 1,000 µg/ml. The percentage conversion of arachidonic acid to PGE_2 was 0.24±0.01%/h (mean ±SEM, n = 24). Similarly, $PGF_{2\alpha}$ biosynthesis increased from 0.1±0.02 ng $PGF_{2\alpha}$/µg protein per h (mean ±SEM, n = 4), at no added arachidonic acid, to 3.4 ±0.3 ng $PGF_{2\alpha}$/µg protein per h (mean±SEM, n = 4), at an arachidonic acid concentration of 1,000 µg/ml. The percentage of arachidonic acid conversion to $PGF_{2\alpha}$ was 0.02±0.002%/h (mean±SEM, n = 24).

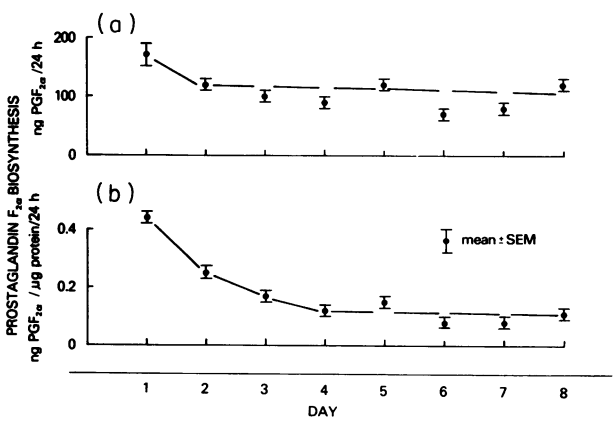

FIGURE 3 $PGF_{2\alpha}$ biosynthesis by rabbit renomedullary interstitial cells during cellular growth in tissue culture. (a) nanograms of $PGF_{2\alpha}$ per flask per 24 h. (b) nanograms of $PGF_{2\alpha}$ per microgram of cellular protein per 24 h (n = 6).

Under the basal conditions at which the various pharmacologic agents were evaluated, control PGE_2 biosynthesis was 0.8±0.06 ng PGE_2/µg cellular protein per h (mean±SEM, n = 38, range: 0.22–1.74).

Nine potential prostaglandin biosynthesis inhibitors were evaluated in the renomedullary interstitial cell tissue culture system (Table I). Indomethacin, a potent prostaglandin synthetase inhibitor, caused a 50% inhibition of PGE_2 biosynthesis at a concentration of 2.7 µM/liter. Among the other inhibitors tested, naproxen, ibuprofen, suprofen, meclofenamate, and acetylsalicylic acid are considered to be inhibitors of the enzyme complex which synthesizes PGE_2 and $PGF_{2\alpha}$ from arachidonic acid (25). The most potent of these agents was naproxen, with a dosage causing 50% inhibition of 40 nM/liter. The relative potencies of indomethacin, naproxen, ibuprofen, suprofen, meclofenamate, and acetylsalicylic acid were 1.0, 67.5, 19.3, 1.8, 1.2, and 0.047, respectively.

Three agents, mepacrine (26), hydrocortisone, and cortisone (27), which are considered to be inhibitors of arachidonic acid release but are also considered to have little or no effect on the prostaglandin synthetase enzyme complex, were evaluated. The reduction in prostaglandin biosynthesis produced by

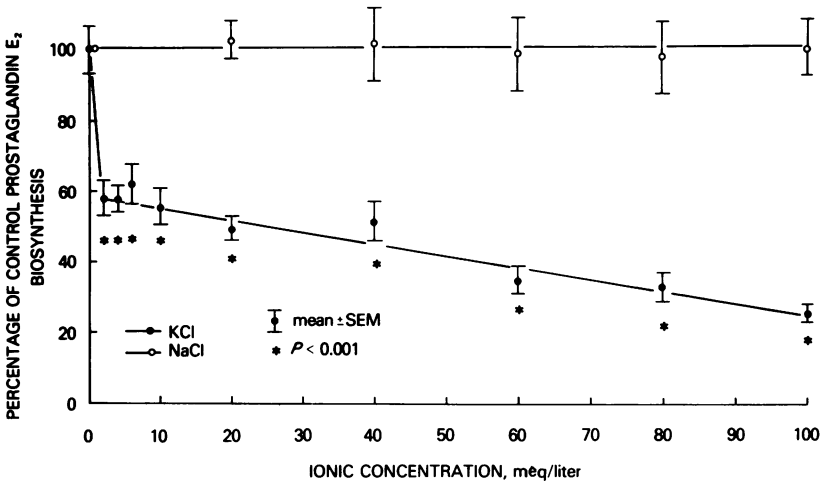

FIGURE 4 Effect of sodium and potassium concentration on PGE_2 biosynthesis by rabbit renomedullary interstitial cells in tissue culture ($n = 8$).

these agents is thought to be secondary to an inhibition of the release of arachidonic acid by phospholipase within the cell (26–28). In the basal, unstimulated state under which mepacrine, hydrocortisone, and cortisone were tested, the 50% inhibitory doses were 9.2, 10, and 19 mM/liter, respectively. The relative potencies of these agents in comparison to indomethacin were 0.00029, 0.00027, and 0.00014, respectively.

The following agents (1 nM/liter–1 mM/liter) were found to have no significant effect on PGE_2 biosynthesis by renomedullary interstitial cells in tissue culture: angiotensin I, epinephrine, norepinephrine, dihydroxyphenylalanine (DOPA), dopamine, serotonin, histamine, fetal bovine serum, dibutyryl cyclic AMP, and dibutyryl cyclic GMP.

With the exclusion of the specific prostaglandin synthesis inhibitors discussed above, the only pharmacologic agent to inhibit PGE_2 biosynthesis was isoproterenol, a beta-adrenergic stimulant. At 30 μM/liter, 27±3% (mean±SEM, $n = 12$, $P < 0.001$) inhibition was observed. This concentration far exceeds the plasma catecholamine concentration; isoproterenol inhibition of PGE_2 biosynthesis does not occur at physiologic concentrations.

Three vasoactive peptides were found to stimulate PGE_2 biosynthesis by renomedullary interstitial cells in tissue culture. Angiotensin II was the most active stimulant of PGE_2 biosynthesis (Fig. 8). Maximum stimulation of PGE_2 biosynthesis was attained at 4 nM/liter, with half-maximal stimulation at 1.9 nM/liter. Maximum PGE_2 biosynthesis was 12.5 ng PGE_2/μg protein per h. Bradykinin, a potent vasodilator and natriuretic agent, produced maximal stimulation of PGE_2 biosynthesis at a concentration of 100 nM/liter. The dosage causing half-maximal stimulation for bradykinin was 32 nM/liter (Fig. 9). Arginine vasopressin also stimulated PGE_2 biosynthesis. Maximum stimulation by AVP was observed at a concentration of 1 μM/liter. The dosage causing half-maximal stimulation for arginine vasopressin was 380 nM/liter (Fig. 10). Rabbit skin fibroblasts produce PGE_2 in tissue culture. In contrast to the renomedullary interstitial cell, arachidonic acid, but not angiotensin II, bradykinin, or ar-

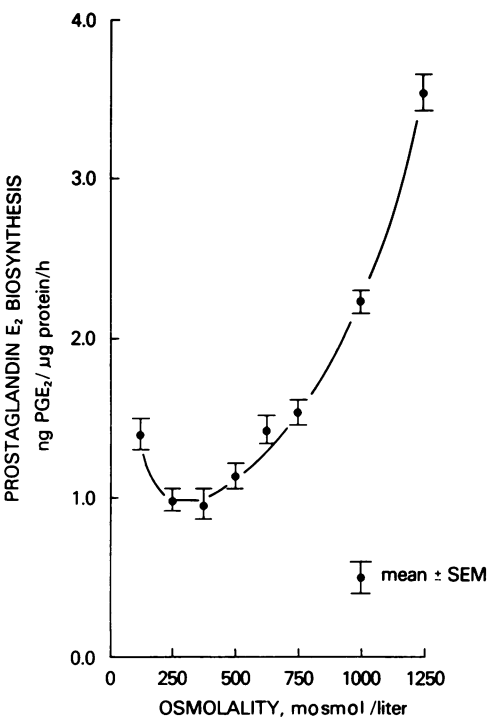

FIGURE 5 Effect of osmolality on PGE_2 biosynthesis by rabbit renomedullary interstitial cells in tissue culture ($n = 6$).

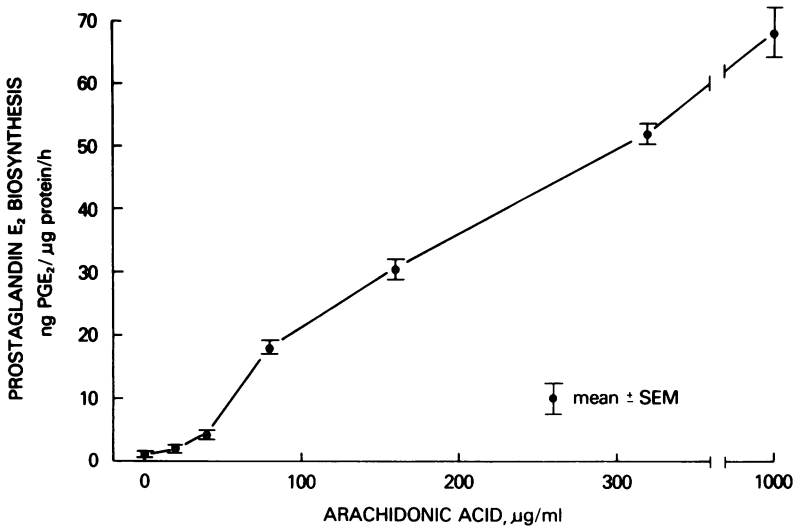

FIGURE 6 Effect of arachidonic acid on PGE_2 biosynthesis by rabbit renomedullary interstitial cells in tissue culture ($n = 4$).

ginine vasopressin, stimulates PGE_2 biosynthesis in the rabbit skin fibroblast in tissue culture.[3]

DISCUSSION

Human vascular endothelium (29), vascular smooth muscle (30), medullary carcinoma of the thyroid (31), rheumatoid synovia (32), renal cell carcinoma (33), fibroblasts (34–37), mouse fibrosarcoma (38), rat renal interstitial cells (39), and rabbit renal interstitial cells (19) have been reported to produce PGE_2 in tissue culture. The unique characteristics of prostaglandin production by the rabbit renomedullary interstitial cell in tissue culture may reflect its role in renal physiology. As a consequence of its unique anatomic location within the renal medulla, the interstitial cell is in close proximity to both tubular fluid and intrarenal blood. The release of PGE_2 by these cells in response to physiologic stimuli may, therefore, derive from blood-borne hormonal stimulation or changes in the renal interstitial milieu as reflected by renal tubular function. The results of this investigation suggest that PGE_2 biosynthesis could be regulated by both these mechanisms.

Studies of the prostaglandin content of renal tissue have revealed high concentrations of prostaglandins in the medulla with relatively low amounts found in the cortex (40–43). Within the medulla, PGE_2 has been found to be the predominant prostaglandin, with PGA_2 and $PGF_{2\alpha}$ levels being only 5–10% those of PGE_2 (40–43). Our studies during cellular growth confirm that PGE_2 is the primary prostaglandin produced by the renal interstitial cell. Although PGE_2 was synthesized at a greater rate during log phase growth, separate studies involving the growth of cells in indomethacin-containing medium (100 μM/liter) failed to alter cellular growth despite almost 100% inhibition of PGE_2 synthesis.[3] Prostaglandins, therefore, are synthetic products of the renal interstitial cell and are unrelated to the essential mechanisms of growth and replication. Hong et al. (34) also observed a diminution of PGE_2 production by the methylcholanthrene-transformed mouse BALB/3T3 fibroblast with increased cell density and a lack of effect of prostaglandin synthesis inhibition on the rate of cellular growth. The mechanism of this decreased synthesis as monolayer confluence is attained is as yet unexplained.

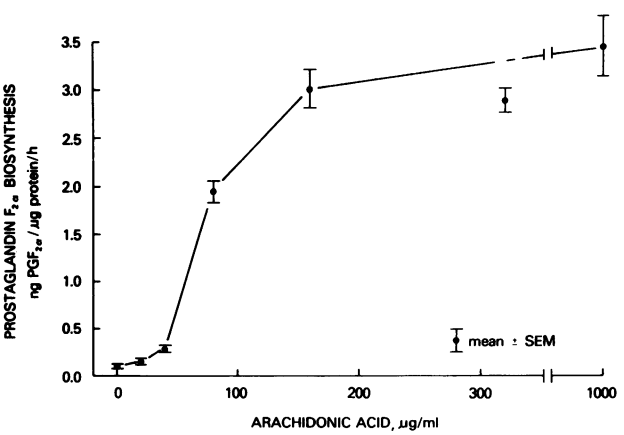

FIGURE 7 Effect of arachidonic acid on $PGF_{2\alpha}$ biosynthesis by rabbit renomedullary interstitial cells in tissue culture ($n = 4$).

[3] R. M. Zusman. Unpublished observations.

TABLE I
Inhibitors of PGE_2 Biosynthesis by Rabbit Renomedullary Interstitial Cells in Tissue Culture

Drug	ID_{50}*	Relative potency
	μM/liter	
Indomethacin	2.7	1.0
Naproxen	0.04	67.5
Ibuprofen	0.14	19.3
Suprofen	1.5	1.8
Meclofenamate	2.3	1.2
Acetylsalicylic acid	58	0.047
Mepacrine	9,200	0.00029
Hydrocortisone	10,000	0.00027
Cortisone	19,000	0.00014

* Dosage causing half-maximal inhibition.

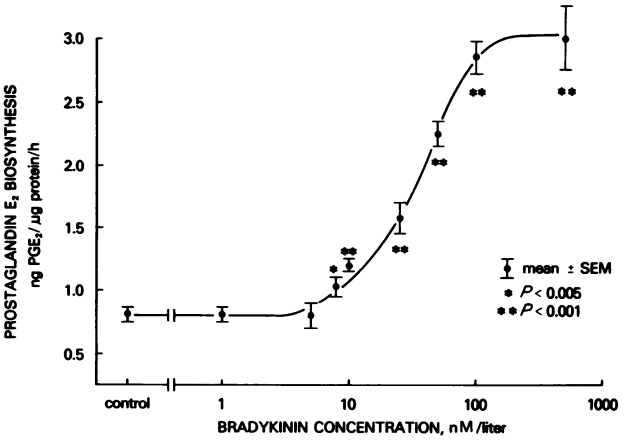

FIGURE 9 Effect of bradykinin on PGE_2 biosynthesis by rabbit renomedullary interstitial cells in tissue culture ($n = 4$).

The conservation of water by the mammalian kidney in states of water deprivation or plasma hyperosmolality is dependent upon the action of antidiuretic hormone (arginine vasopressin) on the renal collecting duct and the establishment of a hypertonic renal interstitium (44). In vitro studies of the effect of PGE on the action of arginine vasopressin in the toad urinary bladder revealed an antagonism by PGE of the vasopressin-induced increase in water permeability (10, 11). Indomethacin, on the other hand, potentiated the action of arginine vasopressin in the urinary bladder of the toad in vitro (13) and in the dog (12) and rat (14) in vivo. Since indomethacin is also an inhibitor of phosphodiesterase (45), this effect may be due to an inhibition of cyclic 3′,5′-AMP destruction, and not due to the inhibition of prostaglandin biosynthesis. Recent studies in the rat, however, have shown that the enhanced antidiuretic action of vasopressin after indomethacin administration is independent of changes in phosphodiesterase activity (14). Arginine vasopressin was found to stimulate PGE_2 biosynthesis by the rat renal papilla in vitro (46) and by the rabbit kidney in vivo (47). Arginine vasopressin stimulates PGE biosynthesis in the toad urinary bladder. Vasopressin-stimulated PGE biosynthesis in the toad bladder inhibits the water permeability response to vasopressin in the toad bladder. Further, inhibitors of prostaglandin biosynthesis, such as indomethacin, ibuprofen, meclofenamic acid, or naproxen, enhance vasopressin-stimulated water flow in the toad urinary bladder (48). In view of these studies, the increase in PGE_2 biosynthesis in response to arginine vasopressin suggests that prostaglandins produced by the renal interstitial cell *in situ* play a role in water homeostasis via modulation of the effects of antidiuretic hormone. Indeed, recent studies in patients with diabetes insipidus indicate that prostaglandin synthesis inhibition after indomethacin administration results in an augmentation of the therapeutic effects of antidiuretic hormone (49).

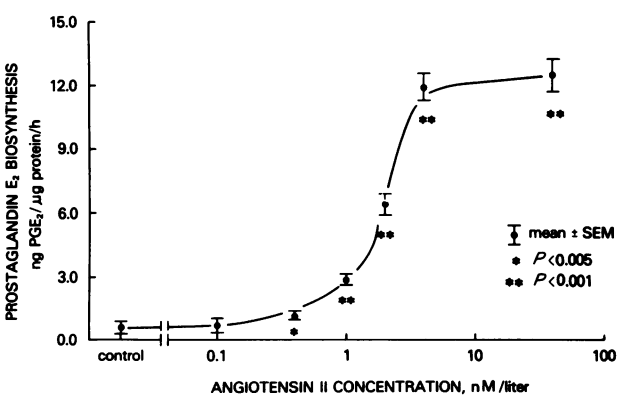

FIGURE 8 Effect of angiotensin II on PGE_2 biosynthesis by rabbit renomedullary interstitial cells in tissue culture ($n = 4$).

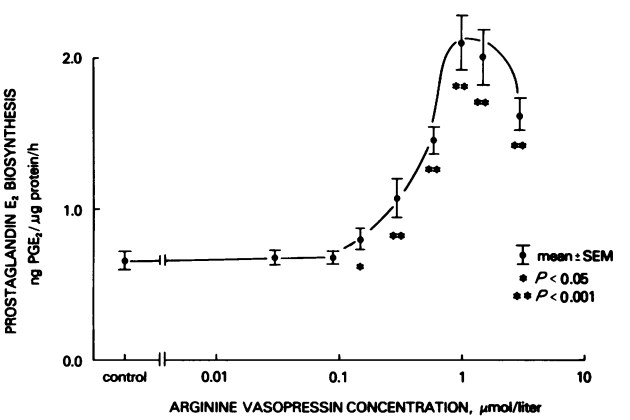

FIGURE 10 Effect of arginine vasopressin on PGE_2 biosynthesis by rabbit renomedullary interstitial cells in tissue culture ($n = 4$).

In similar studies involving a prostaglandin-producing medullary carcinoma of the thyroid in tissue culture, buffer osmolality had no effect on PGE_2 biosynthesis.[3] Thus, it is possible that with regard to prostaglandin production, the renomedullary interstitial cell is unique in its response to osmolality.

Three general classes of compounds were found to inhibit the production of PGE_2 by the renal interstitial cells in tissue culture. First, the non-steroidal anti-inflammatory agents, characterized by indomethacin, were extremely potent inhibitors of PGE_2 biosynthesis. Second, potassium chloride was an extremely potent inhibitor of PGE_2 biosynthesis. This effect was not secondary to changes in the ionic content of the buffer in which the cells were incubated, since equivalent amounts of sodium chloride had no effect on PGE_2 biosynthesis. Third, mepacrine, a phospholipase inhibitor (26), and cortisone and hydrocortisone, which have recently been shown to inhibit hormone-stimulated arachidonic acid release (27), were found to be inhibitors of basal PGE_2 biosynthesis. We have recently shown that mepacrine inhibits hormone-stimulated arachidonic acid release in the rabbit renal interstitial cell without affecting arachidonic acid conversion to PGE_2 (50).

In vivo studies indicate that the renin-angiotensin and kallikrein-kinin systems interact via renal prostaglandins. Angiotensin-induced renal vasoconstriction is potentiated by prostaglandin synthesis inhibition (4). Arachidonic acid or PGE infusions are associated with increased plasma renin activity (51, 52), whereas indomethacin administration results in a fall in both basal and stimulated plasma renin activity (53, 54). Bradykinin results in prostaglandin release in the intact kidney and is accompanied by renal vasodilation, diuresis, and natriuresis (5). These bradykinin-induced effects are prevented by prostaglandin synthesis inhibition (1).

The results of this study lead us to conclude that the actions of angiotensin II, bradykinin, and antidiuretic hormone in the kidney may be mediated, in part, by changes in PGE_2 biosynthesis by the renal interstitial cell and by release of PGE_2 into the renal vasculature and urine. The rabbit renomedullary interstitial cell in tissue culture is a model system for the study of hormone-stimulated prostaglandin biosynthesis.

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