Prostaglandin Synthesis by Rat Glomerular Mesangial Cells in Culture

EFFECTS OF ANGIOTENSIN II AND ARGININE VASOPRESSIN

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ABSTRACT Arginine vasopressin (AVP) and angiotensin II (ANG II) reduce the glomerular filtration rate and ultrafiltration coefficient. Vasodilatory prostaglandins (PG) antagonize these effects. AVP and ANG II also cause mesangial cell contraction. Therefore, possible PG stimulation by these peptides and two vasopressin analogues was studied in cultured rat glomerular mesangial cells. The effect of altered calcium availability on PG production was also studied.

Glomeruli from 75–100-g Sprague-Dawley rats were cultured in supplemented nutrient media for 28 d and experiments were performed on the first passage. Mesangial cell morphology was confirmed by electron microscopy. Cells produced $PGE_2 \gg PGF_{2\alpha} > 6\text{-keto-}PGF_{1\alpha}$ > thromboxane B_2 when incubated with the divalent cation ionophore, A23187, or arachidonic acid (C20:4). ANG II and AVP selectively stimulated PGE_2 at threshold concentrations of 10 nM ANG II and 100 pM of AVP. The effects of the antidiuretic analogue 1-desamino-8-D-arginine vasopressin (dDAVP) and the antipressor analogue [1-(β-mercapto-ββ-cyclopentamethylene propionic acid)-4-valine, 8-D-arginine]-vasopressin (d$[CH_2]_5$VDAVP), were studied. Neither compound stimulated PGE_2 and preincubation with d$(CH_2)_5$VDAVP abolished, and dDAVP blunted, AVP-enhanced PGE_2 production. Incubation in verapamil, nifedipine, or zero calcium media blocked peptide-stimulated PGE_2 production. Increasing extracellular calcium or adding A23187 increased PGE_2 synthesis.

Selective stimulation of PGE_2 by ANG II or AVP in mesangial cells suggests a hormone-sensitive phospholipase and a coupled cyclooxygenase capable of synthesizing only PGE_2. Since neither vasopressin analogue stimulated PGE_2, but both blocked AVP-enhanced PGE_2 production, we conclude that these cells respond to the pressor activity of AVP. This is a calcium-dependent process. Selective stimulation of PGE_2 by ANG II and AVP may modulate their contractile effects on the glomerulus.

INTRODUCTION

Glomerular mesangial cells in culture resemble smooth muscle cells, and contract in response to angiotensin II (ANG II)[1] and arginine vasopressin (AVP), peptides with potent pressor activity (1, 2). Since these cells are the only known contractile cells in the glomerulus, and by autoradiography tritiated ANG II injected into the renal artery is concentrated in the mesangium (3), they are thought to be responsible for the decrements in glomerular surface area (4), ultrafiltration coefficient (K_f) and single nephron glomerular filtration rate (SNGFR) after infusion of ANG II or AVP (5, 6). Therefore, it has been postulated that the mesangial cells may regulate glomerular filtration rate (GFR) by altering the glomerular capillary surface area, one of the determinants of K_f.

Both AVP and ANG II stimulate renal synthesis of prostaglandins (PG) in vivo and in vitro (7–9). Rat glomerular epithelial and mesangial cells in culture pro-

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[1] *Abbreviations used in this paper:* ANG II, angiotensin II; AVP, arginine vasopressin; d$(CH_2)_5$VDAVP, [1-(β-mercapto-ββ-cyclopentamethylene propionic acid)-4-valine, 8-D-arginine] vasopressin; dDAVP, 1-desamino-8-D-arginine vasopressin; DMSO, dimethyl sulfoxide; GFR, glomerular filtration rate; K_f, ultrafiltration coefficient; 6k$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; PG, prostaglandin(s), with the corresponding letter (E, F) for each series; SNGFR, single nephron GFR; TXB_2, thromboxane B_2.

duce radioimmunoassayable PGE_2, thromboxane B_2 (TXB_2), $PGF_{2\alpha}$, and 6-keto (6k) $PGF_{1\alpha}$ (10, 11) and PG synthesis is stimulated by ANG II in rat glomerular mesangial cells (12). ANG II has been found to selectively stimulate PGE_2 production in cultured rat glomerular epithelial cells (13).

The physiologic importance of glomerular PG is unclear, however, cyclooxygenase inhibition has been found to enhance the action of ANG II on SNGFR and glomerular capillary plasma flow rate (14). Therefore, the stimulation of vasodilatory PG, specifically PGE_2, by the pressor peptides may serve to modulate the pressor effect of ANG II and AVP on either vascular smooth muscle, thereby affecting renal blood flow, or on mesangial cell contraction, affecting filtration surface area.

Because of the central role of calcium in the regulation of cell function and hormone-mediated cellular response, considerable attention has focused on the involvement of calcium in cell biochemistry and physiology through the use of calcium entry blockers or calmodulin inhibitors. AVP and ANG II stimulation of PG production occurs by activation of phospholipase(s), a calcium-dependent enzyme (15). The divalent cation ionophore, A23187, is thought to stimulate PG production by increasing availability of calcium to phospholipase A_2 or C (16). Ausiello and Zusman (17) have shown that either the absence of Ca^{++} from the extracellular medium or the presence of verapamil, a calcium entry inhibitor, equally suppresses AVP and ANG II stimulation of PGE_2 synthesis and radioactive arachidonate release from phospholipids in renal medullary interstitial cells.

Our objectives in this study were (*a*) to evaluate the PG synthetic activity of cultured rat glomerular mesangial cells by measuring basal, arachidonate (C20:4)-stimulated and calcium ionophore-stimulated PG production; (*b*) to evaluate AVP and ANG II stimulation of PG synthesis; (*c*) to assess the effect of vasopressin analogues on AVP-enhanced PG production, and (*d*) to determine the role of calcium channels or alteration of extracellular calcium concentration in the response of peptide-stimulated PGE_2 synthesis.

METHODS

Glomerular isolation and culture. Kidneys were removed from ether-anesthetized 75–100-g Sprague Dawley rats killed by exsanguination. Subsequent glomerular isolation was performed using sterile conditions under a laminar flow hood. The renal capsules were removed and cortices dissected away from the medulla. The cortical segment was finely minced into 1–2-mm fragments and gently pressed through a 106-μM sieve, suspended in phosphate-buffered saline (PBS; Gibco Laboratories; Grand Island, NY) to which penicillin, streptomycin sulfate, and fungizone (Gibco Laboratories) were added. The mixture was vigorously pipetted with a siliconized glass pipette. This suspension was then poured through a 75-μM sieve and washed with PBS. The glomeruli collected on the upper surface of the 75-μM sieve and were resuspended in PBS in a conical siliconized glass tube. After the glomeruli settled to the bottom, the supernate was discarded and the glomeruli were resuspended in media for culture. Glomerular purity was assessed by light microscopy and always exceeded 95%. The glomeruli were then plated into Costar 35-mm tissue culture dishes (Costar, Data Packaging, Cambridge, MA) at 3,000 glomeruli/well.

The incubation medium was RPMI 1640 (Gibco Laboratories or Biofluids, Rockville, MD) buffered with 15 mM Hepes (Gibco Laboratories), supplemented with 15% decomplemented fetal bovine serum (Gibco Laboratories), 0.66 U/ml insulin, (Sigma Chemical Co., St. Louis, MO), penicillin (60 U/ml), streptomycin sulfate (60 μg/ml), and fungizone (50 μg/ml) (Gibco Laboratories).

In a 95% air, 5% CO_2 environment at 37°C, glomerular attachment was ~30% and epithelial cells grew from the glomeruli within 1–2 d. Media were changed every 2 d. Since epithelial cells grow rapidly with a peak of cell growth on days 6–8, whereas mesangial growth predominates by day 28, subculture was performed at the end of the 4th wk in culture by using 0.25% trypsin-EDTA (18). Experiments were performed on days 14–16 of the first subculture, before the cells reached confluence and at a time when the passaged cells have hormone receptors (19).

Morphologic confirmation. Identity of these cells was confirmed by electron microscopy. The monolayer was washed free of media and fixed in a 2.5% glutaraldehyde, 4% sucrose, 0.05 M cacodylate buffer (pH 7.4) for 2–6 h. After fixation, the tissue was washed, scraped, and pelleted in 0.1 M cacodylate buffer and was postfixed in 1.0% osmium tetroxide in 0.2 M *S*-collidine buffer. It was then rinsed with distilled water and stained en block in 1% aqueous uranyl acetate, rinsed again and dehydrated in an ascending series of acetone and embedded in Spurr (a mixture of vinylcyclohexene dioxide, D.E.R. 736, nonenyl succinic anhydride, and dimethylaninoethanol. The resulting blocks were cut on an ultramicrotome. Sections of 1-μm thickness were studied by light microscopy to select the proper blocks for electron microscopy. Thin sections were examined with a Siemens Elmiskop 101 electron microscope (Siemens Corp., Iselin, NJ) with magnification ranging from 700 to 50,000.

Contraction experiments. Several hours before these experiments, the culture medium was changed to Ca^{++}- and Mg^{++}-free Hanks' balanced salt solution supplemented with 10% fetal bovine serum to facilitate relaxation of the mesangial cells, which remained adherent to the dish (1). Contraction was studied by phase-contrast microscopy at room temperature by incubating the cells in either 100 nM AVP or 1 μM ANG II in RPMI 1640 without fetal bovine serum.

PG biosynthesis in response to arachidonic acid, calcium ionophore (A23187) ANG II, AVP, or vasopressin analogues. All experiments were performed at 37°C in a 95% air, 5% CO_2 environment, on first subcultures. Cells studied beyond the first passage respond only minimally to the peptides. Culture dishes were washed twice with culture media without fetal bovine serum and allowed to equilibrate for 1 h. At the beginning of the experiment, media were aspirated and 2 ml of fresh media were added to each dish followed by a basal 30-min incubation. 1 ml of this media was then removed and C20:4 (5 μg/ml, Sigma Chemical Co.) in 0.05% ethanol, A23187 (1 μg/ml, Eli Lilly & Co., Indianapolis, IN) in 0.8% dimethyl sulfoxide (DMSO), ANG II (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA) or AVP (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA) in 1 ml media were added. Appropriate ethanol, DMSO, and media

controls were used. At the end of 30 min the remaining media were removed. The supernates from the initial 30-min (basal) and the following 30-min (experimental) incubations were stored frozen at −35°C until radioimmunoassay (RIA) was done. Calculation of stimulated PG synthesis was made by subtracting the amount of PG synthesized during the basal period from that during the experimental period and was expressed as nanograms per milligram of protein per 30 min. Cells were dissolved in 1 N NaOH and protein content was determined by the Bio-Rad method (20). Statistical comparison was made by the *t* test for paired samples.

The synthetic AVP analogues, 1-desamino-8-D-arginine vasopressin (dDAVP) (antidiuretic but not pressor) and [1-(β-mercapto-β,β-cyclopentamethylene propionic acid)-4-valine, 8-D-arginine] vasopressin (d[CH_2]$_5$VDAVP), (neither antidiuretic nor pressor, but a potent antipressor) were assessed for their effects on AVP-stimulated PGE_2 synthesis (21, 22). Cells were prepared as in the previous experiments, except after the 30-min basal period, dDAVP (gift from Dr. L. Kinter, Smith Kline, & French Laboratories, Philadelphia, PA), d(CH_2)$_5$VDAVP, (gift from Dr. M. Manning, Ohio Medical College, Toledo), or medium alone was added to the wells, and incubated for another 15–30 min. 1 ml of media was aspirated and saved, after which another milliliter of media containing either dDAVP, d(CH_2)$_5$VDAVP, AVP, or both dDAVP and AVP or d(CH_2)$_5$VDAVP and AVP were added. Incubation was continued for an additional 30 min at which time supernatant medium was removed and stored for RIA. For these and the following experiments using multiple combination of conditions, statistical evaluation was made by analyses of variance.

Effect of calcium entry blockers on peptide-stimulated PGE_2 synthesis. Experiments were performed, similar to the AVP analogue experiments just described, except that the cells were incubated for 15–30 min in media containing verapamil HCl in 0.05% ethanol (Knoll Pharmaceutical Co., Whippany, NJ), nifedipine in 0.7% DMSO (Pfizer Inc., New York), media alone, or media with vehicle, after which 1 ml of media was removed and replaced with the appropriate calcium-entry blocker with and without ANG II or AVP for the final 30 min.

Effect of extracellular calcium concentration on AVP- or ANG II-stimulated PGE_2 synthesis. For these experiments, cells were first washed in Earle's balanced salt solution supplemented with amino acids and vitamins (Gibco Laboratories) either with zero or 1.8 mM Ca^{++} and allowed to equilibrate for 1 h. After the 1-h preincubation, examination by light microscopy confirmed that the cells in the two groups were equally adherent to the dishes. Basal 30-min incubations were performed followed by ANG II or AVP incubation for another 30 min in either zero or 1.8 mM Ca^{++} media.

RIA. Unextracted samples were assayed for PGE_2 with PGE_2 antisera from Institute Pasteur, Paris (23). $PGF_{2\alpha}$, 6k$PGF_{1\alpha}$, and TXB_2 were also measured in selected experiments. Antibodies to $PGF_{2\alpha}$ and 6k$PGF_{1\alpha}$ were prepared in our laboratory. Antisera to TXB_2 were obtained from Dr. William Campbell, Dallas, TX. The cross-reactivities of these antibodies >1% are: anti-PGE_2, 3.2% with PGE_1; anti-$PGF_{2\alpha}$, 63% with $PGF_{1\alpha}$; anti-6k$PGF_{1\alpha}$, 14% with $PGF_{1\alpha}$ to 2% with PGE_1 and $PGF_{2\alpha}$; anti-TXB_2, none. The radioactive ligands [^{3}H]PGE_2, [^{3}H]$PGF_{2\alpha}$, [^{3}H]TXB_2, and [^{3}H]6k$PGF_{1\alpha}$ were purchased from New England Nuclear, Boston, MA. Culture media not exposed to cells, incubated as in the experiments, were used as blank controls and nonspecific displacement never exceeded 5% of the bound radioactivity.

RESULTS

Morphology and cell contraction. Under phase-contrast microscopy, the mesangial cells appear flat and stellate (Fig. 1). Mesangial cells, maintained in Ca^{++}- and Mg^{++}-free media for 3–4 h, are depicted in Fig. 1 A. When 1 μM of ANG II or 100 nM of AVP were added, ~50% of the cells contracted (Fig. 1 B). Contraction began at 6–7 min after the addition of peptide at room temperature. Reintroduction of media alone containing Ca^{++} and Mg^{++} caused some cells to contrast also, but not as much, this occurred over a longer time period, and the cells stayed contracted. 15 min

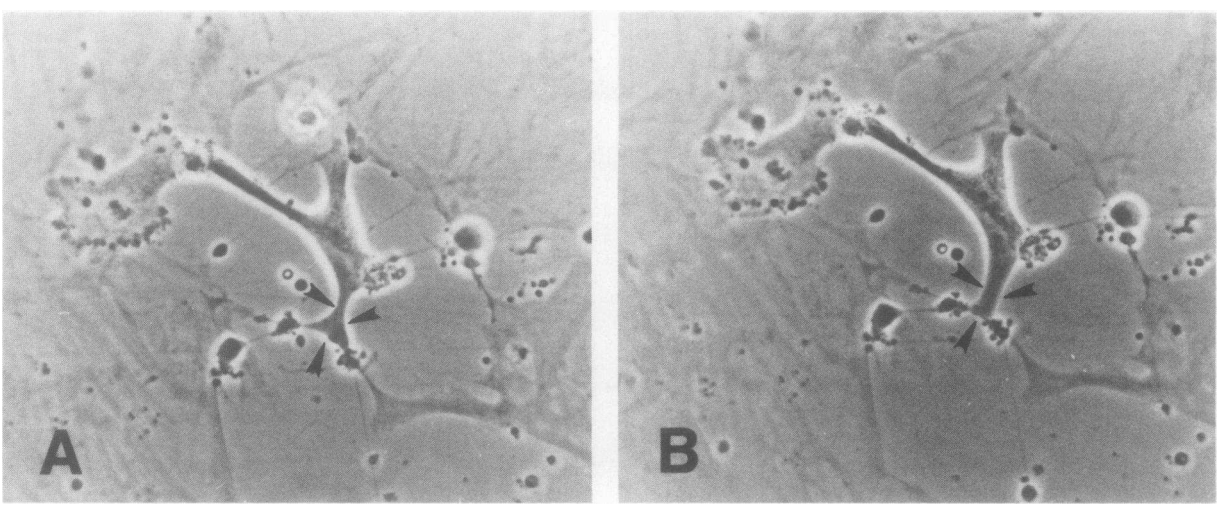

FIGURE 1 Phase-contrast microscopy of a glomerular mesangial cell in culture. Fig. 1 A shows the cell in its resting state. Fig. 1 B, taken 10 min after the addition of 100 nM of AVP. The cytoplasmic extension, highlighted by arrows, changes configuration by contraction. (×500).

after addition of the peptide, the cells resumed their normal morphology. During contraction, the cells lost their flat appearance, becoming rounded, and cytoplasmic processes moved closer to the cell body.

The mesangial cells, as viewed by electron microscopy, are shown in Fig. 2. The cells contain large bundles of microfilaments typical of glomerular mesangial cells in culture. Representative six-well dishes from the three separate primary subcultures were submitted for morphologic confirmation by transmission electron microscopy. Of 50 micrographs (×42,000) examined from each subculture, epithelial cell contamination was ruled out, as assessed by the absence of cells with microvilli or intercellular junctions.

PG synthesis by mesangial cells. Basal and C20:4- or A23187-stimulated PG synthesis by the mesangial cells is summarized in Fig. 3. PGE_2 was the most abundant cyclooxygenase product under both basal and stimulated conditions. In the basal state PGE_2 production was fourfold that of $PGF_{2\alpha}$ and 10-fold greater than PGI_2, as measured by its stable hydrolysis product, $6kPGF_{1\alpha}$.

When PG production was stimulated by the calcium ionophore, or by the addition of exogenous C20:4, the production of the three prostaglandins increased, but TXB_2 was detectable only with arachidonate stimulation (Fig. 3).

Effect of ANG II and AVP on prostaglandin synthesis. Figs. 4 and 5 depict the relationship between ANG II or AVP concentration and prostaglandin biosynthesis. 100 pM of AVP significantly stimulated PGE_2 production and PGE_2 synthesis progressively increased up to AVP concentrations of 1 μM. $PGF_{2\alpha}$ synthesis was increased only at 1 μM AVP and to a much lesser extent than PGE_2. $6kPGF_{1\alpha}$ production was not enhanced.

Similar results were obtained when cells were incubated with ANG II (1 nM–1 μM) (Fig. 5). The threshold ANG II concentration required for PGE_2 stimulation was 10 nM. There was no stimulation of $PGF_{2\alpha}$ or $6kPGF_{1\alpha}$ by ANG II.

The data in Fig. 6 summarize the effect of vasopressin analogues on AVP-mediated PGE_2 synthesis. Whereas AVP 100 nM increased PGE_2 production four-

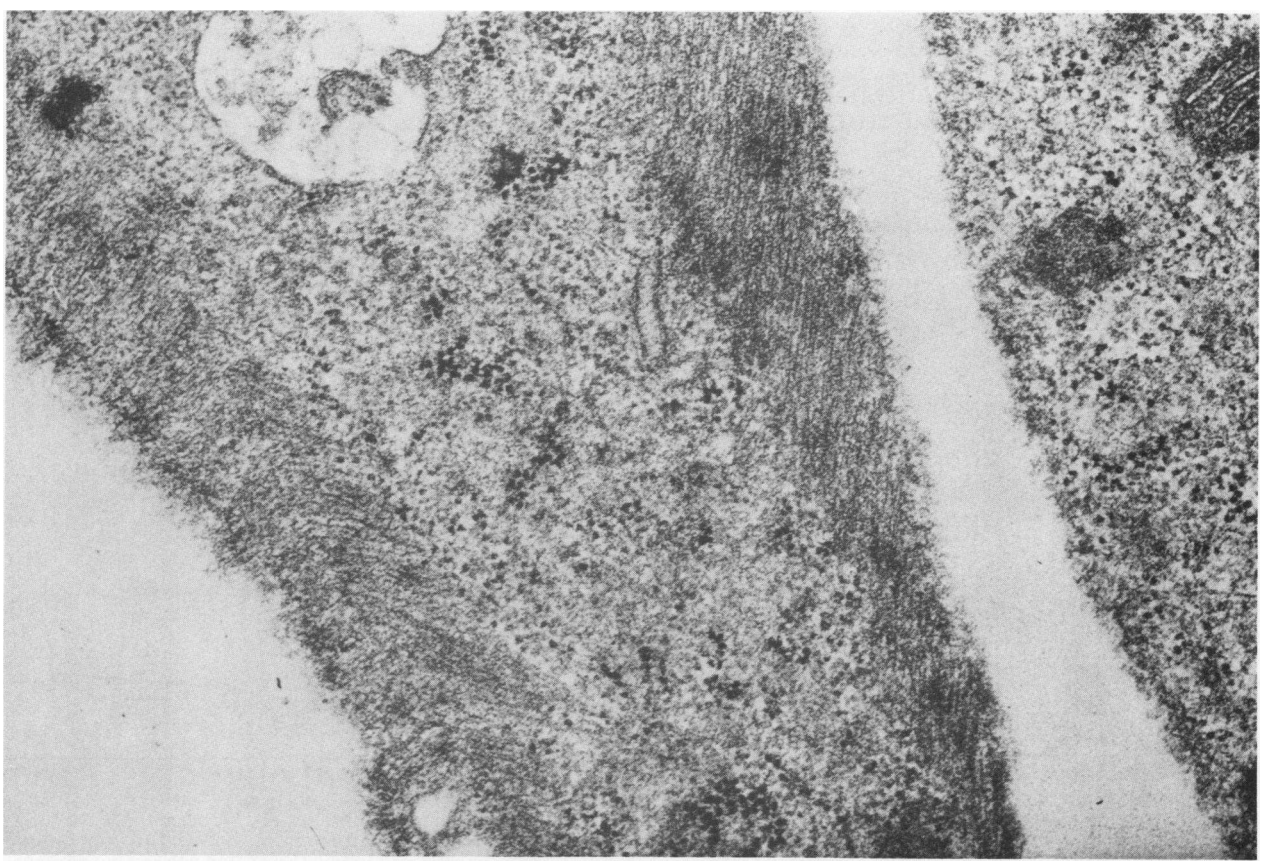

FIGURE 2 Electron microscopic photograph of a cultured mesangial cell. There is an abundant network of myofilaments, thought to be the contractile elements of the cell. (×42,000).

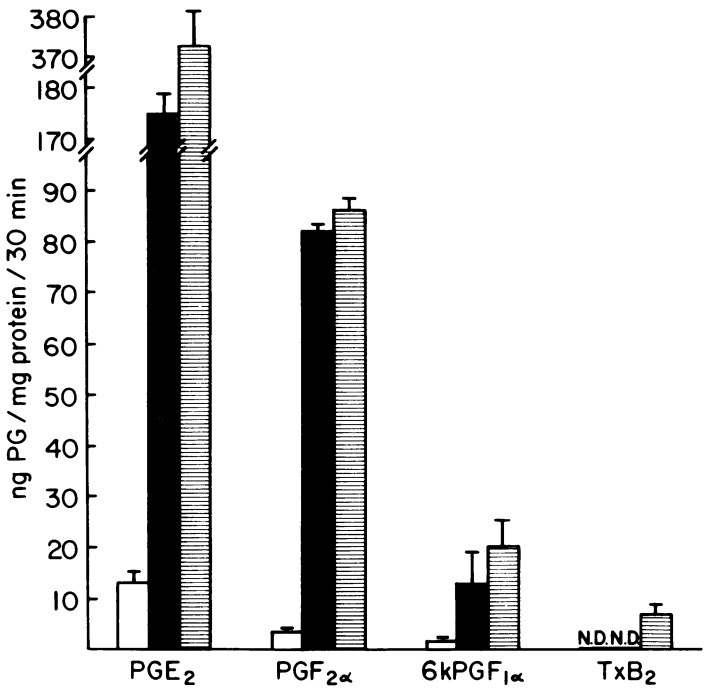

FIGURE 3 PG synthesis by cultured mesangial cells. The bars represent mean±SEM for three experiments from different glomerular subcultures. In each experiment, five separate culture dishes were used. Open bars (□) represent basal production. Dark bars (■) depict PG synthesis after the addition of A23187, 1 μg/ml. Striped bars (▤) represent the results with arachidonate incubation, 5 μg/ml. Total n = 15.

fold, neither dDAVP nor d$(CH_2)_5$VDAVP altered PGE_2 production from basal values. Preincubation with the antipressor analogue d$(CH_2)_5$VDAVP completely abolished subsequent stimulation of PGE_2 by AVP. Preincubation with dDAVP significantly decreased AVP-stimulated PGE_2 production by >50%.

Effects of calcium entry blockers or extracellular calcium concentration on ANG II- and AVP-stimu-

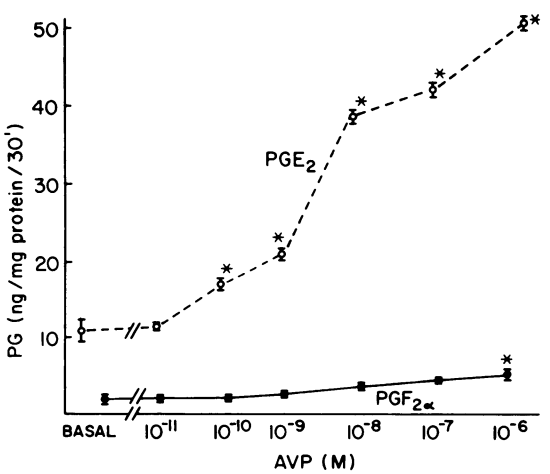

FIGURE 4 AVP-stimulated PG synthesis by cultured mesangial cells. Points represent the mean±SEM for three experiments from different glomerular subcultures, total n = 10. *$P < 0.01$ (peptide-stimulated vs. basal production).

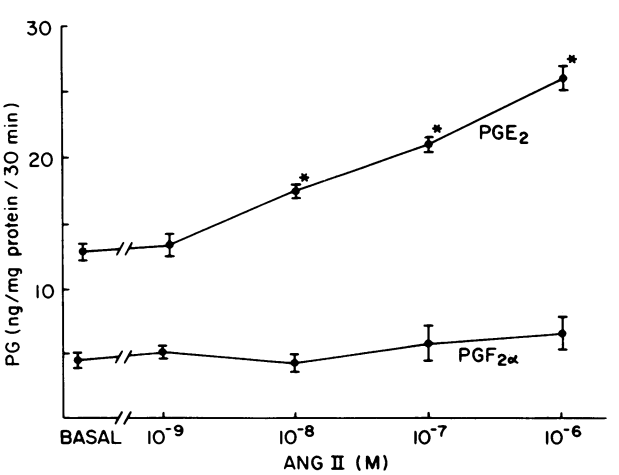

FIGURE 5 ANG II-stimulated synthesis in cultured mesangial cells. Points represent mean±SEM for three experiments from different glomerular subcultures, n = 3 per experiment; *$P < 0.01$.

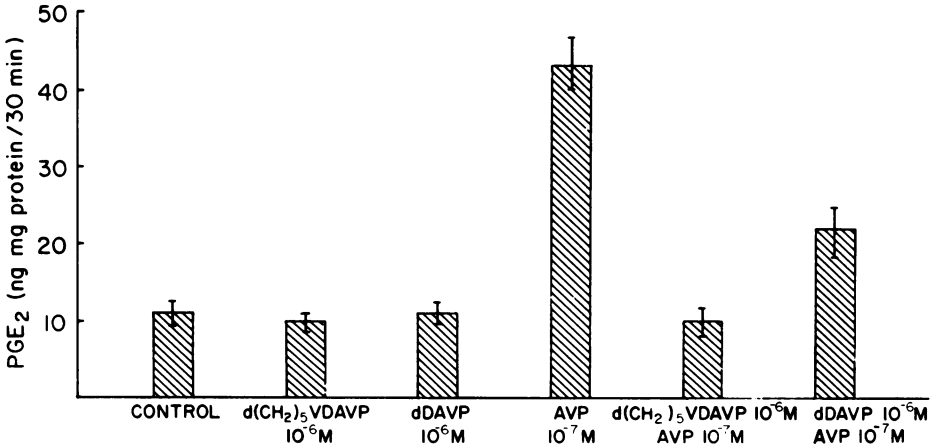

FIGURE 6 Effect of AVP analogues on AVP-mediated PGE_2 synthesis. The bars represent mean±SEM for three experiments from different glomerular subcultures, $n = 5$ per experiment. For AVP alone vs. control, $P < 0.01$.

lated PGE_2 production. When calcium entry was blocked by verapamil or nifedipine (extracellular $Ca^{++} = 0.6$ mM) or when extracellular Ca^{++} was removed from the incubation media, both AVP- and ANG II-stimulated PGE_2 synthesis were inhibited. Preincubation of mesangial cells in verapamil 10 μM, completely abolished the effects of AVP on PGE_2 at 1 and 10 nM AVP, and inhibited production by >50% at 100 nM AVP (Fig. 7). Results with nifedipine, 10 nM, (extracellular $Ca^{++} = 1.8$ mM) or reduction of extracellular Ca^{++} to zero are shown in Fig. 8. Nifedipine did not alter basal PGE_2 production and completely abolished AVP-enhanced PGE_2 at all AVP concentrations tested. Removal of Ca^{++} from the media slightly decreased basal PGE_2 synthesis and also completely inhibited AVP-stimulated PGE_2.

The dose response of PGE_2 to AVP in the control mesangial cells was greater in the experiments depicted in Fig. 8 in which extracellular Ca^{++} concentration was 1.8 mM than that found in the experiments depicted in Figs. 4, 6, and 7, in which extracellular Ca^{++} was 0.6 mM. Nifedipine, 10 nM or verapamil, 10 μM, also blocked completely the ANG II-mediated enhancement of mesangial PGE_2 synthesis (Fig. 9). Removal of extracellular Ca^{++} similarly antagonized ANG II stimulation of PGE_2 synthesis.

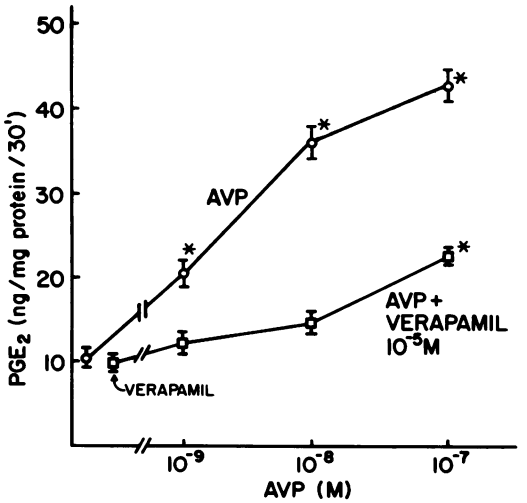

FIGURE 7 Effect of verapamil on AVP-stimulated PGE_2 synthesis by cultured mesangial cells. Points represent mean±SEM in three experiments from different glomerular subcultures. Five separate culture dishes were used per experiment, total $n = 15$; $^*P < 0.01$. (AVP vs. control; AVP plus verapamil vs. verapamil alone).

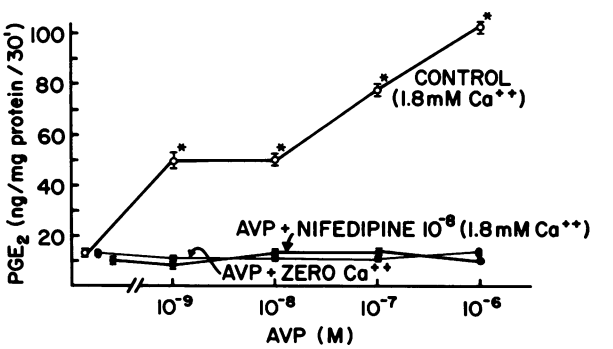

FIGURE 8 Effect of nifedipine or calcium on AVP-stimulated PGE_2 synthesis. Points represent mean±SEM of two experiments from separate subcultures, total $n = 6$; $^*P < 0.01$. (Peptide alone, peptide with nifedipine, or peptide in zero Ca^{++} vs. paired control without peptide.)

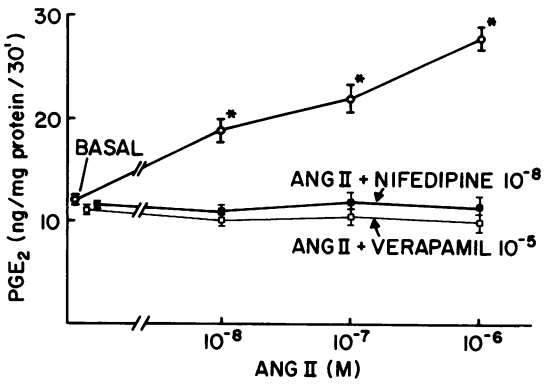

FIGURE 9 Effect of calcium entry blockers on ANG II-stimulated PGE_2 synthesis. Points represent mean±SEM for two experiments from separate subcultures, $n = 3$ per experiment; $^*P < 0.01$. (Peptide alone, with nifedipine, or verapamil vs. paired control without peptide.)

DISCUSSION

Glomerular mesangial cells stimulated by C20:4 or A23187 synthesize large amounts of PG, especially PGE_2. The production of PGE_2 (370 ng/mg protein per 30 min) by rat glomerular mesangial cells is greater than in rat renal medullary interstitial cells (107 ng/mg protein per 1 h) or rat glomerular epithelial cells (25 ng/mg protein per 30 min) (12, 24). PG production by cultured rat glomerular mesangial cells in our experiments differs somewhat from that found by Sraer et al. (10–12). We both found that production of PGE_2 was the greatest in the basal as well as the stimulated state. However, whereas we found that C20:4 incubation stimulated cyclooxygenase products in the following order: $PGE_2 \gg PGF_{2\alpha} > 6kPGF_{1\alpha} > TXB_2$, Sraer et al. reported that TXB_2 was the second most abundant prostanoid.

We evaluated the effects of two constrictor peptides, ANG II and AVP, on mesangial prostaglandin synthesis. ANG II and AVP reduce renal blood flow, GFR, and the K_f in the rat (5, 6). Decrements of K_f, induced by these peptides, may be secondary to mesangial contraction and subsequent loss of filtration surface area in the glomerulus (25). Furthermore, cyclooxygenase inhibitors such as indomethacin or meclofenamate substantially augment these actions of ANG II and AVP on the glomerulus (26). Based on these reports we hypothesized that ANG II and AVP increase mesangial synthesis of vasodilatory PG, which act to modulate the constrictor action of the peptides on the glomerulus. Our results support this hypothesis, namely that the mesangium responds to ANG II and AVP with augmented production of PGE_2. This response is elicited at 100 pM AVP and 10 nM ANG II, and three- to five-fold increments were detected at higher concentrations of the pressor peptide. It is unknown whether peptide-stimulated PGE_2 synthesis antagonizes ANG II- or AVP-induced mesangial contraction, although Ausiello (1) reported that exogenous PGE_1 does not affect pressor peptide-mediated contraction of cloned mesangial cells. However, based on the aforementioned studies using cyclooxygenase inhibitors, it is reasonable to attribute a regulatory role to PGE_2 synthesis in glomerular mesangial and epithelial cells. In addition, captopril, which has been found to improve GFR (27, 28), stimulates PGE_2 production five- to 10-fold with a smaller increase in $6kPGF_{1\alpha}$ in isolated glomeruli (29).

Whereas we found selective stimulation of PGE_2 by ANG II, Sraer et al. (12) found production of both PGE_2 and $PGF_{2\alpha}$ to be similarly enhanced by 10 nM ANG II. Sraer did not, however, study PG production at different concentrations of ANG II. We did, and found that selective stimulation of PGE_2 continued from 10 nM ANG II through to 1 μM ANG II.

Glomerular epithelial cells in culture also increase PGE_2 production in response to ANG II (13). Stimulation of whole, isolated rat glomeruli with ANG II yields less predictable results with either slight augmentation of PG synthesis (30) or no detectable changes (31). The activation of phospholipase, and hence PG synthesis, by the trauma of glomerular separation and isolation may explain the poor responsiveness of whole glomeruli to peptide stimuli. However, in glomeruli isolated by similar methods, the converting enzyme inhibitors captopril (SQ 14225) and SQ 20881) directly and selectively stimulated the vasodilatory PG, PGE_2 and $6kPGF_{1\alpha}$, in the absence of either angiotensin or bradykinin (29).

It is interesting that ANG II and AVP selectively increase PGE_2 synthesis in both glomerular epithelial and mesangial cells. This selectivity of PG stimulation is not attributable to the absence of other enzymes that convert endoperoxides to $PGF_{2\alpha}$, PGI_2, or TXA_2 since C20:4 or A23187 enhance the synthesis and release of these other cyclooxygenase products (Fig. 3). It seems likely that the membrane receptors for AVP and ANG II stimulate a specific phospholipase, which is coupled to a pool of cyclooxygenase and PGE_2 isomerase, separate from the other synthetic enzymes. Schwartzman et al. (32, 33), have reached similar conclusions using isolated and perfused kidney. They have demonstrated a hormone-sensitive (bradykinin, ANG II) and a hormone-insensitive pool of phospholipase with tighter coupling of the hormone-sensitive phospholipase and its released arachidonate to the cyclooxygenase and PGE_2 synthesis (33).

Whereas AVP also selectively stimulated PGE_2 synthesis, dDAVP did not, suggesting that it is the pressor, not the antidiuretic, action of AVP that is associated with its stimulatory ability. Preincubation with

$d(CH_2)_5VDAVP$, a potent antipressor antagonist, completely blocked AVP-stimulated PGE_2. dDAVP blocked PGE_2 production by 50%, suggesting that it does bind to the receptor. These findings are similar to those of Beck et al. (34) in the renal medullary interstitial cell.

Since agents that interfere with calcium entry or calcium binding block the action of ANG II on renal vasculature (35), uterine smooth muscle (36), and glomeruli (37), we further assessed Ca^{++} dependence of ANG II and AVP actions on the glomerular mesangial cell. Our studies confirm the integral role of Ca^{++} as a mediator of the peptide-stimulated PG production. Incubation of the cells with the Ca^{++} ionophore, A23187, enhanced PGE_2 production, as did increasing extracellular Ca^{++} from 0.6 to 1.8 mM. Removal of Ca^{++} from the incubation medium, during AVP or ANG II incubation, abolished peptide-stimulated PGE_2 synthesis. Blocking Ca^{++} entry into the mesangial cell with nifedipine or verapamil similarly inhibited AVP or ANG II-mediated PGE_2 production. These alterations of peptide-stimulated PGE_2 production, effected by changing Ca^{++} availability, probably result from changes in activity of phospholipase(s) since this enzyme in renal slices (38), platelets (39), and leukocytes (40) is calcium dependent and often rate limiting for PG synthesis.

We conclude that mesangial cells have the capability to synthesize large amounts of PG, especially PGE_2. The constrictor peptides, AVP and ANG II, selectively augment PGE_2 production. This PGE_2 stimulation is a Ca^{++} dependent process. The physiologic implication of this is unclear, but it is possible that the effects of ANG II and AVP on renal blood flow and GFR are modulated or antagonized by vasodilatory PG, specifically those synthesized by epithelial and mesangial cells. Experiments with isolated rat glomeruli, in which we measured glomerular planar surface area as an index of mesangial (i.e., glomerular) contraction, have confirmed that PG antagonize ANG II since ANG II-mediated glomerular contraction was potentiated by PG inhibition and decreased by PG stimulation with C20:4 (unpublished observations).

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