Eicosanoids, Mesangial Contraction, and Intracellular Signal Transduction

PAOLO MENE', MICHAEL S. SIMONSON and MICHAEL J. DUNN

Departments of Medicine and Physiology, Case Western Reserve University School of Medicine, Division of Nephrology, University Hospitals of Cleveland, Cleveland, Ohio 44106, USA

MENE', P., SIMONSON, M.S. and DUNN, M.J. *Eicosanoids, Mesangial Contraction, and Intracellular Signal Transduction*. Tohoku J. Exp. Med., 1992, 166 (1), 57-73 — The glomerular mesangial cell is a specialized pericyte with multiple functional capabilities including contraction. Mesangial contraction may reduce the glomerular filtration surface area and hence the ultrafiltration coefficient, K_f . Cultured mesangial cells convert arachidonic acid into biologically active eicosanoids which are either contractile (thromboxane A_2 [TxA_2], prostaglandin $F_{2\alpha}$ [$PGE_{2\alpha}$]) or relaxant (PGE_2 , PGI_2). The addition of TxA_2 analogues, PGE_2 or sulfidopeptide leukotrienes (LTC_4 and LTD_4) stimulated contraction of cultured mesangial cells with threshold responses at approximately 1 nM and maximum responses at 1 μ M. PGE_2 and PGI_2 antagonized mesangial contraction induced by TxA_2 analogues. Contraction was enhanced by inhibiting mesangial cyclooxygenase with nonsteroidal antiinflammatory drugs (NSAID). Contractile eicosanoids stimulated phospholipase C thereby elevating intracellular inositol trisphosphate and cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$). Vasorelaxant prostanoids stimulated adenylate cyclase, increasing intracellular cyclic AMP. We conclude that eicosanoids control mesangial contractility by regulating $[Ca^{2+}]_i$ and cAMP. NSAID increase mesangial reactivity by blocking the inhibitory effects of endogenous vasodilator eicosanoids, with potential consequences on glomerular hemodynamics. ——— calcium; cyclic AMP; mesangial cells; prostaglandins; thromboxane

The glomerular mesangium is an intercapillary network of modified smooth muscle cells embedded in extracellular matrix (Latta 1973; Kreisberg et al. 1985). Cultured cells of mesangial origin contract in response to the vasoactive peptides, angiotensin II (ANG II) and arginine vasopressin (AVP) (Ausiello et al. 1980; Mahieu et al. 1980). This prompted the suggestion that mesangial cells may regulate glomerular blood flow and filtration in synergism with the afferent and efferent arterioles, by modifying capillary surface area and hence the ultrafiltration coefficient, K_f (Brenner et al. 1986). Although there is no direct

Address correspondence to: M.J. Dunn, M.D., Dept. of Medicine, Div. of Nephrology, University Hospitals of Cleveland, 2074 Abington Road, Cleveland, Ohio 44106, USA.

proof of such hemodynamic function of mesangial cells, various observations are consistent with this model, including measurements of selective changes of K_f upon infusion of vasoactive agents (Blantz et al. 1976; Ichikawa and Brenner 1979; Schor et al. 1981) or in glomerulonephritis (Maddox et al. 1975). Moreover, studies in freshly isolated rat and human glomeruli indicate the presence of a contractile apparatus mediating rapid dimensional changes of the tuft upon exposure to vasoconstrictors such as angiotensin II (ANG II) (Savin 1986; Scharschmidt et al. 1986a). It is likely that mesangial cells mediate this mechanical activity, given their contractile phenotype and central location within the capillary loops, suited to application of radial forces (Latta 1973; Kreisberg et al. 1985). Glomeruli are also contracted by stable analogues of the vasoconstrictor metabolite of arachidonic acid (AA), thromboxane A_2 (TxA_2) (Loutzenhiser et al. 1986; Scharschmidt et al. 1986a). It seems therefore legitimate to speculate that the impairment of renal hemodynamics induced by infusion of these compounds in experimental animals (Feigen et al. 1977; Gerber et al. 1979) might in part result from contraction of the glomerular mesangium.

TxA_2 is the predominant cyclooxygenase metabolite of platelets and macrophages (Hamberg et al. 1975; Scott et al. 1982). It is released at the site of vascular injury or inflammation, such as in the course of experimental nephritis (Lianos et al. 1983), ureteral obstruction (Okegawa et al. 1983), renal allograft rejection (Coffman et al. 1985), and lupus nephritis in man (Patrono et al. 1985). A direct hemodynamic effect of TxA_2 in these disease models is demonstrated by reversal of renal vasoconstriction upon administration of TxA_2 -synthetase inhibitors or receptor antagonists. Alternative sources of increased TxA_2 are glomerular epithelial and mesangial cells, also capable of metabolizing AA to TxA_2 , although the synthesis of other prostaglandins (PG), such as PGE_2 and $PGE_{2\alpha}$ predominates (Kreisberg et al. 1982; Scharschmidt and Dunn 1983).

Lipoxygenase metabolites of AA also have potent effects on vascular smooth muscle and chemotactic properties on leukocytes (Hammarström 1983; Lewis and Austen 1984). Leukotrienes (LT) are abundantly produced by leukocytes and possibly resident cells in certain forms of experimental glomerulonephritis (Lianos et al. 1985; Stork et al. 1986). Mesangial cells are a potential target for LT, as the glomerulus expresses specific binding sites for LTC_4 (Ballermann et al. 1985), and contraction of the mesangium may play a role in the renal response to intravenous infusion of LTC_4 in the rat (Rosenthal and Pace-Asciak 1983; Badr et al. 1984).

We therefore investigated the effects of various vasoconstrictor eicosanoids on cultured mesangial cells, as well as their interactions with PGE_2 and PGI_2 , two major vasodilator metabolites of AA in the glomerulus. The cellular mechanisms of action of these agents were also analyzed by monitoring the induction of second messengers that regulate smooth muscle contractility, such as inositol (poly) phosphates, cytosolic calcium and cyclic nucleotides. Finally, we employed

pharmacologic manipulations to modulate mesangial contractility in vitro, in view of potential applications in experimental and human glomerular disease.

Mesangial cell contraction

Contraction studies were performed in cultured rat mesangial cells, established and characterized according to previously published techniques (Mene' and Dunn 1986; Simonson and Dunn 1986). Cells in passages 2 through 7 were employed. Crosssectional area (CSA) of the cells was used as a bidimensional correlate of cell contraction. CSA was measured by computer enhancement of phase-contrast photomicrographs of the cells exposed to the various eicosanoids for 40 min, as described elsewhere (Mene' and Dunn 1986; Simonson and Dunn 1986).

Effects of TxA_2 and endoperoxide analogues. A progressively larger number of rat mesangial cells contracted in response to increasing concentrations of the endoperoxide/ TxA_2 analogues U44069 (9, 11-dideoxy- 9α , 11α -epoxymethano-PGF $_{2\alpha}$) and U-46619 (9, 11-dideoxy- 11α , 9α -epoxymethano-PGF $_{2\alpha}$, Upjohn, Kalamazoo, MI), to a maximum of approximately 35% at $1\ \mu\text{M}$ (Fig. 1). Less than 8% of the cells displayed a significant reduction of CSA during control incubations for 40 min with the vehicle of the TxA_2 analogues (EtOH, final concentration $<0.01\%$). The onset of contraction was detectable as early as 5 min after the addition of each agonist. Contraction progressed for 30 to 40 min, at which time CSA stabilized or spontaneously increased. The process was fully reversible upon removal of the agonist(s), and repeatable in the same cells that contracted initially, indicating specificity of the response to the agonists and the lack of cytotoxicity of vasoconstrictor prostanoids. Moreover, 87% of agonist-treated, trypsinized cells excluded Trypan Blue at the end of 30 min incubations, as opposed to 85.2% in control experiments ($p = \text{NS}$). No cells on coverslips were stained at the end of the incubations with U-46619. All experiments were performed at room temperature, although contraction was enhanced at 37°C .

Authentic TxA_2 generated by sheep platelet microsomes preincubated with AA also stimulated contraction of approximately 30% of the cells (Fig. 1). Measurement of total TxB_2 generation at the end of each experiment revealed average concentrations in the nanomolar range. The initial concentration to which the cells were exposed could not be estimated, but it is likely that a 10-fold dilution of the microsomes/AA mixture during addition to the cells greatly slowed the formation of TxA_2 , since 10 min preincubation of the microsomes yielded 2.42 nM TxB_2 , as compared to an average recovery of 0.77 ± 0.08 nM. TxB_2 did not stimulate contraction in 4 experiments at 1 nM and $1\ \mu\text{M}$.

The apparent insensitivity of a certain percentage of the cells to TxA_2 is a common observation in many studies employing similar morphometric techniques (Latta 1973; Barnett et al. 1986; Mene' and Dunn 1986; Simonson and Dunn 1986; Singhal et al. 1986). Unlike smooth muscle cells (Chamley-Campbell et al. 1979), mesangial cells have been shown to retain contractility in culture, with

Fig. 1. Stimulation of contraction of cultured rat mesangial cells by authentic TxA₂ generated by platelet microsomes preincubated with arachidonic acid, and by the thromboxane A₂/endoperoxide analogues U-44069 and U-46619, 1 pM to 1 μ M. Panel A, percent cells contracting $\pm$ s.e., $n=4-6$ experiments each group; * $p<0.05$, ** $p<0.01$ vs. controls, chi square tests. Panel B, time course of contraction in responding cells, mean reductions of cross-sectional area $\pm$ s.e. vs. basal; NR, non-responding cells. (From *Transactions of the American Clinical and Climatological Association*, **98**, 71-79, 1986, with permission).

stable and reproducible responses to various stimuli, that are rate-limited by adhesion to non-compliant culture surfaces. Actual shortening, or wrinkling of substrates, indicative of isometric contraction, occurs in a majority of the cells grown on flexible materials, such as poly-HEMA or silicon rubber-coated plates (Latta 1973; Venkatachalam and Kreisberg 1985; Singhal et al. 1986). The direct relationship between concentration of the agonists and number of cells contracting in our studies therefore most likely reflects progressive development of tensile forces overcoming tight adhesion to the glass substrates. It is also possible that uneven responses are related to the presence of subsets of cells not expressing receptors for TxA₂ or refractory to contractile stimuli during certain phases of the cell cycle.

Fig. 2. Inhibition of U-44069/U-46619 ($1 \mu\text{M}$)-induced contraction of cultured rat mesangial cells by the thromboxane A_2 -receptor antagonists EP 092 and SQ 27,427 ($10 \mu\text{M}$). Percent cells contracting $\pm$ s.e., $n=3-5$ experiments each group. * $p < 0.05$ vs. agonists alone, chi-square tests. (From Ref. 40, with permission).

Responses to U-44069 and U-46619 were inhibited by a 10-fold molar excess of the structurally different TxA_2 -receptor antagonists EP-092 (Armstrong et al. 1985) and SQ 27, 427 (Greenberg et al. 1984) (gifts of Dr. R.L. Jones of the Dept. of Pharmacology of the University of Edinburgh, Scotland, and Squibb (Princeton, NJ, USA), respectively), added 10 min prior to stimulation of the cells (Fig. 2). The receptor antagonists also blocked completely responses to authentic TxA_2 . The total inhibition of the effects of TxA_2 is in apparent contrast with incomplete blockade of responses to the endoperoxide analogues. This residual contraction in 7.3 to 14.2% of the cells could be explained by binding of the endoperoxide analogues to other eicosanoid receptors. One such site could be a receptor for $\text{PGF}_{2\alpha}$, which in separate experiments also stimulated mesangial cell contraction over a wide dose range, with a maximum of $37.1 \pm 8.6\%$ at $1 \mu\text{M}$, and a time course similar to responses to the endoperoxide analogues.

The endoperoxide analogues significantly stimulated immunoreactive PGE_2 synthesis (U-44069 100.9% and 47.9%, U-46619 141.6% and 50.7% of basal synthesis during 15 min incubations, $1 \mu\text{M}$ and $1 \mu\text{M}$, respectively, $p < 0.05$ vs. controls).

Effects of leukotrienes. Similar to TxA_2 , the sulfidopeptide LTC_4 and D_4 , but not B_4 (gifts from Dr. J. Gleason of Smith, Kline and French Laboratories, Philadelphia, PA, USA), contracted cultured rat mesangial cells. The maximum percentage of responding cells was virtually identical to TxA_2 , although achieved at nanomolar concentrations, with an apparent EC_{50} in the picomolar range (Fig. 3, panel A). Higher doses of $\text{LTC}_4/\text{LTD}_4$ did not stimulate responses in a larger fraction of the cells, but increased the extent of contraction in responding cells, as shown in Fig. 3, panel B. Similar to the time course of responses to TxA_2 and its stable analogues, a significant decrement of CSA was observed within 5 min of

Fig. 3. Stimulation of contraction of cultured rat mesangial cells by leukotrienes C₄, D₄ and B₄. Panel A, percent cells contracting $\pm$ s.e. Panel B, time course of contraction in cells responding to 1 nM/1 μ M LTC₄, mean reductions of cross sectional area $\pm$ s.e. vs. basal, $n=5-6$ experiments each group. NR, non-responding cells. (From *Transactions of the American Clinical and Climatological Association*, **98**, 71-79, 1986, with permission).

addition of both LTC₄ and LTD₄. Contraction induced by 1 μ M LTD₄ was blocked by the receptor antagonist 4R, 5S, 6Z-2-nor-LTD₁, (65.6% inhibition of response at 10 μ M). Unlike other contractile agents such as ANG II, AVP or U-44069/U-46619, LTC₄ had no effect on PGE₂ synthesis at concentrations between 1 pM and 1 μ M. This is in agreement with the data of Barnett et al. (1986), who reported similar effects of LTC₄ and LTD₄ on mesangial contraction in the absence of any significant stimulation of PG synthesis. This may indicate differences between the mechanism of action of LT and various other contractile stimuli, such as ANG II, AVP and TxA₂, which rapidly stimulate phospholipase activity and AA metabolism in these cells (Patrono et al. 1985; Mene' and Dunn 1986).

Modulation of mesangial contraction

Effects of PGE₂ and PGI₂. Given the differential stimulation of PGE₂ by analogues of TxA₂ but not by LT, and the apparently greater potency of the latter on a molar basis, we evaluated whether vasoconstrictor-enhanced PGE₂ synthesis serves any modulatory function on mesangial contraction.

Fig. 4 summarizes the effects U-46619 in rat mesangial cells preincubated with increasing concentrations of PGE₂ for 10 min. PGE₂ dose-dependently inhibited responses to U-46619, an effect shared by the PGI₂ analogue, Iloprost (a gift of Berlex-Schering, Wayne, NJ, and of Dr. K.-H. Thierach at Schering AG, Berlin, FRG) (Schillinger et al. 1986), in the same range of concentrations. PGE₂ had no intrinsic contractile activity (7.2% of the cells at 1 μ M, vs. 5.4% in control experiments). Stimulation of PGE₂ synthesis by addition of 10 μ M AA also inhibited U-46619-induced contraction, to 15.9% vs. 37.2% of the cells ($p < 0.05$) in control experiments. Although significantly reduced, the number of responding cells in these experiments was greater than with 1 μ M PGE₂, which entirely inhibited contraction. A possible explanation is the simultaneous release of other prostanoids with agonist activity on contraction, e.g., PGF_{2 α} and TxA₂. The former particularly is a relevant product of cultured rat mesangial cells (Kreisberg et al. 1982; Scharschmidt and Dunn 1983).

Effects of nonsteroidal antiinflammatory drugs. As PGE₂ and Iloprost potently modulate contractility in vitro, we asked whether basal or agonist-stimulated PGE₂ production has any influence on contractility, and whether the fact that only approximately one-third of the cells are contracted by an agonist could be explained by an internal negative feed-back mechanism. Therefore we

Fig. 4. Inhibition of U-46619-induced contraction of cultured rat mesangial cells by PGE₂ and Iloprost. Percent cells contracting $\pm$ s.e. * $p < 0.05$, ** $p < 0.001$ vs. U-46619 alone, chi-square tests. (From *Circulation Research*, **62**, 916-925, 1988, with permission).

Fig. 5. Potentiation of U-46619-induced contraction of cultured rat mesangial cells by nonsteroidal antiinflammatory drugs (NSAID) (open bars). Inset solid bars, NSAID alone and/or vehicle of U-46619. Percent cells contracting $\pm$ S.E. * p < 0.05, ** p < 0.01 vs. U-46619 alone, chi-square test. ASA, acetylsalicylic acid. (From *Circulation Research*, 62, 916-925, 1988, with permission).

pretreated mesangial cells with one of four nonsteroidal anti-inflammatory drugs (NSAID), that inhibit cyclooxygenase, thereby blocking PG synthesis. As indicated in Fig. 5 indomethacin, meclofenamate, ASA, and piroxicam markedly enhanced U-46619-induced contraction. Notably, NSAID also amplified spontaneous contraction in control incubations, suggesting that the morphology of unstimulated cells in culture is controlled by endogenous synthesis of prostaglandins. Pharmacologic effects on biochemical pathways independent of cyclooxygenase were ruled out by the use of multiple, structurally different drugs. The effects of NSAID were linked to inhibition of PGE_2 , assayed radioimmunologically in simultaneous biochemical studies on mesangial cell monolayers. The ability of the cells to exclude the vital stain Trypan Blue was not affected by NSAID at the concentrations employed, chosen on the basis of >90% inhibition of basal and AA-stimulated PGE_2 synthesis.

These results, taken together with previous work indicating that NSAID enhance and PGE_2 inhibits contraction induced by other agents such as ANG II and platelet activating factor (Foidart and Mahieu 1986; Schlondorff et al. 1984; Scharschmidt et al. 1986b), point to a modulatory role of AA metabolism on glomerular smooth muscle reactivity. Vasodilator PG production may be critical to balance the effects of vasoconstrictor eicosanoids released in the course of glomerular inflammation (Lianos et al. 1983; Stork et al. 1986). Another functional correlate of these studies may be the reduction of renal perfusion, glomerular filtration rate and proteinuria observed after administration of NSAID to subjects with chronic glomerular disease (Kimberly et al. 1978; Dunn 1984;

Ciabattoni et al. 1984 ; Patrono and Dunn 1987 ; Vriesendorp et al. 1986). It has been proposed that local PG synthesis may subserve a compensatory function in this setting, allowing increased filtration in remnant nephrons through relative vasodilation (Ciabattoni et al. 1984). Given the functional antagonism of vasoconstrictors by PG observed in our model, enhanced arteriolar and mesangial contractility may underlie this renal effect of cyclooxygenase inhibitors.

Intracellular signals for contraction/relaxation

Effects of PG on $[Ca^{2+}]_i$ and inositol(poly)phosphates. Since TxA_2 increases $[Ca^{2+}]_i$ in platelets and vascular smooth muscle (Brace et al. 1985 ; Fukuo et al. 1986), and the elevation of $[Ca^{2+}]_i$ stimulates contraction and/or secretion in these cells, we studied the effects of various eicosanoids on $[Ca^{2+}]_i$ of cultured rat mesangial cells. $PGF_{2\alpha}$, U-46619 and PGE_2 dose- dependently stimulated a rapid, transient rise of $[Ca^{2+}]_i$, measured by fluorescence excitation of fura-2-loaded confluent monolayers on plastic coverslips (Fig. 6) (Mene' et al. 1987a, b). The rapid $[Ca^{2+}]_i$ "spike" was followed by a slow return towards prestimulation

Fig. 6. Stimulation of free cytosolic calcium ($[Ca^{2+}]_i$) by $PGF_{2\alpha}$, PGF_2 and U-46619. Fluorescence excitation of fura-2 loaded monolayers of cultured rat mesangial cells, representative experiments. (From Ref. 41, with permission).

values, with elevated levels persistent for >10 min. $\text{PGF}_{2\alpha}$ displayed greater potency at saturating concentrations than PGE_2 and U-46619, with the threshold at $1\text{--}10\ \mu\text{M}$ and peak $[\text{Ca}^{2+}]_i$ responses at $10\ \mu\text{M}$. PGE_1 and the PGI_2 analogue, Iloprost, had no effects upon $[\text{Ca}^{2+}]_i$ at any tested concentration, thereby ruling out a non-specific effect of unsaturated fatty acids. The $[\text{Ca}^{2+}]_i$ transient occurred even after chelation of extracellular Ca^{2+} with EGTA $3\ \text{mM}$, while the tonic phase of responses was suppressed by this maneuver, with immediate return of $[\text{Ca}^{2+}]_i$ to pre-stimulation levels. Sequential additions of increasing concentrations of the same agonist resulted in graded responses. On the other hand, pretreatment with a maximal concentration of any PG inhibited responses to a subsequent addition of the same or other PG as well as to ANG II or AVP, for >10 min. The specificity of $[\text{Ca}^{2+}]_i$ responses was confirmed with TxA_2 -receptor antagonists. Preincubation of the cells for 3 min with a 10-fold molar excess of SQ 27,427 (Fig.7) and (not shown) EP 092 selectively inhibited responses to U-46619, without affecting $\text{PGF}_{2\alpha}$ or PGE_2 -induced $[\text{Ca}^{2+}]_i$ changes, consistent with the presence of separate receptors for each prostanoid.

The effects of $\text{PGF}_{2\alpha}$, PGE_2 and U-46619 on $[\text{Ca}^{2+}]_i$ were accompanied by dose-dependent accumulation of total inositol phosphates (InsP_n), measured after anion exchange chromatography of perchlorate-extracted monolayers labelled with $[^3\text{H}]$ -myoinositol, from a basal of $5,417 \pm 296$ to $36,477 \pm 475$, $36,151 \pm 3,664$ and $8,537 \pm 663$ cpm/well/15 min, respectively. Fig. 8 shows the time course of

Fig. 7. Inhibition of U-46619-induced stimulation of free cytosolic calcium (Ca^{2+}) $_i$ by the thromboxane A_2 -receptor antagonist SQ 27,427 (panels A and C). SQ 27,427 had no effects on responses to PGE_2 (panels B and D). Fluorescence excitation of fura-2 loaded monolayers of cultured rat mesangial cell, representative experiments. (From Ref. 41, with permission).

Fig. 8. Stimulation of inositol phosphate (InsP) accumulation by PGE_{2α} in [³H]-myo-inositol labelled monolayers of cultured rat mesangial cells. Anion exchanges chromatography after perchloric acid extraction; percent of basal $\pm$ S.E., $n = 4$ experiments.

InsP_n formation in response to PGF_{2α}, with a rapid, sustained rise of total InsP₃ and a slower, more marked accumulation of InsP₁ after 15 min.

These data are consistent with activation of phospholipase C by certain eicosanoids, resulting in the release of water-soluble polyols from polyphosphoinositides. Ins-(1, 4, 5)P₃ is most likely responsible for the release of intracellularly stored Ca²⁺ during the early phase of responses to the agonists (Berridge and Irvine 1984). The progressive accumulation of other InsP should parallel the release of diacylglycerol, the endogenous activator of protein kinase C (Berridge and Irvine 1984; Nishizuka 1986). The combined elevation of [Ca²⁺]_i and activation of protein kinase C are probably key events in the contractile response, as Ca²⁺ regulates the interaction of contractile proteins in skeletal and smooth muscle, and protein kinase C can by itself trigger contraction of smooth muscle and cultured mesangial cells (Rasmussen et al. 1987; Troyer et al. 1988).

Effects of PG on intracellular cAMP. cAMP is a known signal transduction mediator for PG of the E and I series in various tissues (Gorman et al. 1977; Friedlander et al. 1983; Oliva et al. 1984), as well as a potent intracellular

TABLE 1. *Effects of PGE₂ and Iloprost on intracellular cAMP of cultured rat mesangial cells^a*

PG concentration (M)	Intracellular cAMP (pmol·mg protein ⁻¹ ·3 min ⁻¹)	
	PGE ₂	Iloprost
0	34.6±3.7	38.6±2.4
-9	40.9±4.8	45.2±2.8
-8	42.6±2.2	51.8±7.2
-7	55.1±2.3	110.4±24.4
-6	60.5±4.1	287.0±61.6
-5	79.6±16.6	308.9±58.5

^aValues are mean±s.e. of $n=6-8$ experiments. Direct radioimmunoassay of the cells and media after 3-min incubations in the presence of 0.1 ml isobutylmethylxanthine.

mediator of smooth muscle relaxation (Adelstein and Hathaway 1979). We therefore studied the effects of eicosanoids on mesangial cell adenylate cyclase, to assess whether cAMP accumulation could explain the inhibitory effects of these prostanoids on contraction. Both PGE₂ and Iloprost dose-dependently stimulated immunoreactive, intracellular cAMP accumulation in the presence of IBMX, as shown in Table 1. cAMP responses peaked at 3 min. U-46619 did not stimulate cAMP at any of the concentrations tested.

To establish whether cAMP accumulation in response to PGE₂ and PGI₂ mediates inhibition of contraction, we inhibited cAMP accumulation by preincubating the cells for 10 min with the false substrate for adenylate cyclase, 2', 5'-dideoxyadenosine (2', 5'-DDA), which blocks agonist-induced cAMP rises in several cell types (Fain et al. 1972; Haslam et al. 1978). 10 min preincubations of the cells with 0.1 mM 2', 5'-DDA, but not with the isomer 3', 5'-DDA, significantly inhibited cAMP accumulation in response to 10 μ M PGE₂ (PGE₂ 79.6±16.6, 2', 5'-DDA + PGE₂ 40.1±4.3, 3', 5'-DDA + PGE₂ 90.8±26.3 pmol/mg prot/3 min±s.e.) and Iloprost (Iloprost 308.9±58.5, 2', 5'-DDA + Iloprost 161.7±26.9, 3', 5'-DDA + Iloprost 244.5±43.9 pmol/mg prot/3 min±s.e.). In cells pretreated with 2', 5'-DDA PGE₂ no longer inhibited U46619-induced contraction (Fig. 9). 2', 5'-DDA also unmasked a latent agonist activity of PGE₂, and enhanced the action of U46619 to stimulate contraction of an additional 40% of the cells that did not respond initially to the endoperoxide analogue.

Contraction induced by PGE₂ had been documented earlier only in cloned cells grown on poly-HEMA treated surfaces by Venkatachalam and Kreisberg (1985). Given the [Ca²⁺]_i stimulation by PGE₂ observed in our fluorimetric studies, it is conceivable that contraction may occur with a mechanism similar to peptides or primarily vasoconstrictor eicosanoids, although antagonized by the concurrent elevation of cAMP documented by us and others (Ausiello et al. 1980;

Fig. 9. Effects of 2',5'-dideoxyadenosine (DDA) on contraction of cultured rat mesangial cells. PGE₂ and U-46619, 1 μ M, added alone or in combination to cells preincubated for 10 min 0.1 mM DDA. Percent cells contracting $\pm$ s.e. * p < 0.001, vs. PGE₂ alone; ** p < 0.01 vs. U-46619 alone; † p < 0.001 vs. U-46619 + PGE₂, chi-square tests. (From *Circulation Research*, **62**, 916-925, 1988, with permission).

Friedlander et al. 1983; Kurtz et al. 1985). cAMP-mediated relaxation seems to predominate in most studies in early passages (Ausiello et al. 1980; Foidart and Mahieu 1986; Scharschmidt et al. 1986b), indicating an effect distal to Ca²⁺-dependent phosphorylation of contractile proteins. Simultaneous activation of phospholipase C and adenylate cyclase by PGE₂ may imply the presence of dual receptors with separate second messengers, similar to other unrelated agents such as AVP, histamine, and serotonin, or coupling of a single receptor to both pathways of signal transduction. The existence of dual receptors for prostanoids has been demonstrated in smooth muscle of various origin (Coleman and Kennedy 1985; Keith and Salama 1987).

CONCLUSION

The present evidence for functional eicosanoid receptors in mesangial cells indicates a novel site of action of vasoactive AA metabolites in the glomerulus, in addition to the afferent and efferent arterioles. Our observations are in agreement with a general model of the mesangial cell as a modified intraglomerular smooth muscle cell, and provide an insight to the intracellular mechanisms controlling the interactions of cytoskeletal and contractile components in these cells. Moreover, contraction relaxation can be pharmacologically manipulated at various levels, employing PG synthesis inhibitors and receptor antagonists, or interfering with intracellular mechanisms of signal transduction. Future work should aim at correlating these observations in culture with glomerular function in vivo, providing direct evidence for a regulatory role of the mesangium on renal hemodynamics.

The data reviewed here may contribute to a better understanding of the role of eicosanoids in the control of the glomerular microcirculation under normal conditions and in settings of enhanced production, such as inflammation or adaptive changes to nephron loss. This could eventually lead to novel approaches to the pharmacologic treatment of experimental and human renal disease.

Acknowledgments

This work was supported by National Heart, Lung and Blood Institute Grant HL-22563. P. Mene' was the recipient of Research Fellowships from the American Heart Association, Northeast Ohio Affiliate and from Consiglio Nazionale delle Ricerche of Italy.

We are indebted to Drs. George Dubyak and Antonio Scarpa for scientific advice and helpful discussion. The excellent technical support of Cheryl Subjoc, Edith Hanzmann, Charles Rettberg and Guy McDermott is also particularly acknowledged.

References

- 1) Adelstein, R.S. & Hathaway, D.R. (1979) Role of calcium and cyclic adenosine 3', 5'-monophosphate in regulating smooth muscle contraction. *Am. J. Cardiol.*, **44**, 783-787.
- 2) Armstrong, R.A., Jones, R.L., Peesapati, V., Will, S.G. & Wilson, N.H. (1985) Competitive antagonism at thromboxane receptors in human platelets. *Br. J. Pharmacol.*, **84**, 595-607.
- 3) Ausiello, D.A., Kreisberg, J.I., Roy, C. & Karnovsky, M.J. (1980) Contraction of cultured rat glomerular cells of apparent mesangial origin after stimulation with angiotensin II and arginine vasopressin. *J. Clin. Invest.*, **65**, 754-760.
- 4) Badr, K., Baylis, C., Pfeffer, J., Pfeffer, M., Soberman, R.J., Lewis, R.A., Austen, K.F., Corey, E.J. & Brenner, B.M. (1984) Renal and systemic hemodynamic responses to intravenous infusion of leukotriene C₄ in the rat. *Circ. Res.*, **54**, 492-499.
- 5) Ballermann, B.J., Lewis, R.A., Corey, E.J., Austen, K.F. & Brenner, B.M. (1985) Identification and characterization of leukotriene C₄ receptors in isolated rat renal glomeruli. *Circ. Res.*, **56**, 324-330.
- 6) Barnett, R., Goldwasser, P., Scharschmidt, L.A. & Schlondorff, D. (1986) Effects of leukotrienes on isolated rat glomeruli and cultured mesangial cells. *Am. J. Physiol.*, **250**, F838-F844.
- 7) Berridge, M.J. & Irvine, R.F. (1984) Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature*, **312**, 315-321.
- 8) Blantz, R.C., Konnen, K.S. & Tucker, B.J. (1976) Angiotensin II effects upon the glomerular microcirculation and ultrafiltration coefficient of the rat. *J. Clin. Invest.*, **57**, 419-434.
- 9) Brace, L.D., Venton, D.L. & LeBreton, G.C. (1985) Thromboxane A₂/prostaglandin H₂ mobilizes calcium in human blood platelets. *Am. J. Physiol.*, **249**, H1-H7.
- 10) Brenner, B.M., Dworkin, K.D. & Ichikawa, I. (1986) Glomerular ultrafiltration. In: *The Kidney*, edited by B.M. Brenner & F.C. Rector, W.B. Saunders, Philadelphia, pp. 124-144.
- 11) Chamley-Campbell, J., Campbell, G.R. & Ross, R. (1979) The smooth muscle cell in culture. *Physiol. Rev.*, **59**, 1-61.
- 12) Ciabattoni, G., Cinotti, G.A., Pierucci, A., Simonetti, B.M., Manzi, M., Pugliese, F., Barsotti, P., Pecci, G., Taggi, F. & Patrono, C. (1984) Effects of sulindac and ibuprofen in patients with chronic glomerular disease. Evidence for the dependence of renal function on prostacyclin. *N. Engl. J. Med.*, **310**, 279-283.

- 13) Coffman, T.M., Yarger, W.E. & Klotman, P.E. (1985) Functional role of thromboxane production by acutely rejecting renal allografts in rats. *J. Clin. Invest.*, **75**, 1242-1248.
- 14) Coleman, R.A. & Kennedy, I. (1985) Characterization of the prostanoid receptors mediating contraction of guinea-pig isolated trachea. *Prostaglandins*, **29**, 363-375.
- 15) Dunn, M.J. (1984) Nonsteroidal antiinflammatory drugs and renal function. *Ann. Rev. Med.*, **35**, 411-428.
- 16) Fain, J.N., Pointer, R.H. & Ward, W.E. (1972) Effects of adenosine nucleotides on adenylate cyclase, phosphodiesterase, cyclic adenosine monophosphate accumulation and lipolysis in fat cells. *J. Biol. Chem.*, **247**, 6866-6872.
- 17) Feigen, L.P., Chapnik, B.M., Flemming, J.E., Flemming, J.M. & Kadowitz, P.J. (1977) Renal vascular effects of endoperoxide analogs, prostaglandins and arachidonic acid. *Am. J. Physiol.*, **233**, H573-H579.
- 18) Foidart, J.B. & Mahieu, P. (1986) Glomerular mesangial cell contractility in vitro is controlled by an angiotensin-prostaglandin balance. *Mol. Cell Endocrinol.*, **47**, 163-173.
- 19) Friedlander, G., Chansel, D., Sraer, J., Bens, M. & Ardaillou, R. (1983) PGE₂ binding sites and PG-stimulated cyclic AMP accumulation in rat isolated glomeruli and glomerular cultured cells. *Mol. Cell Endocrinol.*, **30**, 201-214.
- 20) Fukuo, K., Morimoto, S., Koh, E., Yukawa, S., Tsuchiya, H., Imanaka, S., Yamamoto, H., Onoshi, T. & Kumahara, Y. (1986) Effects of prostaglandins on the cytosolic free calcium concentration in vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.*, **136**, 247-252.
- 21) Gerber, J. G., Ellis, E., Hollifield, J. & Nies, A.S. (1979) Effect of prostaglandin endoperoxide analogues on canine renal function, hemodynamics and renin release. *Eur. J. Pharmacol.*, **53**, 239-246.
- 22) Gorman, R.R., Bunting, S. & Miller, O.V. (1977) Modulation of human platelet adenylate cyclase by prostacyclin (PGX). *Prostaglandins*, **13**, 377-388.
- 23) Greenberg, R., Steinbacher, T. & Haslanger, M.E. (1984) Thromboxane receptor antagonist properties of SQ 27,427 in the anesthetized guinea pig. *Eur. J. Pharmacol.*, **103**, 19-24.
- 24) Hamberg, M., Svensson, J. & Samuelsson, B. (1975) Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. Natl. Acad. Sci. USA*, **72**, 2994-2998.
- 25) Hammarström, S. (1983) Leukotrienes. *Ann. Rev. Biochem.*, **52**, 355-377.
- 26) Haslam, R.J., Davidson, M.M.L. & Desjardin, J.V. (1978) Inhibition of adenylate cyclase by adenosine analogues in preparations of broken and intact human platelets. *Biochem. J.*, **176**, 83-95.
- 27) Ichikawa, I. & Brenner, B.M. (1979) Mechanism of action of histamine and histamine antagonists on the glomerular microcirculation in the rat. *Circ. Res.*, **45**, 737-745.
- 28) Keith, R.A. & Salama, A.I. (1987) Individual variations of prostanoid agonist responses in rabbit aorta: Evidence for the independent regulation of prostanoid receptor subtypes. *Br. J. Pharmacol.*, **92**, 133-148.
- 29) Kimberly, R.P., Bowden, R.E., Keiser, H.R. & Plotz, P.H. (1978) Reduction of renal function by newer nonsteroidal antiinflammatory drugs. *Am. J. Med.*, **64**, 804-807.
- 30) Kreisberg, J.I., Karnovsky, M.J. & Levine, L. (1982) Prostaglandin production by homogeneous cultures of rat glomerular epithelial and mesangial cells. *Kidney Int.*, **22**, 355-359.
- 31) Kreisberg, J.I., Venkatachalam, M. & Troyer, D. (1985) Contractile properties of cultured glomerular mesangial cells. *Am. J. Physiol.*, **249**, F457-F463.
- 32) Kurtz, A., Jelkmann, W., Pfeilschifter, J. & Bauer, C. (1985) Role of prostaglandins in hypoxia-stimulated erythropoietin production. *Am. J. Physiol.*, **249**, C3-C8.

- 33) Latta, H. (1973) Ultrastructure of the glomerulus and juxtaglomerular apparatus. In: *Handbook of Physiology*, Section 8, edited by J. Orloff, R.W. Berliner & S.R. Geiger, Williams and Wilkins, Baltimore, pp. 1-29.
- 34) Lewis, R.A. & Austen, K.F. (1984) The biologically active leukotrienes. Biosynthesis, functions, and pharmacology. *J. Clin. Invest.*, **73**, 889-897.
- 35) Lianos, E.A., Andres, G.A. & Dunn, M.J. (1983) Glomerular prostaglandin and thromboxane synthesis in rat nephrotoxic serum nephritis. Effects on renal hemodynamics. *J. Clin. Invest.*, **72**, 1439-1448.
- 36) Lianos, E.A., Rahman, M.A. & Dunn, M.J. (1985) Glomerular arachidonate lipox-ygenation in rat nephrotoxic serum nephritis. *J. Clin. Invest.*, **76**, 1355-1359.
- 37) Loutzenhiser, R., Epstein, M., Horton, C. & Sonke, P. (1986) Reversal of renal and smooth muscle actions of the thromboxane mimetic U-44069 by diltiazem. *Am. J. Physiol.*, **250**, F619-F626.
- 38) Maddox, D.A., Bennett, C.M., Deen, W. M., Glasscock, R.J., Knutson, D., Daugherty, T.M. & Brenner, B.M. (1975) Determinants of glomerular filtration in experimental glomerulonephritis in the rat. *J. Clin. Invest.*, **55**, 305-318.
- 39) Mahieu, P.R., Foidart, J.B., Dubois, C.H., Dechenne, C.A. & Deheneffe, J. (1980) Tissue culture of normal rat glomeruli: Contractile activity of the cultured mesangial cells. *Invest. Cell Pathol.*, **3**, 121-128.
- 40) Mene' P. & Dunn, M.J. (1986) Contractile effects of TxA_2 and endoperoxide analogues on cultured rat glomerular mesangial cells. *Am. J. Physiol.*, **251**, F1029-F1035.
- 41) Mene', P., Dubyak, G.R., Scarpa, A. & Dunn, M.J. (1987a) Stimulation of cytosolic free calcium and inositol phosphates by prostaglandins in cultured rat mesangial cells. *Biochem. Biophys. Res. Commun.*, **142**, 579-586.
- 42) Mene' P., Abboud, H.E., Dubyak, G.R., Scarpa, A. & Dunn, M.J. (1987b) Effects of PDGF on inositol phosphates, Ca^{2+} and contraction of mesangial cells. *Am. J. Physiol.*, **253**, F458-F463.
- 43) Nishizuka, Y. (1986) Studies and perspectives of protein kinase C. *Science*, **233**, 305-312.
- 44) Okegawa, T., Jonas, P.E., DeSchryver, K., Kawasaki, A. & Needleman, P. (1983) Metabolic and cellular alterations underlying the exaggerated renal prostaglandin and thromboxane synthesis in ureter obstruction in rabbits. *J. Clin. Invest.*, **71**, 81-90.
- 45) Oliva, D., Noe', A., Nicosia, S., Bernini, F., Fumagalli, R., Whittle, B.J.R., Moncada, S. & Vane, J.R. (1984) Prostacyclin-sensitive adenylate cyclase in cultured myocytes: Differences between rabbit aorta and mesenteric artery. *Eur. J. Pharmacol.*, **105**, 207-213.
- 46) Patrono, C. & Dunn, M.J. (1987) The clinical significance of inhibition of renal prostaglandin synthesis. *Kidney Int.*, **32**, 1-12.
- 47) Patrono, C., Ciabattini, G., Remuzzi, G., Gotti, E., Bombardieri, S., DiMunno, O., Tartarelli, G., Cinotti, G.A., Simonetti, B.M. & Pierucci, A. (1985) Functional significance of renal prostacyclin and thromboxane A_2 production in patients with systemic lupus erythematosus. *J. Clin. Invest.*, **76**, 1011-1018.
- 48) Rasmussen, H., Takuwa, Y. & Park, S. (1987) Protein kinase C in the regulation of smooth muscle contraction. *FASEB J.*, **1**, 177-185.
- 49) Rosenthal, A. & Pace-Asciak, C.R. (1983) Potent vasoconstriction of the isolated perfused rat kidney by leukotrienes C_4 and D_4 . *Can. J. Physiol. Pharmacol.*, **61**, 325-328.
- 50) Savin, V. (1986) In vitro effects of angiotensin II on glomerular function. *Am. J. Physiol.*, **251**, F627-F634.
- 51) Scharschmidt, L.A. & Dunn, M.J. (1983) Prostaglandin synthesis by rat glomerular mesangial cells in culture. *J. Clin. Invest.*, **71**, 1756-1764.
- 52) Scharschmidt, L.A., Douglas, J.G. & Dunn, M.J. (1986a) Angiotensin II and eicosanoids in the control of glomerular size in rat and human. *Am. J. Physiol.*, **250**,

- F348-F356.
- 53) Scharschmidt, L.A., Simonson, M. & Dunn, M.J. (1986b) Glomerular prostaglandins, angiotensin II, and nonsteroidal antiinflammatory drugs. *Am. J. Med.*, **81**, Suppl. 2B, 30-42.
 - 54) Schillinger, E., Kraus, T., Lehmann, M. & Stock, G. (1986) Iloprost. In: *New Cardiovascular Drugs*, edited by A. Scriabine, Raven Press, New York, pp. 209-231.
 - 55) Scholondorff, D., Satriano, J.A., Hagege, J., Perez, J. & Baud, L. (1984) Effect of platelet activating factor on prostaglandin E₂ synthesis, arachidonic acid release and contraction of cultured rat mesangial cells. *J. Clin. Invest.*, **73**, 1227-1231.
 - 56) Schor, N., Ichikawa, I. & Brenner, B.M. (1981) Mechanism of action of various hormones and vasoactive substances on glomerular ultrafiltration in the rat. *Kidney Int.*, **20**, 442-451.
 - 57) Scott, W.A., Pawlowski, N.A., Andreach, M. & Cohn, Z.A. (1982) Resting macrophages produce distinct metabolites from exogenous arachidonic acid. *J. Exp. Med.*, **155**, 535-547.
 - 58) Simonson, M.S. & Dunn, M.J. (1986) Leukotriene C₄ and D₄ contract rat glomerular mesangial cells. *Kidney Int.*, **30**, 524-531.
 - 59) Singhal, P.C., Scharschmidt, L.A., Gibbons, N. & Hays, R.M. (1986) Contraction and relaxation of cultured mesangial cells on a silicone rubber surface. *Kidney Int.*, **30**, 862-873.
 - 60) Stork, J.E., Rahman, M.A. & Dunn, M.J. (1986) Eicosanoids in experimental and human renal disease. *Am. J. Med.*, **80**, Suppl. 1A, 34-45.
 - 61) Troyer, D.A., Gonzalez, O.F., Douglas, J.G. & Kreinsberg, J.I. (1988) Phorbol ester inhibits arginine vasopressin activation of phospholipase C and promotes contraction of and prostaglandin production by cultured mesangial cells. *Biochem. J.*, **251**, 901-912.
 - 62) Venkatachalam, M.A. & Kreisberg, J.I. (1985) Agonist-induced isotonic contraction of cultured mesangial cells after multiple passage. *Am. J. Pharmacol.*, **84**, 595-607.
 - 63) Vriesendorp, R., Donker, A.J.M., deZeeuw, D., deJong, P.E., van der Hem, G.K. & Brentjens, J.R.H. (1986) Effects of nonsteroidal anti-inflammatory drugs on proteinuria. *Am. J. Med.*, **81**, Suppl. 2B, 84-94.
-