Eicosanoids and Control of Mesangial Cell Contraction

Paolo Mené and Michael J. Dunn

Contraction of glomerular mesangial cells is stimulated in vitro by the vasoconstrictor metabolite of arachidonic acid, thromboxane A$_2$. To establish the role of mesangial prostaglandin (PG) synthesis in the modulation of contractile responses, we studied the effects of the stable thromboxane A$_2$/endoperoxide analogue U-46619 on cultured rat mesangial cells preincubated with 1) four structurally unrelated, nonsteroidal anti-inflammatory drugs, indomethacin, acetylsaliclycic acid, meclofenamate, and piroxicam, to inhibit the synthesis of PGE$_2$, the major mesangial metabolite of arachidonic acid; 2) exogenous PGE$_2$ and the stable analogue of PGI$_2$, iloprost; and 3) indomethacin in the presence of exogenous PGE$_2$. Computer-assisted image analysis microscopy demonstrated enhancement of spontaneous and agonist-induced contraction by nonsteroidal anti-inflammatory drugs in individual cells grown on a glass substrate, from 37.2 ± 7.3% to a maximum of 75.5 ± 6.4% of the cells with piroxicam, at 1 μM U-46619. PGE$_2$ and iloprost dose-dependently inhibited U-46619–induced contraction, to 5.0 ± 2.8% and 12.5 ± 4.7% of the cells, respectively, at 1 μM U-46619. PGE$_2$, also completely reversed the effects of indomethacin. Both PGE$_2$, and iloprost dose-dependently stimulated intracellular cyclic AMP (cAMP) accumulation during 3-minute incubations, an effect that was blocked by the inhibitor of adenylate cyclase, 2',5'-dideoxyadenosine. The latter reversed the inhibitory action of PGE$_2$, enhancing spontaneous and agonist-induced contractility, thus indicating a modulatory role of cAMP. We conclude that endogenous arachidonate metabolism regulates mesangial cell contraction through elevation of intracellular cAMP. (Circulation Research 1988;62:916–925)

The mesangial cell is a contractile, smooth muscle–like cell of the kidney glomerulus, which has been implicated in the hormonal control of filtration surface area and hence as affecting the ultrafiltration coefficient, $K_f$, through its mechanical properties. Because mesangial cells can be cultured to purity from rodent or human kidneys and still retain contractile properties through multiple passages, they offer a suitable approach to the study of renal smooth muscle reactivity in vitro.

Numerous hormones or vasoactive compounds that affect glomerular hemodynamics stimulate [angiotensin II (Ang II) and arginine vasopressin] or attenuate (isoproterenol and dopamine) contraction of cultured rat mesangial cells, thus indicating a possible in vivo site of action additional to preglomerular and postglomerular resistances. We have recently reported that the vasoconstrictor metabolite of arachidonic acid, thromboxane A$_2$ (TXA$_2$), has biological effects on cultured rat mesangial cells that include mobilization of inositol phosphates, elevation of free cytosolic calcium, and contraction and stimulation of prostaglandin (PG) synthesis. These responses may contribute to the impaired renal hemodynamics after infusion of TXA$_2$ analogues or in disease models characterized by increased intrarenal TXA$_2$ synthesis, including nephrotic serum nephritis, ureteral obstruction, and renal allograft rejection. The observation that TXA$_2$ and other vasoactive agents stimulate the production of PGE$_2$ by mesangial cells, presumably through phospholipase C–mediated agents of polyphosphoinositides, leads to the question of whether this endogenous eicosanoid plays a role in the modulation of contractile responses and whether other vasodilator arachidonic acid metabolites, such as PGI$_2$, or prostacyclin, a product of glomerular endothelial cells, may act locally to control mesangial functions. The issue is relevant because, particularly in glomerular inflammation, both resident and infiltrating cells may contribute considerable amounts of different prostanoids, with potent vasoactive actions in other vascular beds.

In the present investigation, we therefore have studied the effects of 1) pharmacological inhibition of mesangial PGE$_2$ synthesis by nonsteroidal anti-inflammatory drugs (NSAID) and 2) addition of exogenous PGE$_2$ and PGI$_2$ on contraction induced by a stable TXA$_2$ analogue. Because previous studies in other cell types, including platelets, endothelial cells, and smooth muscle cells, indicate the adenylate cyclase–cyclic AMP (cAMP) system as the mediator of the effects of prostaglandins of the E and I series, we have simultaneously evaluated the role of this intracellular signaling system in the functional responses to the prostanoids used in the study.

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Materials and Methods

Cell Culture

Techniques for the isolation of rat glomeruli and the growth of pure lines of mesangial cells have been described in detail previously.\(^7\,8\) In the present investigation, we used 14 independent cell lines noted for purity and contractility to various agonists, including Ang II. Experiments were performed in subcultures 2 through 8. Growth medium was RPMI 1640 (M.A. Bioproducts, Walkerville, Maryland) supplemented with 17% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin (K.C. Biological, Lenexa, Kansas), 5 µg/ml insulin, 5 µg/ml transferrin, 5 ng/ml selenium (ITS Premix, Collaborative Research, Lexington, Massachusetts).

Morphometric Analysis

Computer-assisted image analysis was used as described previously.\(^7\,16\) Briefly, sparsely seeded cells grown on two-well glass chamber-slides (Lab-Tek, Miles Scientific, Naperville, Illinois) were studied 36 hours after subculture. The chamber-slides were placed under the objective of an inverted stage, phase-contrast Nikon Diaphot microscope connected to an Apple Ile computer by a Unicomp MTI video camera. Total magnification was x 200. The RPMI 1640 culture medium was then replaced with 1.8 ml Krebs-Henseleit-HEPES (KHH) buffer containing 0.2% (wt/vol) fatty acid-free bovine serum albumin (Calbiochem, San Diego, California). All agents used were added in KHH from the outset (when preincubation was required) or at zero time in 0.2 ml 10-fold concentrated solution to achieve the final desired concentration. Serial photomicrographs of a field chosen at random, usually containing 10-15 cells with easily discernible boundaries, were obtained at 10, 5, and 0 minutes before addition of the contractile agent or its vehicle in control experiments and then at 5-minute intervals up to 40 minutes. All incubations were at room temperature. Stained and nonstained cells were counted on a hemocytometer after proper dilutions, and results of triplicate determinations expressed as percentage of the total number of cells were studied.

Prostaglandin Synthesis

Monolayers of mesangial cells in 24-well dishes from the same cell lines used in the contraction experiments were washed twice with KHH and incubated for 30 minutes at 37°C in 500 µl KHH with indomethacin, acetylsalicylic acid, meclofenamate, or their vehicle (controls) in the presence or absence of arachidonic acid, 5 µg/ml. At the end of the incubations, media were collected and stored frozen for measurement of the PGE\(_2\) concentration by radioimmunoassay (RIA) without prior extraction as previously described.\(^11\) Values were corrected for the protein content of each well, measured by a modification of Lowry's technique.\(^7\) Antisera were a kind gift from Dr. Alberto Nasjletti (Memphis, Tennessee) and were preliminarily compared in parallel assays with antisera from the Institute Pasteur, Paris, France. Ligands were purchased from New England Nuclear, Boston, Massachusetts.

Intracellular cAMP Synthesis

Monolayers of mesangial cells in 24-well dishes were washed twice with KHH and incubated for 10 minutes in KHH containing 0.1 mM isobutylmethylxanthine (IBMX) with or without 2',5'- or 3',5'-DDA at 37°C. PGE\(_2\) or iloprost was then added to duplicate wells at various final concentrations for 3 minutes. Incubations were stopped by aspiration of the medium was <0.01%. Iloprost stock solutions (1 mM) in normal saline were stored at -70°C and diluted immediately before use in KHH. Iloprost was a generous gift of Berlex, Wayne, New Jersey.

All NSAID were prepared immediately before use and diluted in KHH to the final desired concentration. Indomethacin (Sigma Chemical, St. Louis, Missouri) was dissolved in 20 mM Na\(_2\)CO\(_3\); meclofenamate sodium monohydrate (Warner-Lambert, Ann Arbor, Michigan) was dissolved in normal saline; acetylsalicylic acid lysin salt (Maggiore Farmaceutici, Maggioni, Italy) was dissolved directly in KHH; piroxicam (Pfizer, Groton, Connecticut) was prepared in 2% Na\(_2\)CO\(_3\).

2',5'- and 3',5'-Dideoxyadenosine (DDA) (Pharmacia, Piscataway, New Jersey) were prepared as stock solutions (10 mM) in normal saline and stored frozen until use.

All other chemicals were of the finest grade available from Sigma.

Cell Viability

Cell viability upon exposure to each NSAID and to U-46619 was assessed at the end of 40-minute incubations of trypsinized suspensions of cells by Trypan blue exclusion (0.1%, diluted 1:1 vol/vol in KHH buffer). Viable cells were defined by their ability to exclude Trypan blue during 1-minute incubations at room temperature. Stained and nonstained cells were counted on a hemocytometer after proper dilutions, and results of triplicate determinations expressed as percentage of the total number of cells were studied.
and addition of 0.1N HCl for 2 hours at room temperature. The HCl extracts were then stored frozen for RIA and performed in duplicate after acetylation. Go
to antiser to cAMP was obtained from Research Products International (Mount Prospect, Illinois), with a cross-reactivity of less than 0.001% with all aden
osine and guanosine nucleotides, including cyclic GMP.

Tyrosine methylester cAMP was prepared by the chloramphip T method followed by thin layer chromato
graphy purification. Control samples were incubated to rule out interference of 0.1N HCl, 0.1 mM IBMX, and 0.1 mM 2',5'- or 3',5'-DDA with the assay. Results were normalized for the protein content of each well as determined by a modification of Lowry's technique.

**Statistical Analysis**

Results of morphometric experiments were analyzed by χ² tests with Yates' correction, comparing the number of contracting cells out of the total number of cells analyzed between each of the various experimental conditions. Standard error of the means of the percentages were calculated by analysis of binomial distribution.

Significance of CSA differences between NSAID and control incubations was analyzed by two-way analysis of variance for an unbalanced nested design, followed by individual Tukey's tests.

Overall significance of cAMP responses to PGE, and iloprost with and without DDA isomers was analyzed by one-way analysis of variance followed by individual Tukey's tests (within each curve) and by unpaired t tests (between curves).

**Results**

To establish whether endogenous prostaglandin synthesis has an effect on the contractility of cultured mesangial cells, both basally and in response to an agonist, cells were incubated for 10 minutes in the presence of one of four structurally unrelated NSAID, indomethacin, acetylsalicylic acid, meclofenamate, or piroxicam, followed by exposure to U-46619, 1 μM, for an additional 40 minutes in the continued presence of the NSAID. Results of these experiments are shown in Figure 1 and Table 1. Control incubations with the vehicle of U-46619 resulted in spontaneous contraction of less than 10% of the cells analyzed. All four NSAID increased both spontaneous and U-46619-induced contraction to a maximum of approximately 75% of the cells with piroxicam. The concentrations of the NSAID, except piroxicam, tested directly for dose and effect in the contraction assay, were chosen on the basis of effective inhibition of basal and stimulated mes
gangial cell cyclooxygenase activity, as evaluated by RIA of PGE after 30-minute incubations of the cells with and without arachidonic acid (Table 2). To rule out a possible cytotoxic effect of the drugs, mesangial cell viability was assessed by Trypan blue exclusion after 40-minute incubations in the presence of 1 μM U-46619. None of the NSAID had any effect on cell viability at the concentrations used in the study (control, 6.3% of the cells stained, U-46619 alone 5.9%, indomethacin 5.1%, acetylsalicylic acid 8.2%, meclofenamate 7.9%, and piroxicam 7.5%).

The analysis of the time courses of contraction indicated some degree of enhancement of cell responses by NSAID, achieving statistical significance only with piroxicam, as shown in Figure 2. Particularly at later time points, that is, 20–40 minutes, piroxicam and, to a lesser degree, the other NSAID (as indicated by the maximum percent reductions of CSA in Table 1) increased both the number of responding cells and their average surface reductions, suggesting a modulator function of endogenous prostaglandin synthesis on contractile responses.

**Table 1. Effects of Nonsteroidal Anti-Inflammatory Drugs on U-46619–Induced Contraction of Cultured Rat Mesangial Cells**

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>Cells contracting (%)</th>
<th>Maximum decrease CSA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>3</td>
<td>5.4 (2/37)</td>
<td>17.2 ± 2.8</td>
</tr>
<tr>
<td>Indomethacin 10 μM</td>
<td>2</td>
<td>20.0 (4/20)</td>
<td>13.8 ± 2.1</td>
</tr>
<tr>
<td>Acetylsalicylic acid 10 μM</td>
<td>2</td>
<td>17.6 (3/17)</td>
<td>10.2 ± 0.5</td>
</tr>
<tr>
<td>Meclofenamate 0.1 mM</td>
<td>2</td>
<td>35.0 (7/20)</td>
<td>14.0 ± 2.3</td>
</tr>
<tr>
<td>Piroxicam 10 μM</td>
<td>2</td>
<td>28.5 (4/14)</td>
<td>12.0 ± 0.5</td>
</tr>
<tr>
<td>U-46619 1 μM</td>
<td>5</td>
<td>37.2 (16/43)</td>
<td>21.9 ± 2.2</td>
</tr>
<tr>
<td>+ Indomethacin 10 μM</td>
<td>4</td>
<td>57.6 (34/59)*</td>
<td>24.2 ± 2.1</td>
</tr>
<tr>
<td>+ Acetylsalicylic acid 10 μM</td>
<td>4</td>
<td>57.6 (34/59)*</td>
<td>24.9 ± 1.8</td>
</tr>
<tr>
<td>+ Meclofenamate 0.1 mM</td>
<td>4</td>
<td>58.0 (36/62)*</td>
<td>29.1 ± 2.6</td>
</tr>
<tr>
<td>+ Piroxicam 1 μM</td>
<td>2</td>
<td>29.1 (7/24)</td>
<td>27.7 ± 5.0</td>
</tr>
<tr>
<td>10 μM</td>
<td>3</td>
<td>75.5 (34/45)†</td>
<td>39.6 ± 2.7</td>
</tr>
<tr>
<td>0.1 mM</td>
<td>4</td>
<td>67.4 (58/86)†</td>
<td>34.1 ± 2.3†</td>
</tr>
</tbody>
</table>

Values for cross-sectional area (CSA) are mean ± SEM of the largest percent decrease observed at any time point in each responding cell. n, number of experiments. Percentage of cells contracting (number of cells contracting/total number of cells analyzed) × 100.

* p<0.05; †p<0.01, χ² tests; ‡p<0.01, ANOVA.

**Figure 1. Effects of nonsteroidal anti-inflammatory drugs (NSAID) on spontaneous (solid bars) and U-46619–induced (open bars) contraction of cultured rat mesangial cells. Percent cells contracting ± SEM. *p<0.05; **p<0.01, U-46619 + NSAID vs. U-46619 alone, χ² tests. ASA, acetylsalicylic acid.
We then studied the effects of exogenous PGE₂ on mesangial cell contractility. Exposure of the cells to PGE₂ for 40 minutes did not stimulate significant contraction (7.2% of the cells in five separate experiments, Table 3), while the addition of PGE₂ to cells stimulated with U-46619 resulted in dose-dependent inhibition of contraction, to almost complete suppression at 1 μM. Nearly identical results were obtained with the stable analogue of PGI₂, iloprost, as shown in Figure 3 and Table 3. In additional experiments, summarized in Figure 4, 10 μM exogenous arachidonic acid, which stimulates PGE₂ severalfold in these cells, also mimicked the inhibitory effects of exogenous prostanooids on cell contractility induced by U-46619. When PGE₂ was added to cells pretreated with indomethacin, the enhanced responses to U-46619 were once again inhibited, to the same degree as in previous experiments in cells not pretreated with indomethacin.

To gain insight into the mechanism of action of vasodilator PG in inhibiting mesangial cell contractility, we measured the accumulation of cAMP in monolayers exposed to PGE₂, U-46619, and iloprost in the presence of the phosphodiesterase inhibitor, IBMX. Preliminary experiments (not shown) indicated that intracellular cAMP accumulation peaked at 3 minutes, followed by a rapid decline, even in the presence of IBMX, suggesting efflux of the cyclic nucleotide into the incubation medium. Therefore, 3-minute incubations were chosen for the dose-response studies shown in Figure 5. Both PGE₂ and, more markedly, iloprost stimulated dose-dependent, rapid accumulation of intracellular cAMP. Statistical comparisons with time control with nonstimulated monolayers indicated significant effects at 0.1 μM and higher concentrations, which is consistent with the thresholds for inhibition in contraction experiments. Of interest, U-46619 did not stimulate significant accumulation of cAMP at the concentrations used in the contraction experiments.

To further establish a link between intracellular cAMP and relaxant effects on agonist-induced contraction, we used the cAMP inhibitor DDA, which competes with the endogenous substrate of adenylate cyclase, reducing agonist-stimulated cAMP synthesis. Two isomers were preliminarily tested on PGE₂- and iloprost-induced cAMP accumulation, and only 2',5'-

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**Table 2. Inhibition of Mesangial PGE₂ Synthesis by Nonsteroidal Anti-Inflammatory Drugs**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Basal (5 μg/ml arachidonic acid)</th>
<th>Stimulated (5 μg/ml arachidonic acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin</td>
<td>95.8</td>
<td>81.4</td>
</tr>
<tr>
<td>0.1 μM</td>
<td>98.0</td>
<td>84.8</td>
</tr>
<tr>
<td>1 μM</td>
<td>98.0</td>
<td>94.9</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.1 μM</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 μM</td>
<td>96.2</td>
<td>77.4</td>
</tr>
<tr>
<td>10 μM</td>
<td>15.4</td>
<td>51.6</td>
</tr>
<tr>
<td>Meclofenamate</td>
<td>41.9</td>
<td>69.9</td>
</tr>
<tr>
<td>0.1 μM</td>
<td>75.8</td>
<td>70.2</td>
</tr>
<tr>
<td>10 μM</td>
<td>96.7</td>
<td>88.4</td>
</tr>
</tbody>
</table>

Data are expressed as percent inhibition of PGE₂ synthesis during 30-minute control incubations without nonsteroidal anti-inflammatory drugs; each point is an average of triplicate wells.

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**Table 3. Effects of PGE₂, Iloprost, and Arachidonic Acid on U-46619-Induced Contraction of Cultured Rat Mesangial Cells**

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>Cells contracting (%)</th>
<th>Maximum decrease CSA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-46619 1 μM</td>
<td>5</td>
<td>37.2 (16/43)</td>
<td>21.9 ± 2.2</td>
</tr>
<tr>
<td>PGE₂ 1 μM</td>
<td>5</td>
<td>7.2 (4/55)</td>
<td>21.0 ± 2.2</td>
</tr>
<tr>
<td>U-46619 1 μM + PGE₂ 1 μM</td>
<td>4</td>
<td>5.0 (3/60)†</td>
<td>23.8 ± 4.7</td>
</tr>
<tr>
<td>+ Iloprost 1 μM</td>
<td>3</td>
<td>12.5 (6/48)†</td>
<td>28.2 ± 2.1</td>
</tr>
<tr>
<td>+ Arachidonic acid</td>
<td>5</td>
<td>15.9 (11/69)†</td>
<td>18.6 ± 2.0</td>
</tr>
</tbody>
</table>

PGE₂, prostaglandin E₂.

Values for cross-sectional area (CSA) are mean ± SEM of the largest percent decrease observed at any time point in each responding cell. n, number of experiments. Percentage of cells contracting: (number of cells contracting/total number of cells analyzed) x 100.

All significances are statistically different from U-46619 alone by χ² tests.

* Data from Table 1.
† p<0.05; †† p<0.001.

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**Figure 2. Effects of 10 μM piroxicam on the time course of 1 μM U-46619-induced contraction of cultured rat mesangial cells.** Mean reductions of cross-sectional area ± SEM vs. average of three basal determinations. *p<0.05 vs. U-46619 alone, ANOVA. NR, nonresponding cells.

**Figure 3. Effects of prostaglandin E₂ (PGE₂) and iloprost on U-46619-induced contraction of cultured rat mesangial cells.** Percent cells contracting ± SEM. *p<0.05, **p<0.001 vs. U-46619 alone, χ² tests.
DDA was found effective in inhibiting responses to the prostaglandin. 3',5'-DDA had a small, but not statistically significant, effect only when used against high concentrations of iloprost (Figure 6). 2',5'-DDA was therefore used in the morphometric experiments illustrated in Figure 7 and Table 4. In these studies, 1 µM PGE2 stimulated contraction only when the cells were pretreated with 2',5'-DDA for 10 minutes, an effect that is not easily dissociated from the marked enhancement of spontaneous contraction also observed with 2',5'-DDA (Table 4). The effects of 1 µM U-46619 were also amplified by 2',5'-DDA, indicating a modulatory effect of endogenous PGE2-induced cAMP accumulation during long-term incubations with U-46619. Finally, 2',5'-DDA completely reversed the inhibitory effect of 1 µM PGE2, on U-46619-induced contraction, again suggesting an intermediary role of cAMP synthesis in the action of exogenous PGE2 on mesangial cells. This effect is apparent in the photomicrographs in Figures 8 and 9, which show mesangial cells stimulated with U-46619 in the presence of PGE2 with and without 2',5'-DDA, respectively. In cells that were not exposed to 2',5'-DDA, only minimal contraction was detectable after preincubation with PGE2, while a large fraction of the cells pretreated with 2',5'-DDA displayed restored reactivity to U-46619 despite the addition of PGE2.

**Table 4. Effects of 2',5'-Dideoxyadenosine on Contraction of Cultured Rat Mesangial Cells**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cells contracting (%)</th>
<th>Maximum decrease CSA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDA 0.1 mM</td>
<td>4 (24/55)</td>
<td>28.0 ± 3.2</td>
</tr>
<tr>
<td>PGE2 1 µM + DDA 0.1 mM</td>
<td>4 (20/47)*</td>
<td>26.6 ± 2.0</td>
</tr>
<tr>
<td>U-46619 1 µM + DDA 0.1 mM</td>
<td>3 (50/66)†</td>
<td>35.2 ± 3.3</td>
</tr>
<tr>
<td>U-46619 1 µM + PGE2 1 µM + DDA 0.1 mM</td>
<td>5 (46/89)‡</td>
<td>26.7 ± 2.1</td>
</tr>
</tbody>
</table>

**Discussion**

Stimulation of cultured rat mesangial cells with vasoactive agents such as Ang II, arginine vasopressin, and U-46619 results in the rapid synthesis of PGE2, which has been hypothesized to play a counterregulatory role on mesangial contractility, based on earlier data in freshly isolated rat and human glomeruli. In this investigation, we provide evidence that inhibition of mesangial PGE2 synthesis by NSAID increases significantly both the number of cells contracting and the rate of contraction in response to a PGH2/TXA2 analogue.

The lack of contraction in a certain fraction of the cells, previously described and discussed by numerous investigators, appears largely related to mechanical resistances opposing isotonic contraction in cells plated onto nonflexible substrates. Studies in cells plated on silicone rubber or polyHEMA-coated dishes clearly indicate more homogeneous and faster responses to various agents. Our approach takes advantage of the adhesion of cells to glass surfaces to quantify contractile responses on the basis of the number of cells undergoing CSA reductions out of a large, randomly selected population. In this system, development of greater contractile forces will result in a larger number of cells overcoming the threshold for isotonic contraction. The effects of NSAID indicate that an additional mechanism may account for the apparent

**Figure 4.** Effects of prostaglandin E2 (PGE2), arachidonic acid (AA), and indomethacin on U-46619–induced contraction of cultured rat mesangial cells. Percent cells contracting ±SEM. *p<0.05 vs. U-46619 alone, **p<0.001 vs. U-46619 + indomethacin.

**Figure 5.** Dose-dependent cyclic AMP accumulation in response to eicosanoids in cultured rat mesangial cells. Three-minute incubations in the presence of 0.1 mM isobutylmethylxanthine. *p<0.05 vs. control, ANOVA. PGE2, prostaglandin E2.
unresponsiveness of a certain fraction of the cells, that is, endogenous synthesis of a “relaxant” prostanoid. We used four structurally unrelated compounds to rule out the possibility of a nonspecific, cyclooxygenase-independent mechanism of action. Furthermore, since an interference of carboxylic NSAID with Ca²⁺ transport has been described in several subcellular systems and since cytosolic free Ca²⁺ levels ([Ca²⁺]) are thought to be critical to the regulation of smooth muscle contractility, we used the noncarboxylic oxicam derivative, piroxicam, whose acidic moiety resides in a free enolic proton. Piroxicam does not affect Ca²⁺ transport when tested along with indomethacin and meclofenamate. In our hands, piroxicam displayed even higher potency than the other NSAID used, thus indicating that an effect on Ca²⁺ kinetics is unlikely to be responsible for enhancement of contraction. We also did not have evidence for changes of either basal or stimulated [Ca²⁺] in the presence of NSAID, as evaluated by spectrofluorometry on fura-2–loaded monolayers (P. Mené, G.R. Dubyak, A. Scarpa, and M.J. Dunn, unpublished observations). All four NSAID also displayed a tendency to increase spontaneous contractility, that is, the limited number of cells that undergo detectable surface area changes during 40-min incubations without any agonist. Because no sign of cell toxicity was observed by vital staining at the end of the incubations and because the effects of indomethacin were fully reversed by exogenous PGE₂, it seems likely that basal synthesis of PGE₂ serves a modulatory function on the resting tone of cultured cells. Pharmacological manipulation of both basal and stimulated PGE₂ synthesis results in enhanced excitability and exaggerated mechanical responses to various agonists, as previously described for Ang II.

Additional support for the concept that inhibition of mesangial cell PGE₂ is directly responsible for increased contractility derives from our studies with exogenous vasodilator prostaglandins. Both PGE₂ and the stable analogue of PGI₂, iloprost, dose-dependently inhibited contraction induced by U-46619. Addition of exogenous PGE₂ to indomethacin-treated cells resulted in complete reversal of enhanced contractility, and its effect was also mimicked by arachidonic acid, a potent stimulator of mesangial PGE₂ synthesis, again confirming an inhibitory role of the prostanoid independent of its source. No changes of average CSA reductions were induced by vasodilator prostaglandins, which is consistent with previous results with TXA₂-receptor antagonists, indicating that inhibitors dose-dependently prevent the initial activation of a fraction of the cells without affecting responses once initiated in “nonresponding” cells.

Some of these data are apparently in contrast with the observations by Venkatachalam and Kreisberg, who reported PGE₂-induced contraction of cloned rat mesangial cells grown on polyHEMA-coated plates. Our observations on glass chamber-slides indicate no sig-
significant contraction of the cells induced by PGE₂ alone, which is similar to an earlier report by the same group using noncloned cells grown on conventional surfaces and similar to studies by our laboratory. Foidart and Mahieu, and Okuda and Kurokawa, also indicating relaxation or inhibition of contraction by PGE₂. Likely explanations for this discrepancy are the different sensitivities of cells grown on flexible surfaces, which may respond to weaker agonists with limited contractile forces, undetectable in cells plated on glass or plastic, and the functional changes occurring in cloned cells after multiple passages. In this respect, we believe that our system more closely resembles in vivo conditions, since only early passages are used. Our earlier report of [Ca²⁺], elevations induced by PGE₂, although limited and somewhat inconstant when compared with PGF₉₀ and U-46619 in several cell lines (Mené et al. and P. Mené, G.R. Dubyak, A. Scarpa, and M.J. Dunn, unpublished observations), may also provide indication that PGE₂ has indeed some contractile activity, although no definitive link has been thus far established between [Ca²⁺], and contraction of mesangial cells. More important, though, is the observation by us and others that PGE₂ stimulates adenylate cyclase in these cells, nearly doubling intracellular cAMP levels within 3 minutes of addition in the presence of a phosphodiesterase inhibitor. This finding, confirmed throughout multiple cell lines used in this and similar investigations, suggests that the inhibitory effects of PGE₂ on contraction induced by other agonists, and its elusive contractile properties, are due to the simultaneous generation of an endogenous relaxant mediator, which is also similar to observations in smooth muscle. The data with iloprost, which displays higher
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FIGURE 9. Photomicrographs of cultured rat mesangial cells stimulated with 1 μM U-46619 after 10 minutes preincubation with 1 μM PGE₂ and 0.1 mM 2',5'-dideoxyadenosine. Panel A, 0 time; Panel B, 40 minutes after addition of U-46619. Ten out of 19 cells studied displayed a cross-sectional area reduction >8% of the basal measurement. Magnification, ×165.

potency as a stimulus for adenylate cyclase, are in agreement with this concept, showing dose-dependent inhibition of contraction coincident with the threshold for cAMP accumulation, approximately 0.1 μM.

To confirm a causal relation between cAMP accumulation and inhibition of contraction, we took advantage of 2',5'-DDA, a false substrate for adenylate cyclase that inhibits cAMP accumulation in various cell types. In these experiments 2',5'-DDA, but not the isomer 3',5'-DDA, effectively inhibited cAMP accumulation induced by PGE₂ and iloprost. When used in morphometric studies, 2',5'-DDA unmasked contraction induced by PGE₂, reversed the inhibitory effects of PGE₂ on U-46619–induced contraction, and enhanced the action of the latter compound, suggesting that PGE₂ stimulation by U-46619 mediates an elevation of cAMP, although not detectable during brief 3-minute incubations, thereby partially antagonizing contraction. The 2',5'-DDA also increased spontaneous contractility and to a greater extent than NSAID. This may indicate that basal cAMP levels are also relevant to the morphology of resting cells. In fact, 2',5'-DDA slightly reduced basal cAMP synthesis, although this effect was not statistically significant during short-term incubations. Because stable analogues of cAMP effectively counteracted contraction induced by Ang II in other studies from our laboratory and because stimulation of cAMP by dopamine has been recently shown to inhibit Ang II–induced contraction, it seems appropriate to conclude that cAMP stimulation modulates agonist-induced contraction.

In summary, our studies indicate that both PGE₂ and iloprost exert an inhibitory effect on mesangial cell contraction, which is most likely mediated by an
elevation of intracellular cAMP. While iloprost appears to be a selective agonist of adenylate cyclase, PGE₂ is apparently coupled to a dual signal transduction mechanism, with parallel activation of adenylate cyclase and of the phospholipase C-[Ca²⁺] system. Evidence for PGE₂ receptor subtypes with opposite biological actions in smooth muscle, that is, contraction and relaxation, is consistent with our findings in cultured mesangial cells. Under conventional culture conditions, and possibly in vivo, the inhibitory effects of cAMP prevail over a weak contractile activity, thus suggesting an endogenous negative feedback system preventing marked responses to vasoconstrictor hormones.

Finally, the administration of NSAID to patients with impaired renal hemodynamics or chronic glomerular disease results in marked reductions of renal blood flow and glomerular filtration rate, indicating a functional, compensatory role of renal prostaglandin synthesis. The effects of NSAID on mesangial cells, a model of renal smooth muscle reactivity, may provide an insight into the role and site of action of prostaglandins in the control of cortical perfusion and glomerular function.

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