# Cyclic AMP-associated shape change in mesangial cells and its reversal by prostaglandin $E_2$

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**Cyclic AMP-associated shape change in mesangial cells and its reversal by prostaglandin $E_2$.** The mesangial cell is a glomerular cell type with smooth muscle-like (contractile) properties. The responses evoked in cultured mesangial cells by catecholamines were examined in the presence or absence of prostaglandin $E_2$ ($PGE_2$) with or without a phosphodiesterase inhibitor. Exposure to $10^{-4}$ M norepinephrine, epinephrine, or isoproterenol elevated intracellular cyclic AMP (cAMP) levels in mesangial cells (25th to 30th passages) nearly threefold. If isobutylmethylxanthine (MIX) was also included, the hormones caused marked further increases in cAMP (after a 20-min incubation, control with MIX, 64.2 ± 5.2 pmoles/mg protein; $10^{-4}$ M norepinephrine, 4266 ± 284 pmoles/mg protein; $10^{-4}$ M epinephrine, 5812 ± 173 pmoles/mg protein; and $10^{-4}$ M isoproterenol, 3136 ± 114 pmoles/mg protein). Under both of these circumstances (that is, catecholamines with or without MIX) greater than 50% of the cells underwent a change in shape (that is, had a round cell body with long, thin tapered processes). The cAMP and shape change response was independent of extracellular calcium ions and appeared to be due to $\beta$-adrenergic stimulation. Isoproterenol with MIX stimulated an alteration in morphology and cAMP production at concentrations of $10^{-4}$ M to $10^{-9}$ M. Within 10 min following $\beta$-adrenergic stimulation ($10^{-4}$ M isoproterenol plus MIX) cAMP was maximum; at this time a shape change was first evident. Eighty-five to one hundred percent of the cells had undergone a shape change by 40 min. Dibutyryl cAMP ($10^{-3}$ M) also induced a shape change in cultured mesangial cells. The addition of $PGE_2$ to either morphologically altered cells or to the isoproterenol incubation medium (with or without MIX) prior to treating the cells, resulted in complete restoration to the normal flat appearance of mesangial cells or no shape change, respectively. $PGE_2$ attenuated but did not abolish hormone-induced elevations in intracellular cAMP. Thus, catecholamines caused mesangial cells to change their shape in association with elevations of intracellular cAMP. $PGE_2$ markedly inhibited the shape change as well as markedly attenuated cAMP generation.

**La modification de la forme associé l'AMP cyclique dans les cellules mésangiales et sa interversion par prostaglandine $E_2$.** La cellule mésangiale est un type cellulaire glomérulaire ayant despropriétés voisines du muscle lisse (contractile). Les réponses évoquées dans des cellules mésangiales en culture par les catécholamines ont été examinées en présence ou en l'absence de prostaglandine $E_2$ ($PGE_2$) avec ou sans un inhibiteur des phosphodiestérases. L'exposition à $10^{-4}$ M de noradrénaline, d'adrénaline, ou d'isoprotérénol a élevé les niveaux d'AMP cyclique intracellulaires (cAMP) dans les cellules mésangiales (25ème à 30ème passages) de presque troisfois. Si de l'isobutylméthylxanthine (MIX) était également inclue, les hormones entraînaient des augmentations plus fortes de cAMP (après 20 min d'incubation, contrôles avec MIX, 64,2 ± 5,2 pmoles/mg protéines; $10^{-4}$ M noradrénaline, 4266 ± 284 pmoles/g protéines; $10^{-4}$ M adrénaline, 5812 ± 173 pmoles/mg protéines; et $10^{-4}$ M isoprotérénol, 3136 ± 114 pmoles/mg protéines).

Dans chacune de ces circontances (c'est-à-dire catécholamines avec ou sans MIX), plus de 50% cellules subissaient une modification de forme (c'est-à-dire avaient un corps cellulaire rond, avec des expansions rubannées longues et fines). Les réponses cAMP et de modification de forme étaient indépendantes des ions calcium extracellulaires, et paraîssaient être dues à la stimulation $\beta$-adrénergique. L'isoprotérénol avec MIX stimulait une altération de la morphologie et de la production de cAMP pour des concentrations de $10^{-4}$ M à $10^{-9}$ M. En 10 min, après stimulation $\beta$-adrénergique ($10^{-4}$ M d'isoprotérénol plus MIX), cAMP était maximum; à ce moment, la modification de forme était évidente. Quatre-vingt-cinq à cent pour cent des cellules avaient subi une modification de forme en 40 min. Le dibutyryl cAMP ($10^{-3}$ M) induisait également une modification de forme dans les cellules mésangiales en culture. L'addition de $PGE_2$ soit à des cellules morphologiquement altérées, soit au milieu d'incubation de l'isoprotérénol (avec ou sans MIX), avant de traiter les cellules, entraînait une restauration complète de l'apparence normale, plate, des cellules mésangiales, ou l'absence de modification, respectivement. $PGE_2$ a atténué, mais n'a pas aboli les élévations induites par les hormones du cAMP intracellulaire. Ainsi, les catécholamines faisaient changer de forme les cellules mésangiales en association avec des élévations du cAMP intracellulaire. La $PGE_2$ inhibait de façon marquée la modification de changement, et atténuait sensiblement la génération de cAMP.

Glomerular mesangial cells are smooth muscle-like cells of the glomerulus that respond to vasoactive substances by contracting [1–4]. This contractility appears to regulate glomerular function by altering the caliber of the tortuous glomerular capillaries [2–4, 5]. Antidiuretic hormone (ADH) and angiotensin II (AII) cause glomerular hemodynamic perturbations in vivo [6, 7] and, when directly applied to cultures of mesangial cells, cause a shape change that resembles contraction [1, 8]. This action depends on the availability of extracellular calcium ions [1, 8]. A number of other hormones, including catecholamines, are known to influence glomerular hemodynamics [3, 4, 9], but their modes of action are not known.

Changes in cellular morphology have been reported to occur in many biological events including hormonal stimulation and, in many instances, cAMP has been implicated as the second messenger. For example, rat ovarian granulosa cells have been reported to undergo a dramatic change in cell shape (that is, cell rounding) following hormonal stimulation with follicle-stimulating hormone [10]. This alteration in cellular morphology occurs soon after maximum increases in intracellular cAMP and can be mimicked by dibutyryl cAMP. Other examples of alterations in cellular morphology following treatment with substances that increase intracellular cAMP include: dog thyroid cells treated with thyroid-stimulating hormone [11, 12]; rat sertoli cells

treated with follicle-stimulating hormone or dibutyryl cAMP [13]; and bone cells treated with PTH or dibutyryl cAMP [14]. As reported here, cultured rat mesangial cells undergo a dramatic change in cell shape soon after treatment with agents that stimulate intracellular cAMP. Both stimulation of intracellular cAMP and shape change in mesangial cells with isoproterenol and isobutylmethylxanthine (MIX) are independent of extracellular $Ca^{++}$ and can be modulated by prostaglandin $E_2$ (PGE$_2$). The relevance of these remarkable shape changes to glomerular physiology is presently unclear.

## Methods

### Isolation of glomeruli and culture of glomerular mesangial cells

Typically, six rats have been required to obtain a sufficient number of glomeruli for tissue culture. Animals were anesthetized with 40 mg/kg body weight sodium pentobarbital. The kidneys were perfused in situ via the aorta with Hanks' balanced salt solution (HSS) (Grand Island Biological Co., Grand Island, New York) at a pressure of 120 mm Hg to remove red blood cells. The kidneys were excised, capsules were removed, and cortices were dissected from each kidney. Cortical slices were minced with a razor blade and pressed with a spatula through a stainless steel screen of 60 mesh (250 $\mu$m pore) (W. S. Tyler, Inc., Menton, Ohio) and rinsed with HSS through successive screens of 150 mesh (150 $\mu$m pore) and 200 mesh (75 $\mu$m pore), placed in series [15]. Tissue samples on the 200-mesh screen, consisting of glomeruli, were transferred to a plastic tube containing HSS and washed twice. After the second wash, the glomeruli were resuspended in RPMI 1640 tissue culture medium with 20% fetal calf serum (Grand Island Biological Co.) plus penicillin, streptomycin, and fungizone for explant growth of mesangial cells.

Glomerular mesangial cells were isolated from explants of whole glomeruli according to the differential growth capacities of glomerular epithelial and mesangial cells [16]. With this technique, the first cell type to emerge from the glomerulus was the glomerular epithelial cell; however, after 2 weeks, mesangial cells appeared in the culture and proceeded to overgrow and obliterate the epithelial cells [16]. Upon passaging with trypsin-versene, glomeruli were filtered away and one was left with a pure culture of mesangial cells [16]. Positive identification of this cell type was obtained by published methods [8, 17]; that is, by ultrastructural examination and $^{125}$I-angiotensin II binding capacity [8, 17]. Rat mesangial cells specifically bind $^{125}$I-angiotensin II. Glomerular epithelial cells do not change shape [1] nor do they bind angiotensin II [8]. By these criteria, the cultures were not contaminated with glomerular epithelial cells. Also, immunofluorescence studies to demonstrate factor VIII antigen, a marker for endothelium, were negative indicating that our cultures were not contaminated with glomerular endothelial cells. Detailed hormone-sensitive cyclic nucleotide responses and morphological examination were evaluated in cultured mesangial cells between the 18th and 41st passages. The only change noted with respect to the age of the culture was that the cyclic nucleotide response to hormones and 3-isobutyl-1-methylxanthine (MIX) increased in later passages. Experiments were conducted in 60-mm Corning tissue culture dishes (Corning, Corning, New York); cells were rinsed three times with HSS. Incubations were carried out in HSS with 0.2%

bovine serum albumin (BSA, Fraction V, Sigma Chemical Co., St. Louis, Missouri) with or without $10^{-3}$ M MIX or $10^{-3}$ M theophylline plus vasoactive substances.

### Morphological experiments

The following substances were tested with and without $10^{-3}$ M MIX on mesangial cells for an effect on cell shape: *(1)* prostaglandin (PG)E$_2$ (1 $\mu$g/ml); *(2)* histamine ($10^{-4}$ M); *(3)* serotonin ($10^{-4}$ M); *(4)* norepinephrine ($10^{-4}$ M); *(5)* epinephrine ($10^{-4}$ M); and *(6)* isoproterenol ($10^{-4}$ to $10^{-9}$ M). Theophylline ($10^{-3}$ M) was also used as a phosphodiesterase inhibitor with isoproterenol. Dibutyryl cAMP and cAMP ($10^{-3}$ M) in HSS were also tested for contraction. Also, to determine if elevations in media cAMP were responsible for shape change, we exposed our cells to isoproterenol ($10^{-4}$ M) with MIX for 10 min, washed three times with HSS, chased in hormone-free HSS with BSA for 30 min, and observed for shape change. In some experiments, PGE$_2$ (1 $\mu$g/ml) was added along with the catecholamines and MIX. In other experiments, PGE$_2$ was added to morphologically altered cells and the cells were observed 20 min later. Cells were incubated at 37°C in an atmosphere of 5% CO$_2$ in air. Cells were examined by phase contrast microscopy at 10, 20, 30, 40, 60, 80, 120, and 180 min. To determine if shape change with catecholamines was due to an $\alpha$- or $\beta$-effect, mesangial cells were preincubated with phenoxybenzamine (12 $\mu$M), an $\alpha$-blocker, or d,1-propranolol (12 $\mu$M), a $\beta$-blocker, 15 min prior to hormone and MIX addition. Cells were examined 40 min later. To determine if shape change depended on extracellular $Ca^{++}$, the cells were preincubated in $Ca^{++}$-Mg$^{++}$ depleted HSS (Grand Island Biological Co.) to which Mg$^{++}$ was added back with $10^{-3}$ M ethyleneglycol-*bis*-($\beta$-aminoethyl ether)N,N'-tetracetic acid (EGTA) for 15 min. Isoproterenol and MIX were added in the $Ca^{++}$-depleted HSS. The cells were examined 40 min later. In each of the experimental conditions listed above, at least four dishes of cells were tested.

Cells were examined for maximum shape change by phase-contrast microscopy and considered to be altered in morphology when they assumed a rounded, refractile morphology with long, thin-tapered processes. Only mesangial cells that displayed these features were counted as morphologically altered cells. Cells were examined ultrastructurally after fixation in 2% glutaraldehyde in complete HSS and processing by the usual techniques.

### Cyclic nucleotide experiments

The following substances were tested in the presence and absence of MIX ($10^{-3}$ M): *(1)* PGE$_2$ (1 $\mu$g/ml); *(2)* histamine ($10^{-4}$ M); *(3)* serotonin ($10^{-4}$ M); *(4)* norepinephrine ($10^{-4}$ M); *(5)* epinephrine ($10^{-4}$ M); and *(6)* isoproterenol ($10^{-4}$ to $10^{-9}$ M). Isoproterenol was also tested in the presence of theophylline ($10^{-3}$ M) for a cyclic nucleotide response. To study the effect of $Ca^{++}$-depletion on isoproterenol-MIX-stimulation of cAMP, mesangial cells were preincubated for 15 min in $Ca^{++}$-depleted HSS with $10^{-3}$ M EGTA, after which they were incubated with isoproterenol-MIX in $Ca^{++}$-depleted HSS for 20 min. Incubations were carried out at 37°C in an atmosphere of 5% CO$_2$ in air. Additional studies were done to inhibit the cAMP generation with isoproterenol and MIX by prior incubation of the cells for 15 min with propranolol (12 $\mu$M). The effect of PGE$_2$ (1
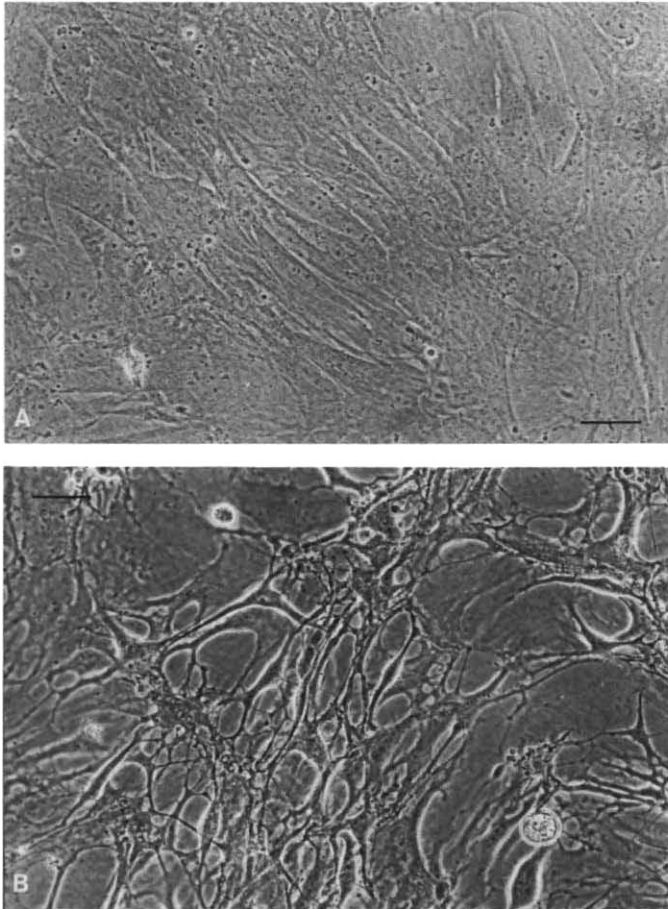
**Fig. 1. A** *Phase-contrast photomicrograph of mesangial cells.* Notice how these cells are flat and spread out. **B** *Phase-contrast photomicrograph of mesangial cells treated with $10^{-4}$ M isoproterenol with $10^{-3}$ M MIX for 40 min.* These cells have markedly altered their shape; namely, they have assumed a more rounded, refractile appearance with long, thin tapered processes. An identical shape change was observed with epinephrine and norepinephrine. Three hours after hormone-MIX addition the cells have reassumed their normal state despite the continued presence of agonists. The *bars* represent 5 μm.

**Fig. 2. A** *Transmission electron micrograph of mesangial cells.* Note the bundles of microfilaments running parallel to the plasma membrane. Note the oval-shaped nucleus. **B** *Transmission electron micrograph of a mesangial cell treated with $10^{-4}$ M isoproterenol with $10^{-3}$ M MIX for 40 min.* Note the rounded appearance of the cell and the accordian-shaped nucleus. Identical changes were observed with epinephrine and norepinephrine. The *bars* represent 1 μm.

μg/ml) on cAMP stimulation by isoproterenol (with and without MIX) was also examined by incubating the cells in HSS with $PGE_2$ and isoproterenol ($10^{-4}$ M) (with and without MIX) for 20 min.

After the incubation period aliquots of media were removed, boiled for 5 min and centrifuged for 2 min at $\times 7000g$ [1]. Supernatants were saved for assays. The dishes were rinsed three times with HSS and 1 ml per dish of cold 5% trichloroacetic acid (TCA) containing tracer amounts of [$^3$H]cAMP (15,000 cpm) was added; the dishes were kept over ice. Cells were scraped off the surface with a rubber policeman and transferred to a centrifuge tube kept on ice. Each dish was rinsed again with 1 ml of TCA with tracer. The TCA extracts were sonicated on ice for 20 sec with a sonicator-cell disruptor (Heat Systems-Ultrasonics, Inc., Plainview, New York). The TCA extracts were centrifuged at $\times 6800g$ for 20 min in a refrigerated centrifuge with a JA-20 rotor (Beckman J221, Beckman Instruments, Inc., Palo Alto, California). The supernatants were collected and acidified with 100 μl of 2 N HCl. The TCA pellets were
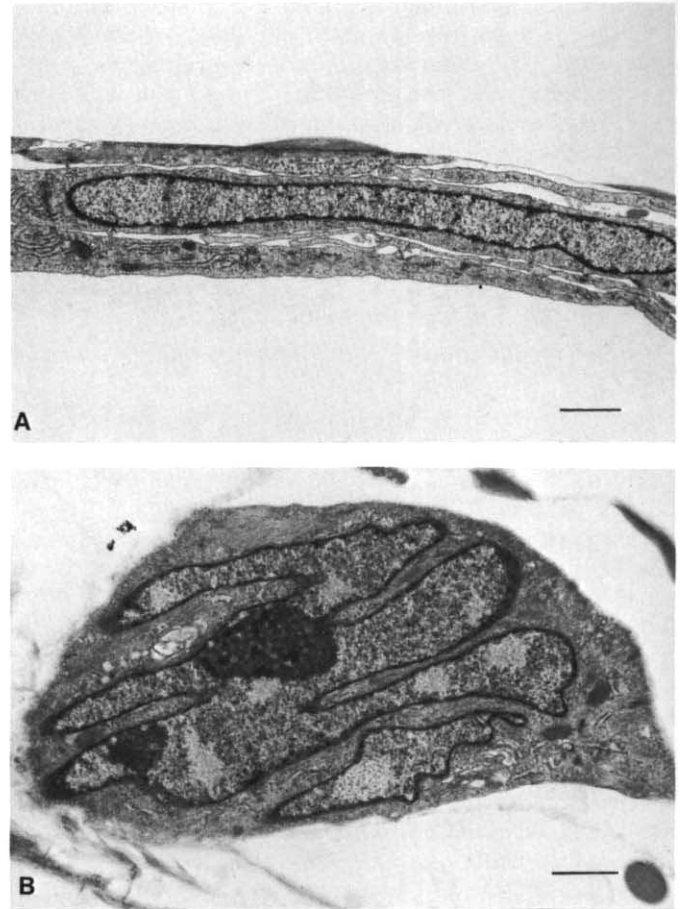
resuspended in 2% sodium carbonate in 0.1 N NaOH buffer for protein quantitation [18]. The TCA was removed from the supernatant by repeated extraction (four times) with water-saturated ethyl ether. Remnants of ether were evaporated by heating, and samples were frozen at $-70°C$ and lyophilized. Lyophilized samples were stored at $-70°C$ until assayed.

cAMP was measured by radioimmunoassay (RIA) adapted from the procedure of Steiner, Parker, and Kipnis [19] using cAMP RIA kits (Becton Dickinson Immunodiagnostics, Orange, New York). Specificity and sensitivity of the antibodies in preliminary experiments were found to be within the manufacturer's specifications. The validity of the cAMP RIA measurements was verified by the linearity of sample dilutions, by cAMP hydrolysis with cyclic nucleotide phosphodiesterase, and by quantitation of added unlabelled cyclic nucleotide to the extracts.

Radioactivity of tritiated tracer nucleotides was determined by liquid scintillation counting. After correction for recovery, the content of cAMP in samples was expressed as picomoles per milligrams of protein.

**Table 1.** Effect of hormones on cAMP in mesangial cells[a]

| Condition | No MIX pmoles/mg protein | | With MIX pmoles/mg protein | |
|---|---|---|---|---|
| HSS alone | 18.5 ± 0.99 | (10) | 64.2 ± 5.2 | (6) |
| HSS + epinephrine ($10^{-4}$ M) | 48.4 ± 2.4 | (4) | 5812 ± 173 | (4) |
| HSS + norepinephrine ($10^{-4}$ M) | 51.3 ± 5.0 | (4) | 4266 ± 284 | (4) |
| HSS + isoproterenol ($10^{-4}$ M) | 46.4 ± 1.8 | (4) | 3136 ± 114 | (8) |
| HSS + PGE$_2$ (1 μg/ml) | 12.1 ± 0.23 | (4) | 33.4 ± 2.7 | (4) |
| HSS + histamine ($10^{-4}$ M) | ND | | 71.9 ± 13.2 | (4) |
| HSS + serotonin ($10^{-4}$ M) | ND | | 52 ± 3.7 | (4) |

Abbreviations: ND, not done; HSS, Hanks' balanced salt solution; MIX, isobutylmethylxanthine.

[a] These studies were performed on 25th to 30th passaged cells. Incubations were carried out for 20 min. Numbers in parentheses indicate the number of determinations. Data are expressed as mean ± SE.

**Table 2.** Dose-response to isoproterenol plus MIX[a]

| Condition | cAMP pmoles/mg protein | % of Cells changing shape |
|---|---|---|
| HSS alone | 18.5 ± 0.99 | 0 |
| HSS + MIX | 47.9 ± 6.1 | 10 to 15% |
| MIX + Isoproterenol ($10^{-4}$ M) | 8034 ± 452 | 90 to 100% |
| MIX + Isoproterenol ($10^{-6}$ M) | 6073 ± 265 | 80 to 90% |
| MIX + Isoproterenol ($10^{-6}$ M) + Propranolol | 41.5 ± 1.7 | <10% |
| MIX + Isoproterenol ($10^{-7}$ M) | 2722 ± 45 | 70 to 75% |
| MIX + Isoproterenol ($10^{-8}$ M) | 412 ± 53 | 40 to 50% |
| MIX + Isoproterenol ($10^{-9}$ M) | 136 ± 5 | 20 to 30% |

Abbreviations are the same as those used for Table 1.

[a] Experiments were performed on 30th passaged cells. Cells were incubated for 10 min for cAMP determinations and 40 min for shape change studies. Data are expressed as mean ± SE for cAMP, and the range of maximally altered cells. $N = 4$, except for HSS alone, where $N = 10$, and HSS plus MIX where $N = 6$.

## Hormone stock solutions

PGE$_2$ was obtained from Sigma Chemical Co., prepared as a 1 mg/ml stock in ethanol, and stored at −20°C. Serotonin and histamine were stored at −20°C. Norepinephrine bitartrate (Levophed, 1 mg/ml), and epinephrine (adrenalin), 1 mg/ml, were obtained in sealed ampules from the hospital pharmacy. Only sealed ampules were used for each experiment. Isoproterenol was obtained from Sigma Chemical Co. and prepared immediately before use.

## Results

MS cells are large flat cells that appear strap-like in culture (Fig. 1A). By electron microscopy they are seen to contain numerous bundles of microfilaments that run parallel to the plasma membrane, with surface dense patches (Fig. 2A) [1]. In many respects they resemble vascular smooth muscle. Maximal concentrations ($10^{-4}$ M) of the catecholamines, norepinephrine, epinephrine, and isoproterenol raised intracellular cAMP content in mesangial cells (25th to 30th passages) two- to threefold (Table 1). PGE$_2$, histamine, and serotonin did not stimulate cAMP production. Forty minutes after the addition of the catecholamines, 50 to 60% of the cells had maximally undergone shape change which resulted in rounded cells with long, thin-tapered processes (Fig. 1B). To maximally elevate cAMP levels in mesangial cells, cells were incubated with hormones in the presence of MIX ($10^{-3}$ M) or theophylline ($10^{-3}$ M). MIX alone increased cAMP content 2.5 times, with approximately 10 to 15% of the cells maximally changing shape (Table 2). MIX plus norepinephrine ($10^{-4}$ M) or epinephrine ($10^{-4}$ M) resulted in a 66- to 90-fold increase in cAMP levels (Table 1) with 85 to 100% of the cells altering their shape (Figs. 1B and 2B). To determine if this shape change was the result of an α- or β-adrenergic effect, cells were preincubated for 15 min with either phenoxybenzamine or propranolol (both at 12 μM) and subsequently challenged with norepinephrine or epinephrine. Less than 10% of the propranolol-treated cells changed shape while there was no inhibition of shape change in cells treated with phenoxybenzamine. Since this alteration in mesangial cell configuration by the catecholamines appeared to be due to β-adrenergic activity, isoproterenol, a pure β-agonist, was chosen for the remaining studies.

Dose response and time course studies for intracellular cAMP and shape change were performed with isoproterenol with either $10^{-3}$ M MIX or $10^{-3}$ M theophylline to maximally elevate cAMP levels. Time course studies with MIX plus isoproterenol ($10^{-4}$ M) determined that cAMP was maximal at 10 min (Fig. 3). At this time the cells exhibited an increase in their refractility and already 10 to 15% of the cells showed maximal shape change (that is, round cell body with long, thin-tapered processes); between 20 to 30 min, half the cells were maximally altered; by 40 min this shape was present in 85 to 100% of the cells (Figs. 1B, 2B, and 3). At 40 min cAMP was still 21 times higher than control (Fig. 3). To determine whether or not large increases in media cAMP were responsible for the observed shape change at 40 min, we measured cAMP in the supernatant after 20 and 40 min of exposure to MIX with and without isoproterenol ($10^{-4}$ M). After 40 min in the presence of MIX and isoproterenol media cAMP rose to only 190 ± 3.3 pmoles/dish (MIX alone 20 min = 6.6 ± 0.70; MIX plus isoproterenol 20 min = 140 ± 4.5; MIX alone 40 min = 10.3 ± 0.70 pmoles/dish). This, along with the lack of cell response to exogenously applied cAMP (see below) led us to conclude that an elevation in intracellular cAMP levels, not extracellular levels, was responsible for initiating shape change. To substantiate this claim, cells exposed to isoproterenol with MIX for 10 min, and washed and chased for 30 min in hormone-free media underwent a shape change similar to those cells that were exposed to hormone continuously (85%). If the hormone-containing medium was removed after 40 min and replaced with fresh hormone-free medium, the cells resumed their flat well-spread morphology within 30 min. Three hours after hormone plus MIX treatment the cells had resumed their normal conformation despite continued presence of agonists. The dose response of isoproterenol plus MIX revealed that cAMP was stimulated from isoproterenol concentrations of $10^{-4}$ M (167-fold stimulation) to $10^{-9}$ M (threefold) (Table 2). Cells also changed shape in a dose-response fashion with half the cells undergoing morphological alterations at approximately $10^{-8}$ M isoproterenol (Table 2).

Theophylline, being a less potent phosphodiesterase inhibitor than MIX, did not cause any shape change by itself. When theophylline was used as the phosphodiesterase inhibitor, iso-
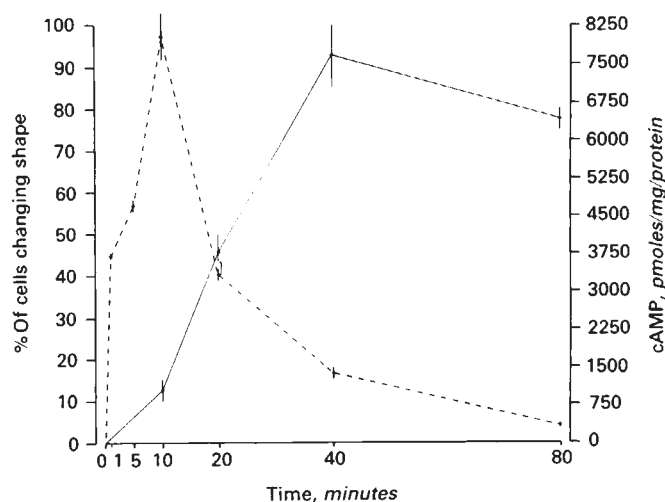
**Fig. 3.** *Time-course curves of cAMP generation (---) and shape-change (—) in mesangial cells treated with $10^{-4}$ M isoproterenol with $10^{-3}$ M MIX.* Each *point* represents the mean ± SE of four dishes.

proterenol-induced cAMP was maximal at 1 min (control 16.0 ± 0.90 pmoles/mg protein vs. isoproterenol 218 ± 15.3 pmoles/mg protein); shape change was also first evident at 10 min (5 to 10% undergoing a maximum change in morphology), 40% of the cells were morphologically altered at 20 min, and 85% exhibited maximal shape changes at 40 min. The cells had fully resumed their flat unaltered state after 160 min in the continued presence of agonists.

To determine whether extracellular $Ca^{++}$ was required for cAMP stimulation and shape change, cells were pretreated for 15 min in $Ca^{++}$-depleted HSS with $10^{-3}$ M EGTA and subsequently incubated in $Ca^{++}$-depleted HSS with isoproterenol and MIX. The cells changed shape and generated cAMP to the same degree as those incubated with hormone and MIX in complete HSS.

The addition of propranolol (a $\beta$-adrenergic blocker) 15 min prior to isoproterenol-MIX treatment blocked the shape change and resulted in a 93% inhibition of cAMP stimulation (Table 2). PGE$_2$ did not stimulate cAMP production in these subcultures of mesangial cells, contrary to the small but statistically significant increases we reported in cloned mesangial cells [1]. In fact, PGE$_2$ with MIX attenuated cAMP production when compared to MIX alone (Table 1). The addition of PGE$_2$ (1 $\mu$g/ml) to rounded cells resulted in their flattening 20 min later. Furthermore, the addition of PGE$_2$ to the isoproterenol or isoproterenol-MIX incubation medium completely blocked a change in cell shape and inhibited cAMP production by 93 and 66%, respectively (Table 3). Thus, PGE$_2$ may be exerting its effect on cell shape by altering certain intracellular pools of cAMP generation in response to certain vasoactive stimuli. When dibutyryl cAMP ($10^{-3}$ M) was added without MIX to cultures of mesangial cells, 85% of the cells underwent a shape change by 40 min. The cAMP ($10^{-3}$ M) addition to the cells, however, resulted in no alteration of morphology.

## Discussion

The results of this study showed that the catecholamines norepinephrine, epinephrine, and isoproterenol caused stimula-

**Table 3.** Effect of PGE$_2$ on cAMP stimulation by isoproterenol[a]

| | No MIX<br>*pmoles/mg protein* | With MIX<br>*pmoles/mg protein* |
|---|---|---|
| Isoproterenol ($10^{-4}$ M) | 721 ± 52 | 5154 ± 337 |
| Isoproterenol ($10^{-4}$ M) + PGE$_2$ (1 $\mu$g/ml) | 52.6 ± 1.5 | 1798 ± 136 |

[a] The larger absolute values obtained here are due to the age of these cultures (that is, 41st passaged cells). Cultures were incubated in the presence of hormone for 20 min. Data are expressed as mean ± SE, $N$ = 4.

tion of cAMP production in mesangial cells which was followed minutes later by a change in cell shape. This hormonal response appeared to be due to a $\beta$-adrenergic effect because propranolol inhibited shape change and cAMP production while phenoxybenzamine, an $\alpha$-blocker, had no effect on this shape change. In addition, PGE$_2$ returned the cells to their prehormone condition as well as inhibited the shape change induced by isoproterenol.

The catecholamine-induced change in cell shape appears to be mediated by cAMP. This conclusion is arrived at by the following observations: *(1)* The effect of the catecholamines is potentiated by phosphodiesterase inhibitors; *(2)* the hormonal-induced change is mimicked by the addition of dibutyryl cAMP; and *(3)* isoproterenol at concentrations that lead to a change in cell shape causes increases in the level of intracellular cAMP that precedes the change in cell shape.

It has been suggested that mesangial cells respond to vasoactive substances to regulate hemodynamics by contraction. An extracellular $Ca^{++}$-dependent shape change that was morphologically indistinguishable from contraction, as described by other investigators [20–23], has already been reported in mesangial cells treated with angiotensin II and AVP [1, 8]. Under the conditions used in those studies to test for contraction, neither agent caused cAMP levels to increase [1]. Only a supramaximal concentration of AVP (200 nM) in the presence of MIX elevated cAMP levels in mesangial cells and this increase was only twofold [1]. Isoproterenol with MIX as reported here caused cAMP levels to increase by approximately two orders of magnitude. Associated with this increase, the cells responded by altering their shape in a similar fashion to that seen after angiotensin II and AVP. This shape change, however, was independent of extracellular $Ca^{++}$. Since cAMP has been shown to cause smooth muscle cell relaxation [24], not contraction, it appears that morphology alone cannot be used to determine the contractile state of a cell. Contraction of smooth muscle in vivo as well as in freshly isolated tissue occurs within seconds following hormonal stimulation. Cultured mesangial cells treated with angiotensin II and AVP change shape in 5 to 10 min [1, 8, 25]. In contrast, the shape change observed with the catecholamines takes place after 10 min reaching a maximum in 40 min. Clearly, the observations in cultured cells are at variance with what would have been expected of contracting mesangial cells in vivo. This delay in response could be explained by either *(1)* a phenotypic change as a result of culture which interferes with normal stimulus-response coupling, or *(2)* the morphological shape change described is not contraction in its true sense. Thus, biochemical determinants (for example, myosin light chain phosphorylation) should also

be used in conjunction with morphological observations to determine the state of contraction of the cell.

A most interesting finding in this study is the modulating role of $PGE_2$. That is, the addition of $PGE_2$ to either mesangial cells that have undergone a shape change or to the isoproterenol-MIX incubation medium, resulted in a rapid reversal (within 20 min) to the normal flat morphology of mesangial cells (Fig. 1A) or no shape change, respectively. $PGE_2$ alone did not stimulate cAMP in mesangial cells and, when added to the isoproterenol-MIX incubation medium, markedly attenuated cAMP production. In a previous study we reported that $PGE_2$ in the presence of MIX stimulated cAMP production in cloned rat mesangial cells [1]. However, this response was highly variable from passage to passage. Such differences between the results could be due to differences in the methods of cell isolation as well as differences in the growth conditions [1]. $PGE_2$ may be exerting its inhibitory effect on cell shape change by modulating intracellular cAMP levels. However, although $PGE_2$ in the presence of MIX plus isoproterenol inhibits cAMP by 66%, the levels of cAMP in the cell should still be high enough to cause shape change (Table 3), which proposes speculation that $PGE_2$ may be exerting its inhibitory effect by modulating certain intracellular pools of cyclic nucleotides or by exerting an effect on another event besides cAMP generation.

## Acknowledgments

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