PROSTAGLANDIN SYNTHESIS BY MESANGIAL AND EPITHELIAL GLOMERULAR CULTURED CELLS

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1. Introduction

Glomeruli isolated from the rat renal cortex synthesize prostaglandins (PGs). Using radioimmunoassay [1,2] and thin layer chromatography [2,3], it has been shown that PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ which is the stable metabolic product of PGI_2 progressively accumulate in the incubation medium of isolated glomeruli. These studies left imprecise the cellular localization within the glomerulus of PG synthesis. Recent work in the laboratory of J.F. and P.M. has led to the culture of homogenous populations of glomerular epithelial and mesangial cells. Using these cells, we show here that rat glomerular mesangial cells are the major site of PGE_2 synthesis.

2. Experimental

2.1. *Materials*

PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ were gifts from Dr J. Pike (Upjohn Co., Kalamazoo, MI) and indomethacin from Merck, Sharp and Dohme (Paris). Arachidonic acid (sodium salt) was obtained from Sigma (St Louis, MO); [^{3}H]PGE_2 and [^{3}H]$PGF_{2\alpha}$ (110–170 Ci/mmol) were purchased from the Radiochemical Center (Amersham). 6-Keto-$PGF_{1\alpha}$ was coupled to histamine using carbodiimide. The iodination with ^{125}I of this conjugate using the chloramine T procedure and the subsequent purification of the tracer were performed as in [4]. Anti-PGE_2, anti-$PGF_{2\alpha}$ and anti-6-keto-$PGF_{1\alpha}$ antiserums were obtained from Institut Pasteur (Paris). These antibodies crossreact only slightly with other PGs and could be considered as specific [5,6]. All other chemicals were analytical grade.

2.2. *Isolation and incubation of rat renal glomeruli*

Renal glomeruli were isolated from Sprague-Dawley rats (150–220 g) by the technique in [7]. In short, cortex from 4 kidneys was dissected and minced to a paste-like consistency. The homogenate, suspended in 20 mM Tris–HCl buffer (pH 7.4) containing 5 mM glucose, 135 mM NaCl, 10 mM KCl and 10 mM $NaCH_3COO$, was pushed successively through a 106 μm-sieve which excluded the tubules and a 50 μm-sieve which retained the glomeruli. The suspension was then passed through a 25-gauge needle and centrifuged at 120 × *g* for 90 s. The supernatant was discarded, the pellet resuspended in the same buffer solution and passed again through the needle and centrifuged. This operation was repeated 5 times. Each individual preparation was checked for purity under light microscopy. Virtually no afferent and efferent arterioles could be detected. Tubular fragments were always 2% of the total number of glomeruli. Isolated glomeruli (500–1000 μg/ml of glomerular protein) were incubated at 37°C in room atmosphere under continuous agitation (100 cycles/min) in 200 μl of buffer similar to that employed in the purification procedure. After 60 min incubation, the preparation was centrifuged at 3000 × *g* for 10 min and the supernatant collected.

2.3. *Cell cultures*

Rat glomerular mesangial and epithelial cells characterized by immunofluorescent microscopy using antimyosine antiserum and by transmission

electron microscopy were isolated and grown in homogenous monolayer cultures as in [8,9]. In short, glomeruli isolated as stated above were cultured at 37°C in plastic flasks containing 5 ml RPMI medium (Flow laboratories, Irvine) supplemented with 10% decomplemented fetal bovine serum, and buffered with 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (Hepes) (pH 7.2). Epithelial cells grow rapidly with a peak of cell division on day 6 whereas mesangial cells grow at a slower rate with a maximum of cell division on day 22. These 2 cell lines were thus separated by performing subcultures at these 2 different times. The conditions of these subcultures were identical to those used for isolated glomeruli. Culture media were changed every 2 days as a rule. Samples were frozen and stored for further PG determinations.

2.4. *Prostaglandin assays*

The concentrations of PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGE_{1\alpha}$ were determined on incubation media of isolated glomeruli immediately at the end of the incubation. These same PGs were also determined at the end of a given experiment on stored and frozen samples of culture media. After acidification to pH 3.5 and extraction with cyclohexane ethylacetate (1:1, v/v), PGE_2 and $PGF_{2\alpha}$ were separated by silicic acid chromatography and measured using specific radioimmunoassay [5]. 6-Keto-$PGF_{1\alpha}$ was extracted according to the same procedure, but directly measured by radioimmunoassay without prior chromatography using ^{125}I-labeled 6-keto-$F_{1\alpha}$ as a tracer and the corresponding specific antiserum at a final dilution of 1:30 000 [6]. In these conditions, 50% inhibition of binding for 6-keto-$PGF_{1\alpha}$, PGE_2 and $PGF_{2\alpha}$ occurred with 22 ng, 350 ng and 2000 ng/tube, respectively. In each of the three radioimmunoassays a blank value obtained with the medium incubated in the absence of cells was subtracted. Each value of PG concentration given in the figures and the tables is the mean of 6 individual determinations performed at increasing dilutions of the culture medium. PG synthesis was currently expressed as ng/ml incubation medium. In some experiments, PG production was related to the number of cells or to the protein content of the flask. Protein determinations were made according to [10].

3. Results and discussion

3.1. *Production of PGs by glomerular cultured cells as a function of time*

PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ production rates by mesangial and epithelial cells, respectively, were measured on media which had been exposed to the cells for 48 h. This 2 day incubation was started at various intervals of time from 0–39 days after subculture had been initiated (fig.1). Synthesis by epithelial cells of these 3 PGs was low but fairly constant whatever the time at which they were studied after subculture. On the contrary, synthesis by mesangial cells of PGE_2 and 6-keto-$PGF_{1\alpha}$ which was also very low when studied from 0–11 days after subculture increased markedly when measured from 11–24 days after, with a peak on day 15. At this time, the production rate was 30- and 20-times greater than with epithelial cells for PGE_2 and 6-keto $PGF_{1\alpha}$, respectively. This was followed by a decreased synthesis of both PGs with return to base line levels on day 39. Synthesis of $PGF_{2\alpha}$ by mesangial cells increased earlier and reached its maximum on day 13.

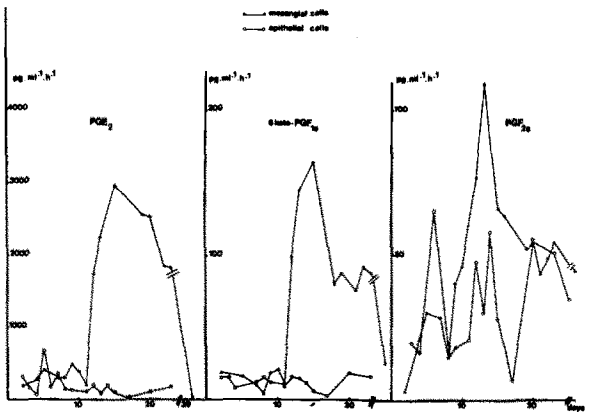

Fig.1. Epithelial cells (○) subcultured 6 days after glomerular explantation and mesangial cells (▲) subcultured 22 days after glomerular explantation were studied at various times (2–39 days) after initiation of the subculture. An individual flask was used for each time studied. Each point on a given day shown on the abscissa represents PG synthesis over the preceding 48 h. All samples were frozen, stored and studied simultaneously at the end of the experiment. Results are the means of 6 individual determinations performed at increasing dilutions of the incubation medium. They are expressed as pg PG synthesized per ml medium and per hour. Note different scales on the ordinate for the 3 PGs.

Table 1
PGE_2, $PGF_{1\alpha}$ and 6-keto-$PGF_{1\alpha}$ production rates by mesangial and epithelial cells on day 20 of the subcultures expressed per ml of culture medium, per mg of tissue protein and per number of cells, respectively

	Mesangial cells			Epithelial cells		
	PGE_2	$PGF_{2\alpha}$	6-keto-$PGF_{1\alpha}$	PGE_2	$PGF_{2\alpha}$	6-keto-$PGF_{1\alpha}$
pg $.ml^{-1}$ $.h^{-1}$	2519 ± 128	71 ± 5	160 ± 6	109 ± 6	56 ± 2	24 ± 1
pg $.mg^{-1}$ $.h^{-1}$	18 253 ± 927	514 ± 40	1159 ± 47	1177 ± 62	579 ± 18	248 ± 3
pg $.(10^6$ cells$)^{-1}$ $.h^{-1}$	54 761 ± 2783	1543 ± 120	3478 ± 141	1890 ± 99	930 ± 28	398 ± 5

Results are means ± SEM of 6 individual determinations

Difference with epithelial cells was not so striking and varied from day to day. When absolute values were considered, synthesis of PGE_2 by mesangial cells appeared markedly predominant since PGE_2 was synthesized by these cells in amounts 15–20-times greater than 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$. Since the amount of PG synthesized/ml incubation milieu depends both on the number of cells present in the flask and on the production per cell, interpretation of these results must take into account the rate of multiplication of the 2 lines of cells. Epithelial cells grow at a faster rate than mesangial cells. A homogenous monolayer made of confluent cells was obtained at day 8 and day 20 of the subculture for epithelial and mesangial cells, respectively. Thus related to the number of cells, production of PGs by epithelial cells compared to that by mesangial cells must be much smaller initially than it appears from fig.1. In order to confirm the marked differences observed from days 11–24 of the subculture, we compared PG production rates by the 2 lines of cells on day 20 of the subcultures when they were both confluent taking into account the number of cells and the amount of cell protein. The differences observed in fig.1 persisted. This demonstrated that PGE_2 and 6-keto-$PGF_{1\alpha}$ synthesis per cell was greater for mesangial than for epithelial cells whereas $PGF_{2\alpha}$ synthesis was in the same range (table 1). The particular pattern of the time-course of PGE_2 production by the mesangial cells can be related to the state of differentiation of these cells. Indeed, the initial percentage of contractile cells after exposure to angiotensin II was 5–12% whereas it reached 33% after day 12 of the subculture [9]. Similarly, the fibrils of myosin detected by immunofluorescent microscopy were less obvious on the early days of the subculture than later [8]. It can be thus considered that the high production rate of PGE_2 by mesangial cells from day 11–24 corresponds to the recovery by these cells of their biological properties.

3.2. *Comparison of PG production by cultured cells and isolated glomeruli*

We have shown [1] that isolated glomeruli synthesize PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ and that this synthesis is maximum in the presence of 5 μg/ml arachidonic acid. Table 2 shows the rates of production of these 3 PGs in basal condition and with arachidonic acid. The values given represent the means ± SEM of individual assays performed on different days over ~5 months during experiments reported [1] or, for the most of them, unpublished. Comparison of these values with those shown on table 1 shows that the rate of synthesis of PGE_2 expressed per mg of tissue

Table 2
PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ production rates by isolated glomeruli

	Basal	With arachidonic acid (5 μg/ml)
PGE_2	2079 ± 201 (34)	7497 ± 655 (27)
$PGE_{2\alpha}$	1642 ± 307 (10)	3526 ± 738 (6)
6-keto-$PGF_{1\alpha}$	1371 ± 197 (5)	2689 ± 82 (5)

Results are the means ± SEM of individual assays (number between brackets) performed on different days. Each of these assays represents the mean of 6 determinations performed at increasing dilutions of the incubation medium. Results are expressed as pg PG synthesized per mg glomerular protein and per hour

protein is greater (2–3-times) in mesangial cells and lower (6–7-times) in epithelial cells than that using entire glomeruli in conditions of maximum stimulation. On the contrary, for $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$, the rates of synthesis by mesangial or epithelial cells were lower than those measured with isolated glomeruli. This comparison has to be interpreted cautiously since experimental conditions were not strictly similar (incubated glomeruli versus cultured cells). It suggests, however, that mesangial cells are the main site of synthesis of PGE_2.

3.3. *Stimulation by arachidonic acid of PG synthesis by cultured glomerular cells*

In order to establish that the extracellular accumulation of PGs reflected new synthesis by the cultured cells, either the precursor of this synthesis, arachidonic acid, or a potent selective inhibitor indomethacin, was added to the culture medium at the start of a 3 h incubation. PG synthesis was studied on days 7 and 12 of subculture for mesangial and epithelial cells, respectively, when the rates of multiplication of both cells lines were maximum. Two flasks were used for each experiment. The culture medium of the 2 preceding days was removed and replaced by fresh serum-free medium with (experimental flask) or without (control flask) arachidonic acid. Fetal bovine serum was omitted in order to suppress any uncontrolled addition of arachidonic acid. After 3 h incubation, media were collected and PG accumulation measured. Addition of arachidonic acid significantly stimulated the production by mesangial cells of the 3 PGs tested (fig.2). The degree of stimulation was markedly greater for PGE_2 (X 12) than for $PGF_{2\alpha}$ (X 3.5) and 6-keto-$PGF_{1\alpha}$ (X 2). Stimulation was also observed at a lesser degree with epithelial cells but only for PGE_2 (X 2) and $PGF_{2\alpha}$ (X 2.5). Addition of indomethacin (1 μM) resulted in inhibition at a similar degree (X 0.30–X 0.50) of the synthesis by the 2 cell lines of the 3 PGs studied.

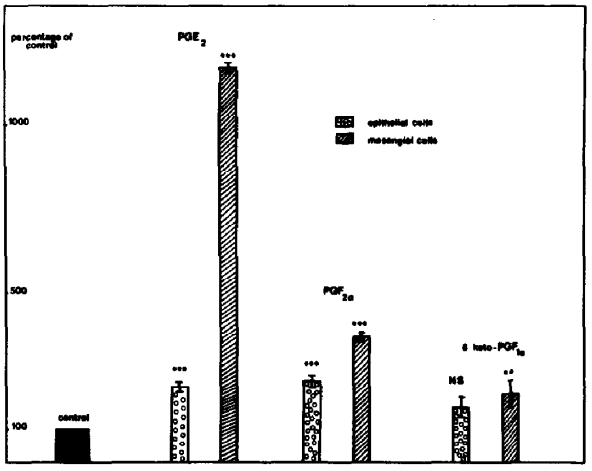

Fig.2. Stimulation of PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ production by mesangial and epithelial cells in the presence of 5 μg/ml arachidonic acid. Results are given as means ± SEM of 6 individual determinations performed at increasing dilutions of the incubation medium. They are expressed as percentages of the corresponding control values. Results of *t* test between control and experimental values (expressed as pg $.ml^{-1}.h^{-1}$) are given. *** = $p < 0.001$; ** = $p < 0.01$; NS, non-significant

4. Conclusions

These findings clearly indicate that glomerular mesangial cells compared to epithelial cells and entire isolated glomeruli are a major site for PGE_2 synthesis. The third cell line present in the glomerulus, endothelial cells, could not be studied due to the lack of an homogeneous population of these cells. The observation that PGE_2 synthesis by mesangial cells was greater than synthesis by entire glomeruli suggests that the contribution of endothelial cells to PGE_2 production by entire glomeruli is not predominant. Synthesis of 6-keto-$PGF_{1\alpha}$ by mesangial cells was also greater than that by epithelial cells. But, its low level in absolute value, its low degree of stimulation by arachidonic acid and the similarity of the production rates per mg of tissue protein obtained using mesangial cells and isolated glomeruli are not in favor of a predominant role of mesangial cells in PGI_2 synthesis. Our findings are in agreement with those of Smith and Bell [1] who localized prostaglandin-forming cyclo-oxygenase in the mesangial cells of ovine and bovine glomerular tufts using immunohistochemical techniques. Since mesangial cells contain bundles of myosin filaments [8] and exhibit a contractile activity [9], the present data suggest that PGE_2 synthesis by the mesangial cells themselves may regulate the role of these cells in glomerular vasoreactivity.

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