

INFLUENCE OF ANGIOTENSIN II AND CATECHOLAMINES ON PROSTANOID SYNTHESIS BY ISOLATED RAT GLOMERULI

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Summary

The relationship between glomerular prostanoids (PGE_2 , $\text{PGF}_{2\alpha}$, TXA_2) and vasoactive agents (angiotensin II, adrenaline, noradrenaline) has been studied in rat renal glomerular suspensions. Angiotensin II stimulates the glomerular production of PGE_2 and TXA_2 (measured by its metabolite TXB_2), but not of $\text{PGF}_{2\alpha}$. The catecholamines had no influence. Non-specific stimulation by arachidonic acid induced an increase in the synthesis of the three prostanoids studied. Specific pools of cellular arachidonic acid may be involved in response to angiotensin II. The inter-relationships between vasoactive agents and glomerular prostanoids may be important in the normal physiology of the glomerular filtration rate.

Introduction

In the mammalian kidney, the glomerular capillary ultrafiltration coefficient (Kf) is one of the major determinants of the glomerular filtration rate (GFR). Locally produced hormones, such as prostanoids may influence the GFR by acting on the ultrafiltration coefficient Kf. For example, recent *in vitro* studies have shown that thromboxane A_2 (TXA_2) induces mesangial cell contraction [1], which may decrease the area component of Kf. Systemic vasoactive agents, such as angiotensin II or noradrenaline, may also stimulate mesangial cell contraction [2]. A possible inter-relationship between systemic and locally synthesized vasoactive agents may, then, be involved in the regulation of GFR. The present experiment was designed to study the possible influence of angiotensin II and catecholamines on the prostanoid synthesis by the isolated rat glomeruli.

Materials and methods

Preparation and isolation of rat renal glomerular suspension Renal glomeruli were isolated from male Sprague-Dawley rats (weighing 180–230g). The kidneys

were removed after perfusion with cold heparinized saline. Cortex from three to four kidneys were dissected and minced to a paste-like consistency. The homogenate was pushed through a 106 microns sieve, which excludes the tubules, and then sieved through a 75 microns sieve, which retained the glomeruli. They were recovered on a tris-buffer solution (pH 7.4) and centrifuged twice at 120G for 90 seconds. Only preparations containing more than 90 per cent of isolated rat glomeruli were used for experiments.

Incubation experiments After the last centrifugation, the supernate was discarded and the pellet resuspended in tris-buffer, pH 7.4. This suspension was equally divided into the incubation tubes and then the following experimental procedures were performed: 1) a pre-incubation period of 40 minutes, at 37°C, in a non-shaking bath, that was stopped in ice-cold water. An aliquot of each tube was gently removed, centrifuged at 3000G for 10 minutes, and the supernate stored at -20°C for further radioimmunoassays; 2) experimental protocol: after removal of the aliquot, the stimulating agent, diluted in the same buffer, was added. Two control tubes (where only tris-buffer was added) were included for each study. The stimulation was performed for 20 minutes, in a shaking bath at 37°C. Incubation was stopped and centrifugation performed as described above. The stimulating agents were angiotensin II (Hypertensine, Ciba), added in increasing concentrations, from 2.5×10^{-10} M to 2.5×10^{-8} M and 10^{-6} M, final dilutions; arachidonic acid (Sigma) added at a final concentration of 5 µg/ml as a non-specific stimulating agent. The difference of concentration of PGE₂, PGF_{2α} and TXA₂ (measured through its metabolite TXB₂) found in the supernate, before and after adding the stimulating agent, was estimated and considered to be the stimulated prostanoid synthesis. PGF_{2α} was measured only in experiments performed angiotensin II and arachidonic acid. Each study was performed at least four times; 3) radioimmunoassay (RIA): PGE₂, PGF_{2α} and TXB₂ were directly measured in the supernate by RIA [3,4].

Antisera to PGE₂, PGF_{2α} and TXB₂, purchased from the Institut Pasteur, Paris, France, have negligible cross reactivity with other prostaglandins [3,4]. Radioactive ligands for PGE₂ and PGF_{2α} were obtained from Amersham Int, UK. In order to eliminate interassay variations, supernates of control and stimulated periods were measured in the same assay. Glomerular proteins were measured according to Lowry et al [5] and prostaglandin production calculated in pg/mg prot/20 min. Results are expressed as mean ± SEM and statistical analysis was performed using the Student's 't' test for paired results.

Results

A II exposure (Table I) Angiotensin II stimulated PGE₂ synthesis at the lowest concentration (2.5×10^{-10} M) 319 ± 25 versus 222 ± 25 pg/mg protein in controls ($p < 0.05$). This stimulation increased with increasing concentration of angiotensin II until a maximum of 520 ± 97 pg/mg/protein ($p < 0.02$). The synthesis of PGF_{2α} was not influenced by angiotensin II. A significant stimulation of TXB₂ was detected at an angiotensin II concentration of 2.5×10^{-9} M, 247 ± 95

TABLE I. Effect of angiotensin II and arachidonic acid on prostanoid synthesis by isolated rat glomeruli (pg/mg prot/20 min)

		PGE ₂	PGF _{2α}	TXB ₂
Control		222±25	398±110	203±48
Angiotensin II (M)	2.5×10 ⁻⁷	520±97**	434±137†	328±75**
	2.5×10 ⁻⁸	458±73**	423±136†	279±50*
	2.5×10 ⁻⁹	401±65*	370±92†	247±95*
	2.5×10 ⁻¹⁰	319±24*	302±82†	179±38†
arachidonic acid	(5μg/ml)	830±153***	702±121****	753±275*

Results are expressed as mean ± SEM

* p<0.05 (experimental versus control)

** p<0.02 (experimental versus control)

*** p<0.01 (experimental versus control)

**** p<0.0025 (experimental versus control)

† Not significant

versus 203±48pg/mg protein (p<0.05). TXB₂ synthesis increased to 328±75pg/mg protein at an angiotensin II concentration of 2.5×10⁻⁷M (p<0.02).

Catecholamine exposure (Table II) At both concentrations, neither adrenaline nor noradrenaline had any effect on PGE₂ and TXB₂ synthesis by isolated rat glomeruli.

TABLE II. Effect of adrenaline and noradrenaline on PGE₂ and TXB₂ synthesis by isolated rat glomeruli (pg/mg prot/20 min)

		PGE ₂	TXB ₂
Control		238±22	228±67
Adrenaline	10 ⁻⁶ M	136±49†	182±33†
	10 ⁻⁸ M	218±38†	231±42†
Noradrenaline	10 ⁻⁶ M	168±32†	177±42†
	10 ⁻⁸ M	261±50†	267±98†

Results are expressed as mean ± SEM

† Not significant (experimental versus control)

Arachidonic acid (Table I) After 20 minutes of incubation, the synthesis of the three prostanoids increased significantly: 830±153 versus 222±25pg/mg protein for PGE₂ (p<0.01); 702±121 versus 398±110pg/mg protein for PGF_{2α} (p<0.0025), and 753±275 versus 203±48pg/mg protein for TXB₂ (p<0.05).

Discussion

The synthesis of prostanoid by isolated rat glomeruli has been recently demonstrated and their role in glomerular physiology discussed [6]. Angiotensin II

enhances PGE₂ synthesis in the whole kidney [6] and in cultured mesangial cells [7,8], but in fresh isolated rat glomeruli the results are controversial: no detectable changes or only slight increases in prostanoid production have been reported [8]. The non-specific traumatic activation of the phospholipase activity, which occurs during preparation of rat glomerular suspension, enhances the basal synthesis of prostanoids and may mask any specific stimulation [8]. Thus, in our experiments, a preincubation period of 40 minutes in a non-shaking bath was performed, in order to allow the phospholipase activity to return to a more quiescent metabolic state. After that, the vasoactive agent was added. As expected arachidonic acid exposure induces an increased synthesis of the three prostanoids. Angiotensin II stimulates the synthesis of PGE₂ and TXB₂, but not of PGF_{2α}. Previous studies have suggested that angiotensin II acts on a specific pool of arachidonic acid, coupled to PGE₂ isomerase, which stimulates only the synthesis of PGE₂ [6].

Our results with an increase of PGE₂ and TXB₂ but not of PGF_{2α}, may be in accordance with this concept of specific activation; however, an inhibition of the glomerular PGE₂-9-ketoreductase activity, diminishing the PGF_{2α} production, cannot be excluded.

The absence of response to catecholamine exposure is in agreement with previous studies performed in isolated rat glomeruli [9] and in cultures of vascular smooth muscle cells [10]. The physiological meaning of these findings remains uncertain. PGE₂ has been suggested to play a role as a modulator of mesangial cell contraction [6] induced by angiotensin II. On the other hand, TXA₂ was shown to mimic the effects of angiotensin II on mesangial cells. At pharmacological doses angiotensin II induced an increased renal synthesis of TXA₂ in different pathological conditions [6]. PGE₂ and TXA₂ have opposite effects on mesangial cell contraction and their synthesis is stimulated by angiotensin II. We may suggest that the control of the glomerular adaptation to haemodynamic changes is a product of the inter-relationship between local and systemic vasoactive agents. Other studies will be needed to further elucidate this concept.

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