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BIDIRECTIONAL ACTION OF EXTRACELLULAR ATP ON INTRACAPILLARY VOLUME OF ISOLATED RAT RENAL GLOMERULI

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Receptors for extracellular nucleotides (P2-purinoceptors) are expressed in renal glomerulus; both on mesangial and endothelial cells. In the present study we have evaluated the potential role of ATP in the regulation of glomerular contraction and relaxation. Using [^{3}H]-inulin we measured the Glomerular Inulin Space (GIS), (that reflects mainly glomerular intracapillary volume), in the presence of ATP and its analogues e.g. 2-methylthio-ATP (P2Y-receptor agonist) and β,γ-methylene-ATP (P2X-receptor agonist). Incubation of the intact glomeruli with ATP or 2-methylthio-ATP or β,γ-methylene-ATP induced a decrease of GIS in similar magnitude as angiotensin II e.g.: about 10% of the basal value. When glomeruli were precontracted with angiotensin II it was observed that both ATP and 2-methylthio-ATP induced an increase of GIS to the basal value, similarly to atrial natriuretic factor. Furthermore, there was no relaxing effect with β,γ-methylene-ATP. We suggest that, these bidirectional changes of the intracapillary volume induced by the extracellular ATP may contribute to regulation of glomerular dynamics.

Key words: *Glomeruli, GIS, ATP, relaxation, contraction, purinoceptors*

INTRODUCTION

Extracellular ATP, as it has been shown, causes vasoconstriction or vasodilatation of renal arteries and these effects are mediated through P2-purinoceptors activation (1). Two classes of P2-receptors, P2X- and P2Y-receptors, exist, both of which have multiple subtypes (2). ATP interacting with P2X receptors (located on smooth muscle cells) results in vasoconstriction, whereas interacting with P2Y receptors (located on endothelial cells) induces vasodilatation (1,2). In renal glomerulus the expression of P2Y-receptors was

shown both on endothelial and mesangial cells (smooth muscle like cells). Exposition of the mesangial cells (cell culture model) to ATP leads to their contraction and increase of $[Ca^{+2}]_i$ (3). However, the activation of P2-receptors located on glomerular endothelial cells leads to increase of $[Ca^{+2}]_i$ and production and release endothelial-derived relaxation factor e.g. nitric oxide (4, 5).

The knowledge about the role of ATP in regulation of glomerular contraction and relaxation is limited until now. Our study analysed the effects of ATP and its analogues e.g. 2-methylthio-ATP (P2Y-receptor agonist) and β,γ-methylene-ATP (P2X-receptor agonist) on dynamics of rat renal glomeruli. We evaluated the contraction and relaxation of glomeruli by measuring changes of Glomerular Inulin Space (GIS).

MATERAL AND METHODS

Isolation of renal glomeruli

The rats (Wistar, male, 200—250g) were decapitated under ether anaesthesia and kidneys were removed and placed in the ice-cold Dulbecco's phosphate buffered saline (PBS) containing (mM): 137 NaCl, 2.7 KCl, 8.1 Na_2HPO_4, 1.5 KH_2PO_4, 0.9 $CaCl_2$, 0.49 $MgCl_2$ and 5.6 glucose at pH 7.4. The renal capsule was removed and the cortex was minced with a razor blade to a paste-like consistence. Minced cortex was dissected and mashed through a gradual nylon sieve (the pore size in sequence: 250, 120 and 70μm).

The final suspension consisted of decapsulated glomeruli which were without afferent and efferent arterioles. The contamination of tubules was checked under light microscopy and was less then 5%. The entire procedure was carried out in ice bath and took no more than 1.5 hours.

Determination of glomerular inulin space

Glomerular inulin space (GIS) was measured according to the previously described (6, 7) and modified method (8, 9). In brief; about 2000 of glomeruli were suspended in 200μl phosphate buffered saline (PBS) containing 1% bovine serum albumin. The samples were preincubated with 0.5μCi [^{3}H]-inulin for 30 minutes at 37°C in shaking water bath (100 cycles/min). Incubation was continued with 1μM Ang II or solvent alone (PBS) for 5 minutes and then with 1μM ANF, 10μM ATP, 10μM 2-methylthio-ATP or 10μM β,γ-methylene-ATP for the next 2 minutes. The reactions were terminated by centrifugation (5 sec at 5000 × g) of the incubation mixture in microtube containing ice-cold silicone oil. The tip of the microtube with glomerular pellet was cut off and the contents were dissolved in 500μl of 0.3% Triton X-100. The supernatant taken from the medium above oil was transferred to the scintillation vial, too. Radioactivity of samples was counted in the liquid scintillation counter. Glomerular Inulin Space (GIS) of the single glomerulus was calculated as follows:

$$GIS = \frac{[^3H]\text{-pellet (cpm)}}{[^3H]\text{-supernatant (cpm/pl)} \times \text{number of glomeruli in the pellet}}$$

Each GIS determination carried out in quadruplicate samples. The results are expressed as a percent of changes of the basal GIS value (641 ± 21pl/glomerulus). The changes of GIS value reflect contraction or relaxation of glomeruli.

Statistics

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by the Dunnett's t-test to determine significance. P values <0.05 were considered to be significant.

RESULTS

Incubation of intact glomeruli with ATP or ATP analogues (*Fig. 1A*) e.g.: 2-methylthio-ATP (P2Y-receptor agonist) or β,γ-methylene-ATP (P2X-receptor agonist) induced a decrease of GIS about 13 ± 0.5, 11 ± 0.5 and 12 ± 0.7% of basal value ($p<0.05$), respectively. A similar decrease of GIS value was observed during incubation of intact glomeruli with the known vasoconstrictor agent — angiotensin II (Ang II). In the second group of experiments (*Fig. 1B*), glomeruli were precontracted with Ang II (5 minutes, 1μM, reduction of GIS about 15 ± 0.3% of basal value). Incubation of Ang II-precontracted glomeruli with ATP or 2-methylthio-ATP induced an increase of GIS to the basal value, similarly as the atrial natriuretic factor (ANF). However, there was no relaxing effect with β,γ-methylene-ATP.

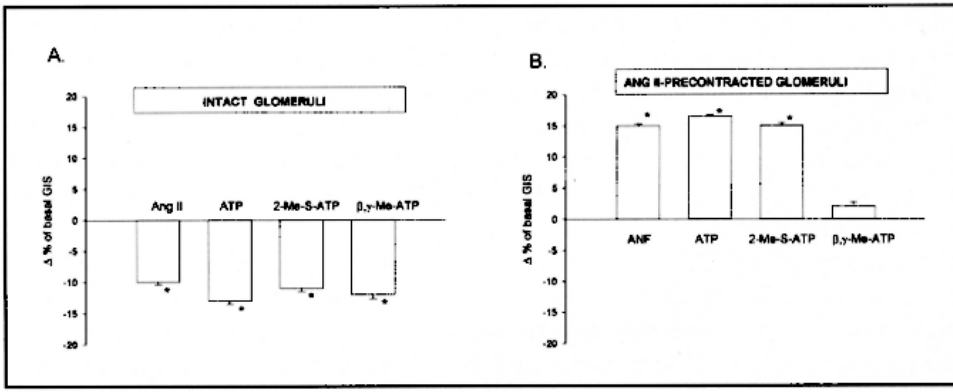

Fig. 1. The effects of the ATP and ATP analogues on the intracapillary volume of isolated intact glomeruli (A) or angiotensin II-precontracted glomeruli (B)

The glomeruli were incubated with 1μM Ang II or solvent alone (PBS) for 5 minutes and then with 10μM ATP, or 10μM ATP analogues (2-methylthio-ATP, β,γ-methylene-ATP) or 1μM ANF for the next 2 minutes. *$p<0.05$ nucleotides vs. control (A-basal GIS value, B-Ang II-reduced GIS value).

DISCUSSION

We have investigated the action of ATP, the natural purinoreceptors agonist, on the extracellular volume of isolated and decapsulated rat renal glomeruli. Using [^{3}H]-inulin (the agent penetrating only the extracellular space) we measured the Glomerular Inulin Space (GIS) which reflects mainly intracapillary space. It was repeatedly shown (7) that reduction of basal GIS value more than 5% reflects contraction of glomeruli whereas increase of GIS value of precontracted glomeruli reflects their relaxation.

In the present experiments we have shown for the first time that ATP and its nonmetabolised analogue 2-methylothio-ATP, a specific agonist of P2Y purinoceptors, may bidirectionaly change the intracapillary volume of renal glomeruli. Firstly, it may reduce GIS similarly like other vasoconstrictor agents such as angiotensin II (Ang II), vasopressin, *Fig. 1A* (7). On the other hand, ATP and 2-methyltio-ATP similarly to vasorelaxant agent — ANF may increase GIS reduced by Ang II (8, 9). However, another ATP analogue e.g. β,γ-methylene-ATP, a specific agonist of P2X purinoceptors was unable to reverse the GIS-reducing effect of Ang II. It was previously shown that P2-purinoceptors (especially P2Y) were expressed on endothelial and mesangial cells (3, 4, 10). Activation of P2Y-purinoceptors located on endothelial cells may lead to relaxation of glomeruli whereas activation of receptors located on mesangial cells may cause glomerular contraction.

How is it possible that ATP and 2-methyltio-ATP may induce either contraction or relaxation? Morphological study indicates (11) that mesangial cells are probably freely surrounded by capillary loops in the intact glomeruli (relaxed glomeruli). Thus, P2Y-purinoceptors located on mesangial and endothelial cells are readily accessible to their ligands. Under these conditions ATP and its analogue 2-methylotio-ATP induce contraction of glomeruli because of a free access to mesangial cells and activation of P2Y-receptors located there. Also another analogue of ATP e.g. β,γ-methylene-ATP contracts intact glomeruli *via* P2X-receptors activation, which are probably located only on mesangial cells. In the opposite situation when glomeruli are precontracted mesangial cells are probably hidden between loops of capillary, which renders then poor access of P2-receptors agonists. Under these conditions ATP and 2-methylotio-ATP induce relaxation of glomeruli because of a free access only to endothelial cells and activation of P2Y-receptors located there. Thus, we postulate the final effect of P2-receptor agonists action on intracapillary volume of glomeruli depends on the their access to the receptors located on mesangial cells. The postreceptors mechanism underlying bidirectional action of purinoceptors agonists is under particular investigation, now.

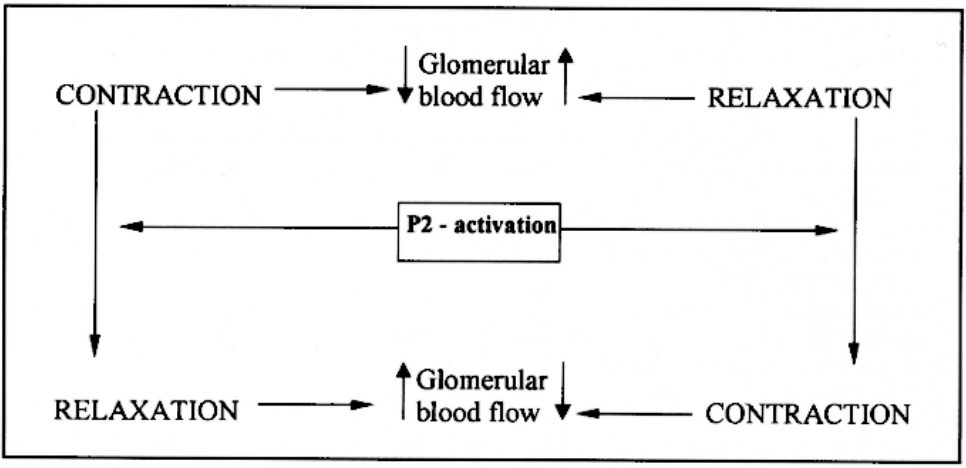

Fig. 2. A scheme illustrating the potential role of extracellular nucleotides in the regulation of the glomerular blood flow.

We suggest that bidirectional changes of glomerular intracapillary volume induced by extracellular ATP *via* P2-purinoceptors activation may contribute to the regulation of single glomerular blood flow may and subsequently to the glomerular filtration rate *(Fig. 2)*.

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