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Effects of diadenosine polyphosphates on glomerular volume

[1,2]Miroslawa Szczepańska-Konkel, *[,1,2]Maciej Jankowski, [1]Anna Stiepanow-Trzeciak & [2]Stefan Angielski

[1]Laboratory of Monitoring Therapy and Pharmacogenetics, Medical University of Gdansk, Debinki 7, 80-211 Gdansk, Poland and [2]Laboratory of Cellular and Molecular Nephrology, Medical Research Centre of the Polish Academy of Science, Poland

1 Diadenosine polyphosphates (P^1,P^3-diadenosine triphosphate, Ap_3A; P^1,P^4-diadenosine tetraphosphate, Ap_4A; and P^1,P^5-diadenosine pentaphosphate, Ap_5A) are vasoactive molecules. The experimental model of isolated rat renal glomeruli was used to investigate their effects on glomerular vasculature. We measured the changes of glomerular inulin space (GIS) as a marker of glomeruli contractility.

2 Ap_4A and Ap_5A induced concentration- and time-dependent reduction of GIS whereas Ap_3A had no effect. The effects of Ap_4A and Ap_5A (both at 1 μM) were prevented by a nonselective P2 receptor antagonist, that is, suramin (10 μM) and P2Y receptor antagonist – reactive blue 2 (50 μM). However, the antagonist of P1 receptor, that is, theophylline (1 μM) and A_1 receptor 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 10 μM) did not affect the responses of glomeruli to Ap_4A or Ap_5A.

3 Ap_3A, in contrast to Ap_4A and Ap_5A, prevented angiotensin II-induced reduction of GIS in a concentration- and time-dependent manner. This effect was partially prevented by suramin and markedly reduced by reactive blue 2 and the specific antagonist of $P2Y_1$ receptor – MRS 2179 (10 μM). However, theophylline and the specific antagonist of A_2 receptor – 3,7-dimethyl-1-propargylxanthine (DMPX; 10 μM) – did not affect Ap_3A action.

4 We indicate that diadenosine polyphosphates changed the glomerular volume *via* activation of P2 receptors. We suggest that extracellular Ap_4A and Ap_5A *via* P2X and P2Y receptors may decrease and Ap_3A *via*, at least in part, $P2Y_1$ receptors may increase filtration surface, which in turn may modify glomerular filtration rate.
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Abbreviations: Ap_nAs, diadenosine polyphosphates; Ap_3A, P^1,P^3-diadenosine triphosphate; Ap_4A, P^1,P^4-diadenosine tetraphosphate; Ap_5A, P^1,P^5-diadenosine pentaphosphate; DMPX, 3,7-dimethyl-1-propargylxanthine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; GIS, glomerular inulin space; MRS 2179, 2′-deoxy-N^6-methyl adenosine 3′5′-diphosphate diammonium salt; PBS, phosphate-buffered saline

Introduction

The naturally occurring diadenosine polyphosphates (Ap_nAs, $n=2$–7) are recognised as vasoactive nucleotides. These molecules consist of two adenosine moieties connected *via* 5′-ribose linkage to both ends of the polyphosphate chain. Ap_nAs are present in all living cells and certain amounts of these are released into extracellular space during platelet aggregation, metabolic stress and neurotransmission (Luthje & Ogilvie, 1988; Schluter *et al*., 1994; Miras-Portugal *et al*., 1998). The effects of Ap_nAs are mediated *via* P1 and P2 receptors or dinucleotide receptors (Hoyle *et al*., 1996; Vahlensieck *et al*., 1996; Pintor *et al*., 1997; Ralevic & Burnstock, 1998; Verspohl *et al*., 1999). P1 receptors (G proteins coupled receptors) are classical receptors for adenosine and are further subdivided, according to convergent molecular, biochemical and pharmacological evidence into the following subtypes: A_1, A_{2A}, A_{2B} and A_3. These receptors have been cloned from several species (Ralevic & Burnstock, 1998). P2 receptors are divided into P2X (ligand-gated ion channels) and P2Y (G proteins coupled receptors). Up to this time, seven mammalian P2X receptors: $P2X_{1–7}$ and eight mammalian P2Y receptors: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11–13}$ and $P2Y_{15}$ have been cloned (Illes & Ribeiro, 2004). There is also pharmacological evidence favouring the hypothesis that biological action of Ap_nAs is mediated by activation of the specific dinucleotide receptor (Pintor & Miras-Portugal, 1995; Diaz-Hernandez *et al*., 2001; Jimenez *et al*., 2002). However, this receptor has not been cloned yet. Vasomotor actions of Ap_nAs depend on the localisation of receptors and number of phosphate groups in the moiety (Ralevic *et al*., 1995; Van der Giet *et al*., 1997; Ralevic *et al*., 2001). Ap_3A (P^1,P^3-diadenosine triphosphate) induces endothelium-dependent vasodilatation in the rat mesenteric arteries (Ralevic *et al*., 1995) and endothelium-independent vasodilatation in rabbit mesenteric arteries (Busse *et al*., 1988). Ap_4A (P^1,P^4-diadenosine tetraphosphate) induces endothelium-independent vasoconstriction and endothelium-dependent vasodilatation in rabbit mesenteric arteries (Busse *et al*., 1988). Ap_5A (P^1,P^5-diadenosine pentaphosphate) induces vasoconstriction in rat mesenteric arteries (Ralevic & Burnstock, 1991) and relaxation in the porcine coronary artery (Sumiyoshi *et al*., 1997).

*Author for correspondence; E-mail: majank@amg.gda.pl
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There is also an increasing interest in the renal effects of Ap_nA (Hohage *et al*., 1996). Ap_3A and Ap_5A induce vasoconstriction of intralobular arteries (Gabriels *et al*., 2000). Bolus injection of Ap_4A, Ap_5A or Ap_6A into the jugular vein decreases the renal blood flow (Khattab *et al*., 1998). It has been shown that Ap_3A, Ap_4A, Ap_5A and Ap_6A can influence the tone of resistance arteries in the human kidney (Steinmetz *et al*., 2003). We have shown that intravenous infusion of Ap_4A reduces glomerular filtration rate (GFR) and evokes natriuresis and diuresis in rat (Szczepanska-Konkel *et al*., 2003). There are also reports describing action of Ap_nAs on renal cells. Ap_3A, Ap_4A, Ap_5A and Ap_6A induce depolarisation of mesangial cell membrane by activation of Cl^- and nonselective conductance (Kleta *et al*., 1995). Diadenosine polyphosphates (Ap_3A–Ap_6A) modulate pH regulation of rat mesangial cells (Schulte *et al*., 1999). Moreover, these nucleotides increase intracellular calcium concentration (Tepel *et al*., 1996) and cause contraction of mesangial cells (Schlatter *et al*., 1995).

These reports have led us to focus our efforts on the investigation of the influence of Ap_nAs on glomerular microvasculature. The diameter of glomerular vessels is one of the factors that may alter GFR. In the present study, we examined the vasomotor properties of Ap_3A, Ap_4A and Ap_5A in isolated rat renal glomeruli. The effects of nucleotides were evaluated based on changes of glomerular [^{3}H]-inulin space (GIS).

Methods

Animals

Experiments were performed on male Wistar rats (weighing 220–250 g). The rats were housed in an animal care facility at the Medical University of Gdańsk and fed the standard pellet diet (Altromin GmbH, Lage, Germany). Experiments were approved by the local Ethics Committee for Animal Research.

Isolation of renal glomeruli

Rats were decapitated under diethyl ether anaesthesia (5 ml l air^{-1}, inhalation for 1 min in a glass bell) and the kidneys were removed and placed in ice-cold phosphate-buffered saline (PBS; pH 7.4, composition in mM) containing NaCl 137, KCl 2.7, Na_2HPO_4 8.1, KH_2PO_4 1.5, $CaCl_2$ 0.9, $MgCl_2$ 0.49 and glucose 5.6. Glomeruli were isolated by gradual sieving technique (Misra, 1972). Briefly, the renal capsule was removed and the cortex was minced with a razor blade to a paste-like consistency and strained through a steel sieve (pore size 250 μm). The mash that passed through this sieve was suspended in ice-cold PBS. Then, the suspension passed through two consecutive steel sieves (120 and 70 μm). The glomeruli retained on the top of the 70 μm sieve were washed off with ice-cold PBS. Glomeruli were resuspended in ice-cold PBS buffer. The final suspension consisted of decapsulated glomeruli devoid of afferent and efferent arterioles. The tubular contamination was less than 5% as assessed under the light microscope. The entire procedure was carried out in an ice bath and took no more than 1 h.

Determination of glomerular inulin space

Glomerular inulin space (GIS) was measured according to the previously described method (Savin, 1986; Fujiwara *et al*., 1989) with our own modifications (Szczepanska-Konkel *et al*., 1991; Jankowski M *et al*., 2001a). Briefly, about 2000 glomeruli were suspended in 200 μl ice-cold PBS containing 1% bovine serum albumin. Samples were preincubated with 0.5 μCi [^{3}H]inulin for 30 min at 37°C in a shaking water bath (1.7 Hz). Incubation was continued with varied concentrations of tested agents for the indicated time. The final volume of incubation mixture was 250 μl. Reactions were terminated by centrifugation in the following manner: suspension of glomeruli (200 μl) was transferred to a microtube containing 100 μl ice-cold silicone oil (Wacker Silicone) and centrifuged for 5 s at $5000 \times g$. Glomeruli were spun through the oil, forming a pellet on the bottom of the tube with incubation medium remaining behind. The tip of the microtube with the glomerular pellet was cutoff with a scalpel blade and content was resuspended in 500 μl 0.3% Triton X-100. Supernatant (20 μl) was taken from the medium above the oil and also transferred to the scintillation vial. After solubilisation, 2 ml of scintillation cocktail was added. Radioactivity of samples was measured in a liquid scintillation counter (LKB Wallace). GIS of a single glomerulus was calculated as follows:

$$\mathrm{GIS} = \frac{[^3\mathrm{H}]\text{pellet}}{[^3\mathrm{H}]\text{supernatant} \times \text{no. of glomeruli in the pellet}}$$

where [^{3}H]pellet is measured in counts per minute (c.p.m.) and [^{3}H]supernatant in c.p.m. divided by picolitres. The number of glomeruli in suspension medium was counted with a light microscope at low magnification. Each GIS determination was carried out in quadruplicate samples.

The results are expressed as picolitres per glomerulus or as a percentage of basal GIS value. Basal GIS value (mean $\pm$ s.e.m.) of pooled calculations ($n = 36$, control GIS before the start of incubation with tested agents) was 632 ± 18 pl per glomerulus.

Experimental protocols

Measurements of GIS were performed according to the following protocols

Group 1. Effects of Ap_3A, Ap_4A and Ap_5A on GIS of intact glomeruli: concentration- and time-dependency For concentration–response curves, glomeruli were incubated for 5 min with Ap_3A, Ap_4A or Ap_5A at concentration range 1 pM–100 μM each. For time–response curves, glomeruli were incubated with 1 μM tested dinucleotides for 0–10 min.

Group 2. Effect of P1 and P2 receptors antagonists on Ap_4A and Ap_5A action on GIS of intact glomeruli Glomeruli were preincubated with theophylline (1 μM), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 10 μM), suramin (10 μM) or reactive blue 2 (50 μM) for 2 min and, thereafter, incubation was continued with 1 μM Ap_4A or Ap_5A for 5 min.

Group 3. Effects of Ap_3A, Ap_4A and Ap_5A on GIS in the presence of angiotensin II: concentration- and time-dependency For concentration–response curves, glomeruli were co-incubated with 1 μM angiotensin II and different

concentrations of Ap_3A, Ap_4A or Ap_5A (1 pM–1 μM) for 5 min. For time–response curves, glomeruli were co-incubated with 1 μM angiotensin II and 1 μM Ap_3A, Ap_4A or Ap_5A for 0–10 min.

Group 4. Effect of P1 and P2 receptors antagonists on Ap_3A action on GIS in the presence of angiotensin II Glomeruli were preincubated with theophylline (1 μM), 3,7-dimethyl-1-propargylxanthine (DMPX; 10 μM), suramin (10 μM), reactive blue 2 (50 μM) or MRS 2179 (10 μM) for 2 min and, thereafter, incubation was continued with 1 μM angiotensin II and 1 μM Ap_3A for 5 min.

Hydrolysis of Ap_3A, Ap_4A and Ap_5A in suspension of glomeruli

The hydrolysis of dinucleotides by glomeruli was determined by measurement of ATP, adenosine, inosine and hypoxanthine concentration in suspension of glomeruli. About 2000 glomeruli were suspended in 200 μl ice-cold PBS and preincubated for 30 min at 37°C in shaking water bath (1.7 Hz). Then, incubation was continued with Ap_3A, Ap_4A or Ap_5A (each at 1 μM) for the indicated time. Reactions were terminated by centrifugation (5 s at 5000 × *g*) of 200 μl of the suspension in the microtube containing 100 μl of ice-cold silicone oil (Wacker Silicone). Supernatant (150 μl) was taken from the medium above the oil and concentration of ATP, adenosine, inosine and hypoxanthine was measured immediately.

Analysis of metabolite formation from Ap_3A, Ap_4A and Ap_5A

The concentration of ATP in suspension of glomeruli was measured using ATP Bioluminescence Assay Kit CLS II as per the manufacturer's instructions according to the published method (Kimmich *et al*., 1975). The concentration of adenosine, inosine and hypoxanthine in suspension of glomeruli was determined using the standard chemiluminescent method (Kather *et al*., 1987). Since adenosine was transformed to inosine, followed by conversion into hypoxanthine, the amount of inosine and hypoxanthine was pooled to that of adenosine to evaluate the total adenosine formation. The incubation medium (pH 8.2) contained 0.2 mM Na_2HPO_4/KH_2PO_4, 2.5 mM EDTA, 25 μM luminol and the following enzymes (all activities in U ml^{-1}): 1.5 peroxidase, 40 xantine oxidase, 50 nucleoside phosphorylase, 2.4 adenosine deaminase. Luminescence was measured in room temperature with a photon-counting luminometer (TD-20/20) with autoinjector system.

Data analysis

Data were analysed statistically using Student's *t*-test or one-way analysis of variance, followed by a Dunnett's multiple comparison test using Sigma Stat 2.01 as appropriate. Differences were considered statistical significant where P was found to be <0.05. Data are shown as the mean ± s.e.m. from n independent experiments.

Materials

Ammonium salts of Ap_3A, Ap_4A, Ap_5A, MRS 2179, scintillation cocktail, suramin, reactive blue 2, theophylline, luminol, nucleoside phosphorylase were purchased from Sigma (St Louis, MO, U.S.A.), DPCPX and DMPX from RBI (Natick, MA, U.S.A.) and peroxidase, xantine oxidase, adenosine deaminase and ATP Bioluminescence Assay Kit CLS II from Roche (Mannheim, Germany). [^{3}H]Inulin was obtained from DuPont NEN Products (Boston, MA, U.S.A.). All other agents were purchased from POCh (Gliwice, Poland).

Results

Hydrolysis of Ap_nA by isolated glomeruli

Ap_3A, Ap_4A and Ap_5A were tested to evaluate their potential degradation by glomerular ecto-nucleotidases to vasoactive metabolites, that is, ATP and adenosine. The results are summarised in Table 1 ($n=3$). Luminescence analysis of the supernatant of glomeruli suspension incubated with 1 μM Ap_3A did not reveal any detectable accumulation of ATP. With 1 μM Ap_4A or Ap_5A, however, there was significant accumulation of ATP after 1-min incubation. The maximal ATP concentration was detected during incubation with Ap_4A 60.5 ± 1.7 nM and it was three times more than with Ap_5A. All tested dinucleotides were degraded to adenosine, inosine and hypoxanthine during 5-min incubation. Total concentrations of these metabolites increased about three-fold compared to basal value (control).

Effects of Ap_3A, Ap_4A and Ap_5A on GIS of intact glomeruli

The actions of Ap_4A and Ap_5A were concentration dependent. Ap_4A and Ap_5A had an apparent EC_{50} of approximately 1 pM (Figure 1, $n=5$–6). By contrast, the actions of Ap_3A were not concentration related in the range used in this study. The reduction of GIS induced by 1 μM Ap_4A or Ap_5A was significant in the 2nd min, reaching a maximum, that is, reduction of GIS about 15% in the 5th min (Figure 2, $n=4$–5).

Effect of P1 and P2 receptor antagonists on responses of glomeruli to Ap_4A and Ap_5A

It is accepted that A_1 receptors mediate vasoconstrictive response to adenosine. Therefore, in order to investigate the

Table 1 Hydrolysis of 1 μM Ap_nA by isolated rat glomeruli

Ap_nA	*ATP* (nM)			*Ado + Ino + Hyp* (μM)
	1 min	*2* min	*5* min	*5* min
Control	ND	ND	ND	0.44 ± 0.04
Ap_3A	ND	ND	ND	1.39 ± 0.16*
Ap_4A	60.5 ± 1.7*	54.1 ± 0.6*	45.4 ± 1.1*	1.31 ± 0.15*
Ap_5A	17.5 ± 0.6*	15.7 ± 0.8*	21.4 ± 0.2*	1.13 ± 0.06*

$n=3$, *$P<0.001$, ND = not detectable.

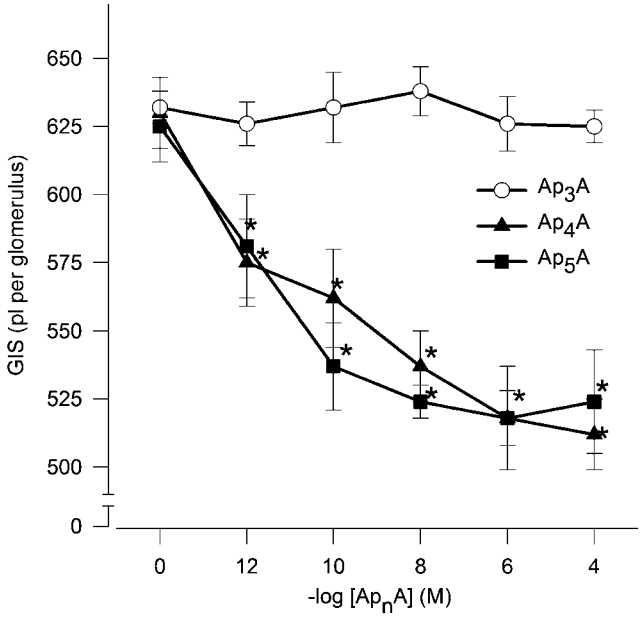

Figure 1 Concentration–response curves illustrating the constrictor effects of Ap_nAs on GIS. Glomeruli were incubated with Ap_3A (open circles), Ap_4A (filled triangles) or Ap_5A (filled squares) at concentration range 1 pM–100 μM for 5 min. Results are expressed as the mean of absolute values $\pm$ s.e.m. for $n = 5$–6 experiments. $^*P < 0.05$ Ap_nAs vs basal value.

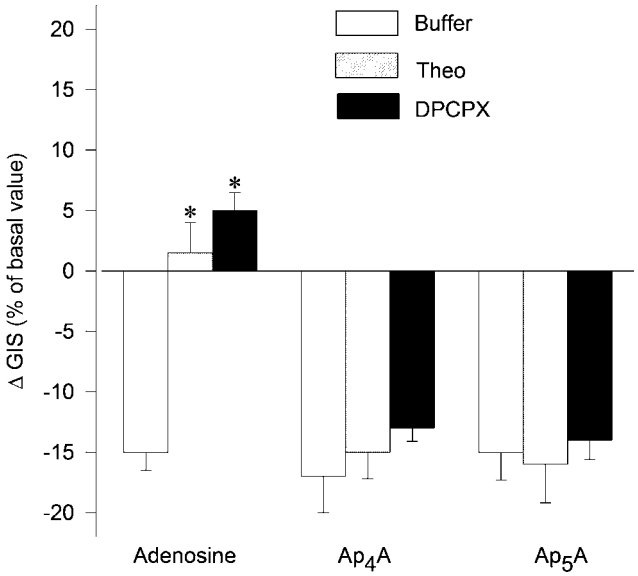

Figure 3 Effects of P1 receptor antagonists on contractile responses of glomeruli to Ap_4A and Ap_5A. Glomeruli were incubated with 1 μM theophylline (Theo, rising right columns), 10 μM DPCPX (selective A_1 receptors antagonist, solid columns) or with buffer (open columns) for 2 min. Incubation was continued for 5 min with Ap_4A, Ap_5A and adenosine (each at 1 μM). Results are expressed as the percentage of changes of basal value $\pm$ s.e.m. for $n = 4$–5 experiments. $^*P < 0.05$ agent + antagonist vs agent + buffer.

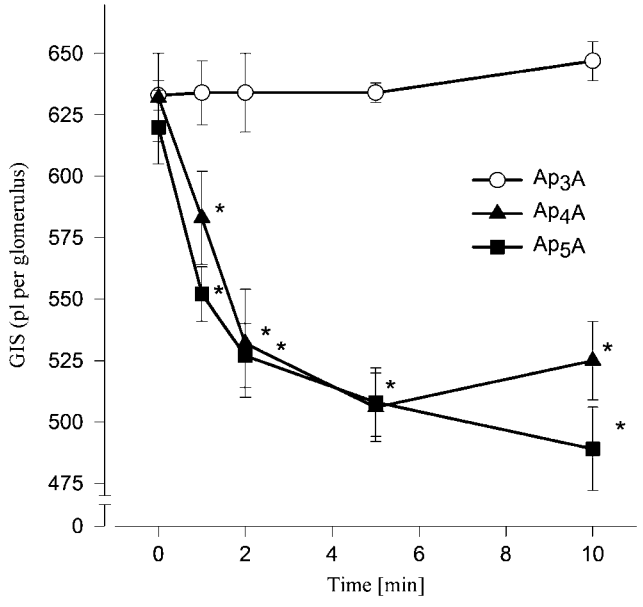

Figure 2 Time–response curves illustrating the constrictor effects of Ap_nAs on GIS. Glomeruli were incubated with 1 μM Ap_3A (open circles), Ap_4A (filled triangles) or Ap_5A (filled squares) for the indicated time. Results are expressed as the mean of absolute values $\pm$ s.e.m. for $n = 4$–5 experiments. $^*P < 0.05$ agent vs basal value.

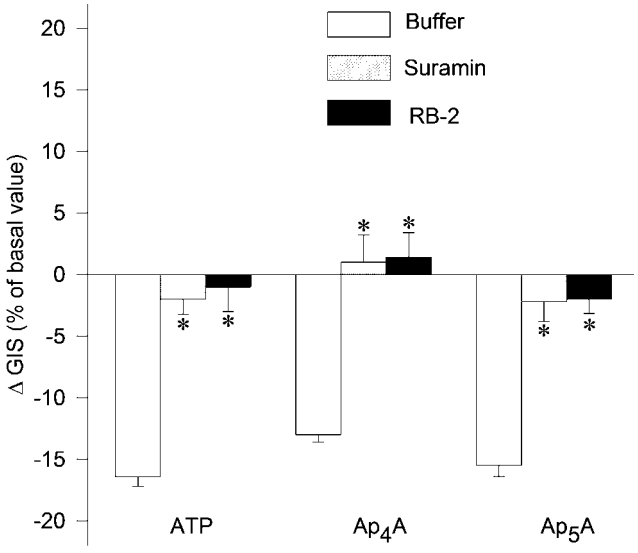

Figure 4 Effects of P2 receptor antagonists on dilation responses of glomeruli to Ap_4A and Ap_5A. Glomeruli were incubated with buffer (open columns), 10 μM suramin (rising right columns) or 50 μM reactive blue 2 (RB-2, solid columns) for 2 min and then with ATP, Ap_4A and Ap_5A (each at 1 μM) for 5 min. Results are expressed as the percentage of changes of basal value $\pm$ s.e.m. for $n = 3$–4 experiments. $^*P < 0.05$ agent + antagonist vs agent + buffer.

potential involvement of A_1 receptors in Ap_nA-induced reduction of GIS, we used the nonselective antagonist of P1 receptor; theophylline (Theo) and selective antagonists of A_1 receptor, that is, DPCPX. In the presence of Theo (1 μM) or DPCPX (10 μM) (Figure 3, $n = 4$–5), responses of glomeruli to Ap_4A and Ap_5A were not significantly affected. The control response to 1 μM adenosine (Ado) was completely abolished. Theo and DPCPX alone did not exert any significant influence on GIS (data not shown).

In another set of experiments, the glomeruli were preincubated with nonselective P2 receptor antagonist, that is,

suramin (10 μM) or relatively selective P2 receptor antagonist, that is, reactive blue 2 (50 μM) to evaluate the involvement of P2 receptors in Ap_4A- and Ap_5A-induced reduction of GIS glomeruli. The responses to Ap_4A, Ap_5A and ATP as well, were almost completely abolished (Figure 4, $n=3$–4, $P<0.05$). Suramin and reactive blue 2 alone did not exert any significant influence on GIS (data not shown).

Effects of Ap_3A, Ap_4A and Ap_5A on GIS in the presence of angiotensin II

To evaluate the potential vasodilatory effects of Ap_nA, the responses of glomeruli to Ap_3A, Ap_4A and Ap_5A were tested in the presence of a vasoconstrictive agent, that is, angiotensin II. It was published that angiotensin II-induced reduction of GIS and ATP prevented the action of angiotensin II in a concentration- and time-dependent manner (Jankowski M *et al*., 2001a). As shown in Figure 5 ($n=5$–6), Ap_3A at concentration range 1 pM–1 μM prevented Ang II-induced reduction of GIS with apparent EC_{50} of approximately 1 pM. Ap_4A and Ap_5A (0.1 nM–1 μM) were not active in the concentration range used in this study. The effect of Ap_3A was maximal in the 2nd min and stable during the 10 min of incubation (Figure 6, $n=5$–6).

Effects of P1 and P2 receptor antagonists on responses of glomeruli to Ap_3A in the presence of angiotensin II

To determine whether glomerular vasodilator response to Ap_3A involves A_2 receptors, Theo (1 μM) and selective A_2 receptor antagonist – DMPX (10 μM) – were used. Following preincubation with Theo or DMPX, the maximal responses to

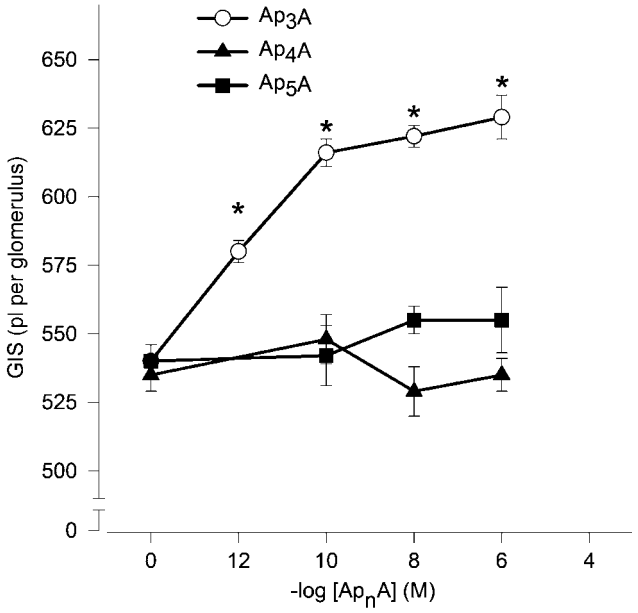

Figure 5 Concentration–response curves illustrating the dilator effects of Ap_nAs on GIS. Glomeruli were co-incubated with 1 μM angiotensin II and tested Ap_nA: Ap_3A (open circles), Ap_4A (filled triangles) and Ap_5A (filled squares) at concentration range 1 pM–1 μM for 5 min. Results are expressed as the absolute values ± s.e.m. for $n=5$–6 experiments. $^*P<0.05$ Ap_nAs + Ang II vs Ang II + buffer.

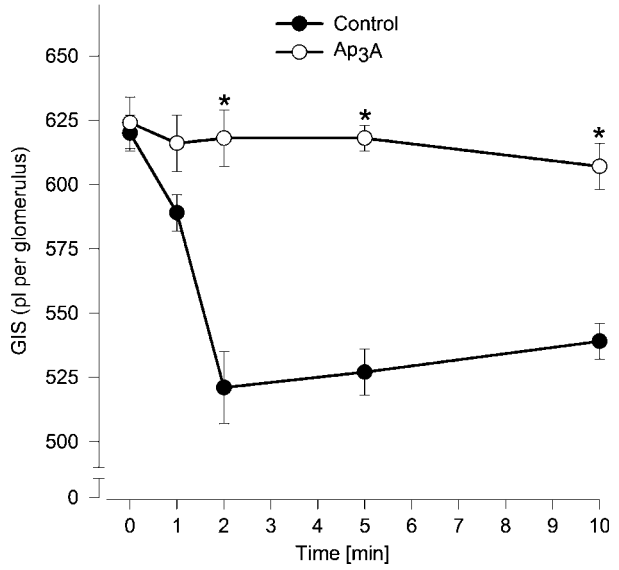

Figure 6 Time–response curves illustrating the dilator effects of Ap_nAs on GIS. Glomeruli were incubated with 1 μM Ap_3A (open circles) or buffer (control; closed circles) in the presence of angiotensin II (1 μM) for the indicated time. Results are expressed as the absolute values ± s.e.m. for $n=5$–6 experiments. $^*P<0.05$ Ap_3A vs control.

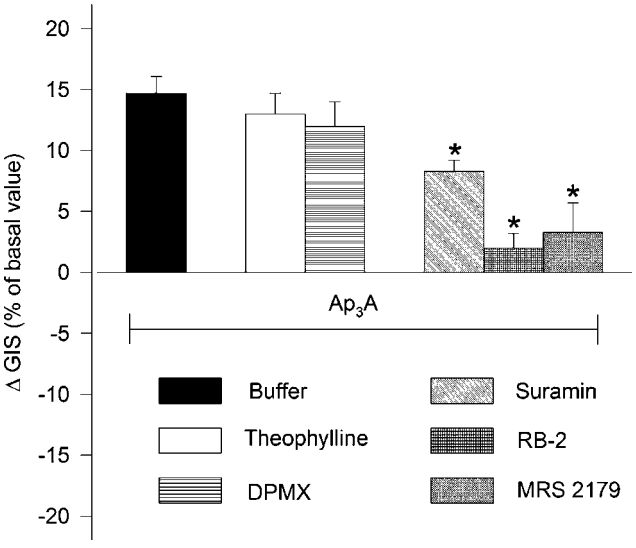

Figure 7 Effects of P1 and P2 receptors antagonists on dilation responses of glomeruli to Ap_3A. Glomeruli were preincubated with buffer (solid column) or P1 receptors antagonists: 1 μM theophylline (Theo, open columns), 10 μM DPMX (selective A_2 receptors antagonist, horizontal lines column) or P2 receptors antagonists: 10 μM suramin (rising left column), 50 μM reactive blue 2 (horizontal cross hatch) and 10 μM MRS 2179 (selective $P2Y_1$ receptors antagonist, diagonal cross hatch) for 2 min. Incubation was continued with angiotensin II (1 μM) and Ap_3A (1 μM) for 5 min. Results are expressed as the percentage of changes of Ang II-treated glomeruli ± s.e.m. for $n=4$–5 experiments. $^*P<0.05$ Ap_3A + antagonists vs Ap_3A + buffer.

Ap_3A were not significantly affected (Figure 7, $n=4$–5). In order to determine whether P2 receptors are involved in Ap_3A action, P2 receptor antagonists were used. The effect of Ap_3A was almost completely abolished following preincubation

of glomeruli with reactive blue 2 (50 μM) or selective $P2Y_1$ receptor antagonist MRS 2179 (10 μM). The response to Ap_3A was partially attenuated by suramin ($P<0.05$).

Discussion

The filtration of plasma, which occurs in renal glomeruli, is a fundamental physiological process. The glomerular tuft comprises capillary network supported by a central mesangium made up of smooth-muscle-derived cells, that is, mesangial cells and the dense fibrillar matrix (Lemley *et al*., 1992). It is known that mesangial cells contain well-developed contractile apparatus (Foidart *et al*., 1980; Anderson *et al*., 1981). Contraction of mesangial cells may lead to reduction of the effective filtration area, which, in turn, may reduce GFR. The studies on regulation of glomerular filtration have provided findings showing that ultrafiltration may be significantly changed as a result of mesangial cell contraction (Savin, 1986; Mene *et al*., 1989). The glomerular endothelial cells also influence the filtration process. These cells, under physiological conditions, are able to produce and release vasoactive substances, for example, nitric oxide, PGI_2. These substances may influence mesangial cells tension and, in turn, GFR (Stockand & Sansom, 1998).

In this study, we investigated the action of diadenosine polyphosphates on contractility of glomeruli by measuring changes of extracellular volume (^{3}H-labelled inulin space) of isolated decapsulated rat glomeruli. It has been shown that most of the extracellular space of decapsulated glomeruli is the intracapillary space that accounts for about 34% of total glomerular volume (Fujiwara *et al*., 1989). The intracapillary volume was calculated (GIS) after the separation of glomeruli from the medium. The basal GIS value we determined was 632 ± 18 pl glomerulus^{-1}. This value was comparable with other estimations (Fujiwara *et al*., 1984; Lewko *et al*., 1997). An increase or decrease of GIS reflects a relaxation or contraction of glomeruli, respectively. The validity of this method was endorsed by a previous study (Fujiwara *et al*., 1984; Kikkawa *et al*., 1986). It was confirmed that a decrease of glomerular diameter about 4% in response to angiotensin II was equivalent to about 10% decrease of GIS.

In the present study, we have investigated responses of isolated rat glomeruli to extracellular Ap_3A, Ap_4A and Ap_5A. We have shown, for the first time, that incubation of intact glomeruli with Ap_4A and Ap_5A leads to concentration- and time-dependent reduction of glomerular volume. However, Ap_3A did not affect the volume of native isolated glomeruli. Conversely, the potential vasodilatory activities of Ap_nA were investigated during co-incubation with angiotensin II (Ang II). This well-known vasoconstrictive agent induces stable in time reduction of GIS by about 20% (Fujiwara *et al*., 1984) or 16% (Figure 6). In our experiments, Ap_3A prevented the action of Ang II in a concentration- and time-dependent manner. However, Ap_4A and Ap_5A did not affect the vasoconstrictive action of Ang II. These observations are in line with findings in various isolated vascular beds and arteries of several species (Busse *et al*., 1988; Van der Giet *et al*., 1997; Gabriels *et al*., 2000; Steinmetz *et al*., 2002). However, on the contrary to our observations, several investigators have shown that Ap_4A and Ap_5A possess the vasodilatory activity (Busse *et al*., 1988; van Ginneken *et al*., 2002). This bidirectional action of Ap_4A and Ap_5A observed by other investigators is probably due to, at least in part, characteristic for particular species and vessels distribution of purinoceptors, which may be simultaneously activated by Ap_nA (Steinmetz *et al*., 2000).

We have previously shown that extracellular ATP possesses unique vasomotor properties and acts bidirectionally on isolated glomeruli. ATP induces cGMP-dependent relaxation of angiotensin II-precontracted glomeruli (Jankowski M *et al*., 2001a) and induces contraction of intact glomeruli (Jankowski *et al*., 2000) with involvement of Rho-kinase in this process (Jankowski M *et al*., 2003). In contrast to ATP, the tested dinucleotides affect the glomerular volume only in one direction; Ap_3A induces only relaxation whereas Ap_4A and Ap_5A induce only contraction of isolated glomeruli.

The effects of dinucleotides on glomeruli can be induced, at least in part, through their vasoactive metabolites, such as mononucleotides and adenosine. It has been reported for several cell types that dinucleotides are degraded by cell surface ecto-nucleotides that may be of importance for biological effects of extracellular dinucleotides (Verspohl *et al*., 1999). We analysed the ability of isolated glomeruli to hydrolyse Ap_nA and we observed significant release of adenosine (from Ap_3A, Ap_4A and Ap_5A) and ATP (from Ap_4A and Ap_5A) during incubation (Table 1). From our previous work with ATP and adenosine in isolated glomeruli, it was suggested that dinucleotides might act through their vasoactive metabolites, that is, adenosine and ATP. We have previously shown that adenosine induces decrease of the glomerular volume and this effect is abolished in the presence of nonselective antagonist of P1 receptor, that is, theophylline (Jankowski M *et al*., 2001b). It is widely accepted that the vasoconstrictor response to adenosine is mediated *via* A_1 receptors (Olivera *et al*., 1989). The expression of A_1, A_{2A}, A_{2B} receptors has been observed on the cells of glomerulus (Inscho, 2001; Vitzthum *et al*., 2004). In order to investigate the potential involvement of adenosine receptors in Ap_nA-induced contraction and relaxation of glomeruli, we used the nonselective antagonist of P1 receptor; theophylline and selective antagonists of A_1 receptor, that is, DPCPX and A_2 receptor, that is, DMPX. Neither theophylline nor DPCPX prevented Ap_4A- and Ap_5A-induced contraction of glomeruli. Similarly, theophylline and DPMA did not prevent Ap_3A-induced relaxation of glomeruli. These results suggest that P1 receptors, in particular A_1 and A_2 receptors, are probably not involved in vasoconstrictive and vasodilatory effects of Ap_nA. However, we cannot fully exclude that adenosine *via* P1 receptors, which appears in medium during incubation, modulates response of glomeruli to Ap_nA. Further studies are needed to investigate the cross-talk between P1 and P2 receptors during incubation of glomeruli with Ap_nA.

It has been previously reported that vascular effects of Ap_nA are mediated, at least in part, *via* P2 receptors. Ap_3A and Ap_5A evoke transient constrictions in vessels of the hydronephrotic rat kidney *via* P2 receptors (Gabriels *et al*., 2000). Ap_5A mediates the decrease of mean arterial blood pressure by activation of $P2Y_1$ receptors (Steinmetz *et al*., 2000). There is also pharmacological evidence showing that activity of Ap_5A in the isolated perfused rat kidney is mediated by P2X receptors (Van der Giet *et al*., 1999). Ap_4A and Ap_5A are full agonists of rat $P2X_3$ receptors and Ap_4A is the partial agonist of rat $P2X_4$ when expressed in *Xenopus laevis* oocytes (Wildman *et al*., 1999). It has been reported that Ap_3A is a

potent agonist of $P2Y_1$ (Pintor *et al*., 1996). Our previous study has shown the functional expression of P2X receptors in isolated glomeruli (Jankowski *et al*., 2000). The expression of $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2X_7$ receptors has been observed in glomeruli (Inscho, 2001; Bailey *et al*., 2004).

The contraction and relaxation of glomeruli induced by Ap_nA can be compared to the glomerular effects of mononucleotides. ATP has been shown to be a potent agonist of P2X and P2Y receptors in the renal glomeruli (Jankowski M *et al*., 2001a; Bailey *et al*., 2004). The role of P2 receptors in response to Ap_nA was analysed using a common P2 receptor antagonists, that is, suramin (nonselective of P2 receptors) and reactive blue 2 (relatively selective of P2Y receptors). Both antagonists abolished the effects of Ap_4A, Ap_5A, and ATP as well, on isolated glomeruli. Conversely, the vasodilatory effect of Ap_3A was partially prevented by suramin and markedly reduced by reactive blue 2. It has been previously shown that Ap_3A is a potent agonist of $P2Y_1$ receptors (Pintor *et al*., 1996). We have shown that the potent agonist of $P2Y_1$ receptors, that is, 2-methythio-ATP induces the relaxation of glomeruli (Jankowski M *et al*., 2001a). Moreover, mRNA for $P2Y_1$ receptors has been detected in isolated glomeruli (Bailey *et al*., 2004). Based on the above evidence we used MRS 2179: selective antagonist of $P2Y_1$ receptor (Baurand *et al*., 2001). In our experiments, MRS 2179 abolished the vasodilatory effect of Ap_3A. This finding strongly suggests that the Ap_3A induces relaxation *via* $P2Y_1$ receptors, which are probably located on endothelial cells. Similar effects were observed in rat mesenteric arterial bed where the effects of Ap_3A were blocked by endothelium removal (Busse *et al*., 1988).

Our experiments show that the effects of Ap_3A, Ap_4A and Ap_5A on glomerular volume are mediated *via* P2 receptors. However, it should be noted that Ap_nA are hydrolysed to mononucleotides, which may act on glomerular cells *via* P2 receptors. To our knowledge, it is not feasible to differentiate between effects evoked by Ap_nA *per se* and ATP and ADP in this experimental model. Hydrolysis of Ap_nA can be affected by agents that are antagonists of P2 receptors, for example, suramin, PPADS, Cibacron Blue. However, application of P2 inhibitors, for example, suramin will potentially obscure pharmacological experiments in which the involvement of P2 receptors in Ap_nA action on isolated glomeruli are investigated – because Ap_nA hydrolysis and P2 receptors are simultaneously blocked (Vollmayer *et al*., 2003).

Taken together, we have shown that Ap_3A, Ap_4A and Ap_5A *per se* or *via* their degradation products, that is, ATP or ADP affect glomerular volume in a concentration of nmol range. Ap_3A *via* $P2Y_1$ receptors increase the glomerular volume but Ap_4A and Ap_5A *via* P2X and P2Y receptors decrease glomerular volume. These actions may subsequently lead to modification of GFR. Accordingly, we have previously shown that intravenous infusion of Ap_4A reduces GFR (Szczepanska-Konkel *et al*., 2003). The concentrations of Ap_3A–Ap_6A in the human plasma are in μmol range under physiological conditions; however, a considerable portion of plasma Ap_nA is protein bound (Jankowski J *et al*., 2003). Thus, only a small portion of Ap_nA can affect their effectors, for example, microvasculature of glomeruli. It is believed that local concentrations of Ap_nA, that is, close to the place of its release, are important determinants of their effects. It can be assumed that local concentration of Ap_nA reaches 10 μM after Ap_nA release from platelets during their aggregation. The local concentration of Ap_nA may be higher in patients with essential hypertension because of elevated concentration of Ap_5A and Ap_6A in their platelets (Hollah *et al*., 2001). The elevated amounts of Ap_nA can be found also in platelets of haemodialysis patients and subsequently higher local concentration of Ap_nA can be reached after formation of a platelet thrombus. Therefore, Ap_nA are taken into consideration as an additional atherogenic factor in haemodialysis patients because of growth-stimulating effects on vascular smooth muscle cells (Jankowski J *et al*., 2001). Similarly, diadenosine polyphosphates are mesangial cell growth factors (Heidenreich *et al*., 1995) and may take part in pathogenesis of glomerulonephritis where platelet aggregation is one of the essential steps in the development of glomerular damage (Johnson *et al*., 1990; Harada *et al*., 2000; Rost *et al*., 2002).

The local concentration of Ap_4A in plasma is increased also during ischaemia. Ap_4A are released from myocardial granules into the blood stream of coronary vessels during ischaemia of the heart and play a role in cardioprotective function (Jovanovic *et al*., 1996; Luo *et al*., 2004). Ap_nAs may be released together with norepinephrine after sympathetic stimulation and hence may modulate the neural regulation of glomerular and tubular function (DiBona & Kopp 1997; Burnstock, 2004). This may be pathologically pronounced in patients with increased activity of sympathetic system, for example, essential hypertension (Hollenberg *et al*., 1975; Schlaich *et al*., 2004) or nephrotic syndrome (Herman *et al*., 1989).

In summary, the results of this study show that extracellular nucleotides, that is, Ap_3A, Ap_4A and Ap_5A similar to vasoactive mononucleotides change glomerular volume. Ap_4A and Ap_5A *via* P2X and P2Y receptors may decrease and Ap_3A *via*, at least in part, $P2Y_1$ receptors may increase filtration surface, which in turn may modify GFR. It may be speculated that the physiological role of Ap_nAs may be the regulation of GFR *per se* or that they play the role of a long-term source of mononucleotides affecting glomerular function. Further studies are needed to determine the participation of particular cells of glomerulus in these actions. The present observations give an opportunity to develop new pharmacological tools that could be used to modulate renal function in different pathophysiological situations.

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References

ANDERSON, J.M., GIMBRONE, M.A. & ALEXANDER, R.W. (1981). Angiotensin II stimulates phosphorylation of the myosin light chain in cultured vascular smooth muscle cells. *J. Biol. Chem.,* **256,** 4693–4696.

BAILEY, M.A., TURNER, C.M., HUS-CITHAREL, A., MARCHETTI, J., IMBERT-TEBOUL, M., MILNER, P., BURNSTOCK, G. & UNWIN, R.J. (2004). P2Y receptors present in the native and isolated rat glomerulus. *Nephron Physiol.,* **96,** 79–90.

BAURAND, A., RABOISSON, P., FREUND, M., LEON, C., CAZENAVE, J.P., BOURGUIGNON, J.J. & GACHET, C. (2001). Inhibition of platelet function by administration of MRS 2179, a P2Y1 receptor antagonist. *Eur. J. Pharmacol.,* **412,** 213–221.

BURNSTOCK, G. (2004). Cotransmission. *Curr. Opin. Pharmacol.,* **4,** 47–52.

BUSSE, R., OGILVIE, A. & POHL, U. (1988). Vasomotor activity of diadenosine triphosphate and diadenosine tetraphosphate in isolated arteries. *Am. J. Physiol.,* **254,** H828–H832.

DIAZ-HERNANDEZ, M., PINTOR, J., CASTRO, E. & MIRAS-PORTUGAL, M.T. (2001). Independent receptors for diadenosine pentaphosphate and ATP in rat midbrain single synaptic terminals. *Eur. J. Neurosci.,* **14,** 918–926.

DIBONA, G.F. & KOPP, U.C. (1997). Neural control of renal function. *Physiol. Rev.,* **77,** 75–197.

FOIDART, J., SRAER, J., DELARUE, F., MAHIEU, P. & ARDAILLOU, R. (1980). Evidence for mesangial glomerular receptors for angiotensin II linked to mesangial cell contractility. *FEBS Lett.,* **121,** 333–339.

FUJIWARA, Y., KIKKAWA, R., KITAMUTA, E., TAKAMA, T. & SHIGETA, Y. (1984). Angiotensin II effects upon glomerular intracapillary volume in the rat. *Renal Physiol.,* **7,** 344–348.

FUJIWARA, Y., KITAMUTA, E., UEDA, N., FUKUNAGA, M., ORITA, Y. & KAMADA, J. (1989). Mechanism of action of angiotensin II on isolated rat glomeruli. *Kidney Int.,* **36,** 985–991.

GABRIELS, G., ENDLICH, K., RAHN, K.H., SCHLATTER, E. & STEINHAUSEN, M. (2000). *In vivo* effects of diadenosine polyphosphates on rat renal microcirculation. *Kidney Int.,* **57,** 2476–2484.

HARADA, H., CHAN, C.M., LOESCH, A., UNWIN, R. & BURNSTOCK, G. (2000). Induction of proliferation and apoptotic cell death via P2Y and P2X receptors, respectively, in rat glomerular mesangial cells. *Kidney Int.,* **57,** 949–958.

HEIDENREICH, S., TEPEL, M., SCHLUTER, H., HARRACH, B. & ZIDEK, W. (1995). Regulation of rat mesangial cell growth diadenosine phosphates. *J. Clin. Invest.,* **95,** 2862–2867.

HERMAN, P.J., SAWIN, L.L. & DIBONA, G.F. (1989). Role of renal nerves in renal sodium retention of nephritic syndrome. *Am. J. Physiol.,* **256,** F823–F829.

HOHAGE, H., REINHARDT, C., BORUCKI, U., ENCK, G., SCHLUTER, H., SCHLATTER, E. & ZIDEK, W. (1996). Effects of diadenosine polyphosphates on renal function and blood pressure in anesthetised Wistar rats. *J. Am. Soc. Nephrol.,* **7,** 1216–1222.

HOLLAH, P., HAUSBERG, M., KOSCH, M., BARENBROCK, M., LETZEL, M., SCHLATTER, E. & RAHN, K.H. (2001). A novel assay for determination of diadenosine polyphosphates in human platelets: studies in normotensive subjects and in patients with essential hypertension. *J. Hypertens.,* **19,** 237–245.

HOLLENBERG, N.K., ADAMS, D.F., SOLOMON, H., CHENITZ, W.R., BURGER, B.M., ABRAMS, H.L. & MERILL, J.P. (1975). Renal vascular tone in essential and secondary hypertension. *Medicine,* **54,** 29–44.

HOYLE, C.H., ZIGANSHIN, A.U., PINTOR, J. & BURNSTOCK, G. (1996). The activation of P1- and P2-purinoceptors in the guinea-pig left atrium by diadenosine polyphosphates. *Br. J. Pharmacol.,* **118,** 1294–1300.

ILLES, P. & RIBEIRO, J.A. (2004). Molecular physiology of P2 receptors in the central nervous system. *Eur. J. Pharmacol.,* **483,** 5–17.

INSCHO, E.W. (2001). P2 receptors in regulation of renal microvascular function. *Am. J. Physiol.,* **280,** F927–F944.

JANKOWSKI, J., HAGEMANN, J., YOON, M.S., VAN DER GIET, M., STEPHAN, N., ZIDEK, W., SCHLUTER, H. & TEPEL, M. (2001). Increased vascular growth in hemodialysis patients induced by platelet-derived diadenosine polyphosphates. *Kidney Int.,* **59,** 1134–1141.

JANKOWSKI, J., JANKOWSKI, V., LAUFER, U., VAN DER GIET, M., HENNING, L., TEPEL, M., ZIDEK, W. & SCHLUTER, H. (2003). Identification and quantification of diadenosine polyphosphate concentrations in human plasma. *Arterioscler. Thromb. Vasc. Biol.,* **23,** 1231–1238.

JANKOWSKI, M., SZCZEPAŃSKA-KONKEL, M., KALINOWSKI, L. & ANGIELSKI, S. (2000). Bidirectional action of extracellular ATP on intracapillary volume of isolated rat renal glomeruli. *J. Physiol. Pharmacol.,* **51,** 491–496.

JANKOWSKI, M., SZCZEPAŃSKA-KONKEL, M., KALINOWSKI, L. & ANGIELSKI, S. (2001a). Cyclic GMP-dependent relaxation of isolated rat renal glomeruli induced by extracellular ATP. *J. Physiol.,* **530.1,** 123–130.

JANKOWSKI, M., SZCZEPAŃSKA-KONKEL, M., KALINOWSKI, L. & ANGIELSKI, S. (2001b). The role of P2Y-receptors in the regulation of glomerular volume. *Med. Sci. Monit.,* **7,** 635–640.

JANKOWSKI, M., SZCZEPAŃSKA-KONKEL, M., KALINOWSKI, L. & ANGIELSKI, S. (2003). Involvement of Rho-kinase in P2Y-receptor-mediated contraction of renal glomeruli. *Biochem. Biophys. Res. Commun.,* **302,** 855–859.

JIMENEZ, A.I., CASTRO, E., DELICADO, E.G. & MIRAS-PORTUGAL, M.T. (2002). Specific diadenosine pentaphosphate receptor coupled to extracellular regulated kinases in cerebellar astrocytes. *J. Neurochem.,* **83,** 299–308.

JOHNSON, R.J., GARCIA, R.L., PRITZL, P. & ALPERS, C.E. (1990). Platelets mediate glomerular cell proliferation in immune complex nephritis induced by anti-mesangial cell antibodies in the rat. *Am. J. Physiol.,* **136,** 369–374.

JOVANOVIC, A., ZHANG, S., ALEKSEEV, A.E. & TERZIC, A. (1996). Diadenosine polyphosphate-induced inhibition of cardiac KATP channels: operative state-dependent regulation by a nucleoside diphosphate. *Pflugers Arch.,* **431,** 800–802.

KATHER, H., WIELAND, E. & WAAS, W. (1987). Chemiluminescent determination of adenosine, inosine and hypoxantine/xantine. *Anal. Biochem.,* **163,** 45–51.

KHATTAB, M., HOHAGE, H., HOLLACH, P.E., RAHN, K.H. & SCHLATTER, E. (1998). Effects of diadenosine polyphosphates on systemic and regional hemodynamics in anesthtized rats. *Kidney Blood Pressure Res.,* **21,** 42–49.

KIKKAWA, R., KITAMURA, E., FUJIWARA, Y., ARIMURA, T., HANEDA, M. & SHIGETA, Y. (1986). Impaired contractile responsiveness of diabetic glomeruli to angiotensin II: A possible indication of mesangial dysfunction in diabetes mellitus. *Biochem. Biophys. Res. Commun.,* **136,** 1185–1190.

KIMMICH, G.A., RANDLES, J. & BRAND, J.S. (1975). Assay of picomole amounts of ATP, ADP, and AMP using the luciferase enzyme. *Anal. Biochem.,* **69,** 187–206.

KLETA, R., HIRSH, J., HEIDENREICH, S., SCHLUTER, H., ZIDEK, W. & SCHLATTER, E. (1995). Effects of diadenosine polyphosphates, ATP and angiotensin II on membrane voltage and membrane conductances of rat mesangial cells. *Pflugers Arch.,* **430,** 713–720.

LEMLEY, K.V., ELGER, M., KOEPPEN-HAGEMANN, I., KRETZLER, M., NAGATA, N., SAKAI, T., UIKER, S. & KRIZ, W. (1992). The glomerular mesangium: capillary support function and its failure under experimental conditions. *Clin. Invest.,* **70,** 843–856.

LEWKO, B., WENDT, U., SZCZEPAŃSKA-KONKEL, M., STEPINSKI, J., DREWNOWSKA, K. & ANGIELSKI, S. (1997). Inhibition of endogenous nitric oxide synthesis activates particulate guanylyl cyclase in the rat renal glomeruli. *Kidney Int.,* **52,** 654–659.

LUO, J., JANKOWSKI, V., GUNGAR, N., NEUMANN, J., SCHMITZ, W., ZIDEK, W., SCHLUTER, H. & JANKOWSKI, J. (2004). Endogenous diadenosine tetraphosphate, diadenosine pentaphosphate, and diadenosine hexaphosphate in human myocardial tissue. *Hypertension,* **43,** 1055–1059.

LUTHJE, J. & OGILVIE, A. (1988). Catabolism of Ap_4A and Ap_3A in whole blood. The dinucleotides are long-lived signal molecules in the blood ending up as intracellular ATP in the erythrocytes. *Eur. J. Biochem.,* **173,** 241–245.

MENE, P., SIMONSON, M.S. & DUNN, M.J. (1989). Physiology of the mesangial cell. *Physiol. Rev.,* **69,** 1347–1424.

MIRAS-PORTUGAL, M.T., GUALIX, J. & PINTOR, J. (1998). The neurotransmitter role of diadenosine polyphosphates. *FEBS Lett.,* **430,** 78–82.

MISRA, R.P. (1972). Isolation of glomeruli from mammalian kidneys by graded sieving. *Am. J. Clin. Pathol.,* **62,** 135–139.

OLIVERA, A., LAMAS, S., RODRIGUEZ-PUYOL, D. & LOPEZ-NOVOA, J. (1989). Adenosine induces mesangial cell contraction by an A_1-type receptor. *Kidney Int.,* **38,** 1300–1305.

PINTOR, J., KING, B.F., MIRAS-PORTUGAL, M.T. & BURNSTOCK, G. (1996). Selectivity and activity of adenine dinucleotides at recombinant $P2X_2$ and $P2Y_1$ purinoceptors. *Br. J. Pharmacol.,* **119,** 1006–1012.

PINTOR, J. & MIRAS-PORTUGAL, M.T. (1995). A novel receptor for diadenosine polyphosphates coupled to calcium increase in rat midbrain synaptosomes. *Br. J. Pharmacol.,* **115,** 895–902.

PINTOR, J., PUCHE, J.A., GUALIX, J., HOYLE, C.H. & MIRAS-PORTUGAL, T. (1997). Diadenosine polyphosphates evoke Ca2+ transients in guinea-pig brain via receptors distinct from those from ATP. *J. Physiol.,* **504.2,** 327–335.

RALEVIC, V. & BURNSTOCK, G. (1991). Effects of purines and pyrimidines on rat mesenteric arterial bed. *Circ. Res.,* **69,** 1583–1590.

RALEVIC, V. & BURNSTOCK, G. (1998). Receptors for purines and pyrimidines. *Pharmacol. Rev.,* **50,** 413–492.

RALEVIC, V., HOYLE, CH. & BURNSTOCK, G. (1995). Pivotal role of phosphate chain length in vasoconstrictor *versus* vasodilatator actions of adenine dinucleotides in rat mesenteric arteries. *J. Physiol.,* **483,** 703–713.

RALEVIC, V., JANKOWSKI, J. & SCHLUTER, H. (2001). Structure–activity relationship of diadenosine polyphosphates (Ap(n)As), adenosine polyphospho guanosines (Ap(n)Gs) and guanosine polyphospho guanosines (Gp(n)Gs) at P2 receptors in the rat mesenteric arterial bed. *Br. J. Pharmacol.,* **134,** 1073–1083.

ROST, S., DANIEL, C.H., SCHULZE-LOHOFF, E., BAUMERT, H.G., LAMBRECHT, G. & HUGO, C.H. (2002). P2 receptor antagonist PPADS inhibits mesangial cell proliferation in experimental mesangial proliferative glomerulonephritis. *Kidney Int.,* **62,** 1659–1671.

SAVIN, V.J. (1986). *In vitro* effects of angiotensin II on glomerular function. *Am. J. Physiol.,* **251,** F627–F634.

SCHLAICH, M.P., LAMBERT, E., KAYE, D.M., KROZOWSKI, Z., CAMPBELL, D.J., LAMBERT, G., HASTINGS, J., AGGARWAL, A. & ESLER, M.D. (2004). Sympathetic augmentation in hypertension. Role of nerve firing, norepinephrine reuptake, and angiotensin neuromodulation. *Hypertension,* **43,** 169–175.

SCHLATTER, E., ANKORINA, I., HAXELMANS, S. & KLETA, R. (1995). Effects of diadenosine polyphosphates, ATP and angiotensin II on cytosolic Ca^{2+} activity and contraction of rat mesangial cells. *Pflugers. Arch.,* **430,** 721–728.

SCHULTE, E.A., HOHENDAHL, A., STEGEMANN, H., HIRSCH, J.R., SALEH, H. & SCHLATTER, E. (1999). Natriuretic peptides and diadenosine polyphosphates modulate pH regulation of rat mesangial cells. *Cell Physiol. Biochem.,* **9,** 310–322.

SCHLUTER, H., OFFERS, E., BRUGGEMANN, G., VAN DER GIET, M., TEPEL, M., NORDHOFF, E., KARAS, M., SPIEKER, C., WITZEL, H. & ZIDEK, W. (1994). Diadenosine phosphates and the physiological control of blood pressure. *Nature,* **367,** 186–188.

STEINMETZ, M., BIERER, S., HOLLAH, P., RAHN, K.H. & SCHLATTER, E. (2000). Heterogeneous vascular effects of AP5A in different rat resistance arteries are due to heterogeneous distribution of P2X and P2Y1 purinoceptors. *J. Pharmacol. Exp. Ther.,* **294,** 1182–1187.

STEINMETZ, M., GABRIELS, G., LE, T.V., PIECHOTA, H.J., RAHN, K.H. & SCHLATTER, E. (2003). Vasoactivity of diadenosine polyphosphates in human small renal resistance arteries. *Nephrol. Dial. Transplant.,* **18,** 2496–2504.

STEINMETZ, M., JANSSEN, A.K., PELSTER, F., RAHN, K.H. & SCHLATTER, E. (2002). Vasoactivity of diadenosine polyphosphates in human small mesenteric resistance arteries. *J. Pharmacol. Exp. Ther.,* **302,** 787–794.

STOCKAND, J.D. & SANSOM, S.C. (1998). Glomerular mesangial cells: electrophysiology and regulation of contraction. *Physiol. Rev.,* **78,** 723–744.

SUMIYOSHI, R., NISHIMURA, J., KAWASAKI, J., KOBAYASHI, S., TAKAHASHI, S. & KANAIDE, H. (1997). Diadenosine polyphosphates directly relax porcaine coronary arterial smooth muscle. *J. Pharmacol. Exp. Ther.,* **283,** 548–556.

SZCZEPAŃSKA-KONKEL, M., LANGNER, G., BEDNARCZUK, G., STIEPANOW-TRZECIAK, A., JANKOWSKI, M. & ANGIELSKI, S. (2003). Renal haemodynamics and natriuretic responses to intravenous administration of diadenosine tetraphosphate (Ap_4A) and nicotinamide adenine dinucleotides (NAD) in rat. *J. Physiol. Pharm.,* **54,** 163–173.

SZCZEPAŃSKA-KONKEL, M., REDLAK, M. & ANGIELSKI, S. (1991). Glibenclamide-sensitive K^+ channels are responsible for angiotensin II hypersensitive contraction and atrial natriuretic factor refractoriness of glomeruli in low-sodium rats. *Biochem. Biophys. Res. Commun.,* **181,** 871–876.

TEPEL, M., HEIDENREICH, S., SCHLUTER, H., BEINLICH, A., NOFER, J.R., WALTER, M., ASSMANN, G. & ZIDEK, W. (1996). Diadenosine polyphosphates induce transplasma membrane calcium influx in cultured glomerular mesangial cells. *Eur. J. Clin. Invest.,* **26,** 1077–1084.

VAHLENSIECK, U., BOKNIK, P., KNAPP, J., MULLER, F.U., NEUMANN, J., HERZIG, S., SCHLUTER, H., ZIDEK, W., DENG, M.C., SCHELD, H.H. & SCHMITZ, W. (1996). Negative chronotropic and inotropic effects of exerted by diadenosine hexaphosphate (AP6A) via A1-adenosine receptors. *Br. J. Pharmacol.,* **119,** 835–844.

VAN DER GIET, M., CINKILIC, O., JANKOWSKI, J., TEPEL, M., ZIDEK, W. & SCHLUTER, H. (1999). Evidence for two different P2X-receptors mediating vasoconstriction of Ap5A and Ap6A in the isolated perfused rat kidney. *Br. J. Pharmacol.,* **127,** 1463–1469.

VAN DER GIET, M., KHATTAB, M., BORGEL, J., SCHLUTER, H. & ZIDEK, W. (1997). Differential effects of diadenosine phosphate on purinoceptors in the rat isolated perfused kidney. *Br. J. Pharmacol.,* **120,** 1453–1460.

VAN GINNEKEN, E.E., RONGEN, G.A., RUSSEL, F.G. & SMITS, P. (2002). Diadenosine pentaphosphate vasodilates the forearm vascular bed: inhibition by theophylline and augmentation by dipyridamole. *Clin. Pharmacol. Ther.,* **71,** 448–456.

VERSPOHL, E.J., JOHANNWILLE, B., KAISERLING-BUDDEMEIER, I., SCHLUTER, H. & HAGEMANN, J. (1999). Diadenosine polyphosphates in cultured vascular smooth-muscle cells and endothelium cells – their interaction with specific receptors and their degradation. *J. Pharm. Pharmacol.,* **51,** 1175–1781.

VITZTHUM, H., WEISS, B., BACHLEITNER, W., KRÄMER, B.K. & KURTZ, A. (2004). Gene expression of adenosine receptors along the nephron. *Kidney Int.,* **65,** 1180–1190.

VOLLMAYER, P., CLAIR, T., GODING, J.W., SANO, K., SERVOS, J. & ZIMMERMANN, H. (2003). Hydrolysis of diadenosine polyphosphates by nucleotide pyrophosphatases/phosphodiesterases. *Eur. J. Biochem.,* **270,** 2971–2978.

WILDMAN, S.S., BROWN, S.G., KING, B.F. & BURNSTOCK, G. (1999). Selectivity of diadenosine polyphosphates for rat P2X receptor subunits. *Eur. J. Pharmacol.,* **367,** 119–123.

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