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Differential effects of diadenosine phosphates on purinoceptors in the rat isolated perfused kidney

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1 The activation of various purinoceptors in rat renal vasculature by P^1,P^2-diadenosine pyrophosphate (Ap_2A), P^1,P^3-diadenosine triphosphate (Ap_3A), P^1,P^4-diadenosine tetraphosphate (Ap_4A), P^1,P^5-diadenosine pentaphosphate (Ap_5A), P^1,P^6-diadenosine hexaphosphate (Ap_6A) was studied by measuring their effects of perfusion pressure of a rat isolated perfused kidney.

2 The vasoconstrictive response to Ap_5A was completely due to P_{2X} purinoceptor activation, that to Ap_4A and Ap_6 was P_{2X} purinoceptor mediated to a large extent, as evidenced by the inhibitory effects of suramin and pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid tetrasodium (PPADS).

3 The vasoconstrictive effects of Ap_2A and Ap_3A were mostly due to stimulation of A_1-receptors, as shown by the inhibitory effect of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX).

4 The vasoconstrictive response to Ap_6A was partially insensitive to A_1 and P_{2X} purinoceptor blockers.

5 In raised tone preparations Ap_2A and Ap_3A evoked vasodilatation, which was blocked by the A_2 receptor blocker, 3,7-dimethyl-1-propargylxanthine (DMPX).

6 In raised tone preparations Ap_4A evoked vasodilatation when the P_2-purinoceptors were blocked by suramin.

7 The activation of different purinoceptor subtypes by diadenosine phosphates critically depends on the number of phosphate groups.

Keywords: Purinoceptors; diadenosine phosphates; P_2-receptors; P_1-receptors; rat isolated perfused kidney; vasoconstriction; vasodilatation

Introduction

In recent years, diadenosine phosphates have received considerable attention because there is evidence indicating that these agents play an important role as extracellular messengers (Hoyle, 1990; Pohl *et al*., 1991; Pintor *et al*., 1993; Schlüter *et al*., 1994; Heidenreich *et al*., 1995). Several diadenosine phosphates have been isolated from human tissue, including diadenosine tri-, tetra-, penta- and hexaphosphate (Lüthje & Ogilvie, 1983; Schlüter *et al*., 1994).

Diadenosine phosphates have different actions on the vasculature depending on the number of phosphate groups. Ap_3A and Ap_4A are potent vasodilators in the mesenteric and coronary vasculature (Pohl *et al*., 1991; Ralevic *et al*., 1995), whereas Ap_5A and Ap_6A appear to show vasoconstrictor properties (Schlüter *et al*., 1994). These different actions lead to the question, which receptors are activated by diadenosine phosphates in the vasculature. There have been several findings of receptor activation by diadenosine phosphates in mesenteric vessels (Ralevic *et al*., 1995) and umbilical vessels (Davies *et al*., 1995). Diadenosine phosphates are proposed to elicit extracellular effects via P_1- and P_2-purinoceptors.

In the present study we examined the vasoconstrictive and vasodilator effects of diadenosine phosphates in renal arterial vessels and identified the receptors involved in the different effects. In this preparation A_1, A_2, P_{2X} and P_{2Y} receptors (Inscho *et al*., 1991; Churchill & Ellis, 1993) are present and have been shown to mediate vasoconstriction and vasodilatation to various purine compounds.

Methods

Preparation of the rat isolated perfused kidney

Adult male Wistar-Kyoto-Rats (four- to six-months-old) were anaesthetized with urethane (1.4 g kg^{-1} body weight, intraperitoneally). The abdominal cavity was opened by a midventral incision. The aorta and the left kidney were carefully isolated from adhesive tissue by blunt dissection. Ligatures were placed around the left renal artery and the infrarenal aorta. A polyethylene catheter was placed in the distal aorta. Immediately after the insertion of the catheter, 500 u of heparin sodium were injected. Then perfusion was started. The catheter was gently advanced into the left renal artery without interruption of flow. The kidney was excised and immediately mounted in the perfusion system.

Perfusion system

The perfusion procedure followed generally the description given by Hofbauer *et al*. (1973). The preparation was perfused at a constant flow rate of 8 ml min^{-1} by a peristaltic pump. The perfusate was Tyrode solution of the following composition (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, $NaHCO_3$ 12, NaH_2PO_2 0.42 and glucose 5.6 gassed with 95% O_2-5% CO_2 and maintained at 37°C. Responses were measured as changes in perfusion pressure (mmHg) with a pressure transducer (Statham Transducer P23Gb, Siemens) on a side arm of the perfusion catheter, connected to a bridge amplifier (Hugo Sachs, Freiburg, FRG), and recorded on a polygraph. Preparations were allowed to equilibrate for 30 min before experimentation.

Basal-tone preparation

Vasoconstrictor responses of preparations to doses of the diadenosine phosphates, α,β-methylene adenosine 5′-triphosphate (ATP), **R**(−) N^6- (2-phenylisopropyl) adenosine and ATP were assessed at basal tone. For each substance dose-response curves were constructed, with 5 min being allowed to elapse between consecutive doses. This procedure allowed dose-response curves for several agonists to be constructed for the same preparation. A significant degree of cross-desensitization or auto-desensitization was not detected. The unspecific

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P_2-purinoceptor antagonist suramin (100 μM), the P_2-purinoreceptor antagonist pyridoxal-phosphate-6-azophenyl-2;4-disulphonic acid (PPADS, 10 μM) and the A_1- receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (10 μM) were added to the perfusate 30 min before challenge with mono- or dinucleotides.

Raised-tone preparation

Vasodilator response of preparations to doses of the diadenosine phosphates, 2-methylthio-ATP, 5′-N-ethylcarboxamidodenosine (NECA) were assessed at raised-tone, a rat perfused kidney in which the tone was increased by continuous perfusion with angiotensin II (200 nM). The resistance of vasodilator responses to desensitization and reproducibility of responses with time allowed dose-response curves for several agonists to be constructed for the same preparation. The P_2-purinoceptor antagonist suramin (100 μM), the A_2-receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX, 10 μM) and N^G-nitro-L-arginine methyl ester (L-NAME, 50 μM) were added to the perfusate 30 min before challenge with adenine mono- or dinucleotides. The antagonists and the nitric oxide (NO)- synthase inhibitor did not significantly attenuate the elevated tone.

Materials

All mono- and diadenosine phosphates, angiotensin II and noradrenaline were applied as 100 μl bolus into a valve proximal to the preparation. Drug dilutions were performed daily from stock solutions of 10 mM (concentrates stored frozen) in bidistilled water or dimethyl sulphoxide (DMSO) unless indicated otherwise. Heparin (sodium salt), suramin (hexasodium salt), α,β-methylene ATP (α,β-MeATP), 2-methylthioATP (2-meSATP, tetrasodium salt), 5′-N-ethylcarbosamidoadenosine (NECA), **R**(−)N^6-(2-phenylisopropyl) adenosine (**R**(−)-PIA), 3,7-dimethyl-1-propargylxanthine (DMPX), pyridoxal-phosphate-6-azophenyl-2 and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) were from Research Biochemicals Inc. P^1, P^2-diadenosine pyrophosphate (Ap_2A), P^1, P^3-diadenosine triphosphate (Ap_3A), P^1, P^4-diadenosine tetraphosphate (Ap_4A), P^1, P^5-diadenosine pentaphosphate (Ap_5A), P^1, P^6-diadenosine hexaphosphate (Ap_6A) and all other drugs were from Sigma. Before use Ap_2A, Ap_3A, Ap_4A, Ap_5A and Ap_6A were purified according to a procedure described by Heidenreich *et al.* (1995).

Statistics

Responses were measured as changes in perfusion pressure (mmHg) and results presented as the means ± s.e.mean. Statistical analysis was performed with the Mann-Whitney test. The *P* values obtained with this test were corrected for multiple comparisons with Bonferroni's correction, where appropriate. All *P* values presented are two-tailed. *P* values <0.05 were considered significant.

Results

Vasoconstrictor responses in basal tone preparation

The baseline perfusion pressure of the rat isolated perfused kidneys decreased by 10–15 mmHg during the first and by 6 mmHg during the second hour of perfusion. Vascular reactivity to vasoactive agents did not diminish during this time. After the equilibration period, the baseline pressure was 62 ± 5 mmHg ($n=67$). Addition of suramin (100 μM) to the perfusate caused an increase of perfusion pressure of 9 ± 3 mmHg, whereas after addition of DPCPX (10 μM) or PPADS (10 μM) to the perfusate baseline pressure did not significantly change.

At basal tone the nucleotides caused dose-dependent vasoconstriction (Figure 1a and b). The dose-response curves were not parallel and the maximal contractions induced varied considerably, which makes calculation of potency ratios difficult, but by estimation of the concentration required to induce

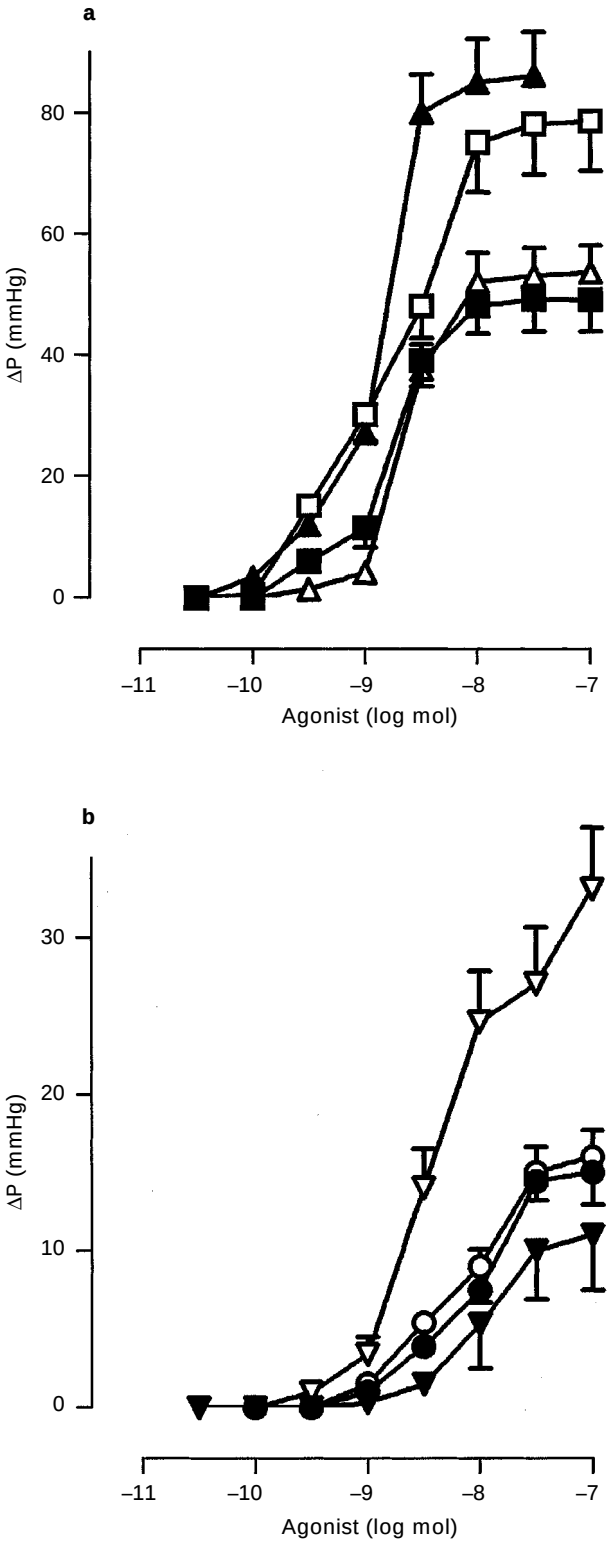

Figure 1 Changes in perfusion pressure in the rat isolated perfused kidney induced by (a) Ap_4A (■), Ap_5A (□), Ap_6A (△), α,β-MeATP (▲), (b) Ap_2A (●), Ap_3A (○), ATP (▽) or **R**(−)-PIA. (▼). Each point is the mean of at least 5 determinations and vertical lines show s.e.mean. Significant differences from baseline perfusion pressure for Ap_4A, Ap_5A, Ap_6A and ATP doses $\geqslant 10^{-9.5}$ mol and for **R**-PIA, Ap_2A and Ap_3A $\geqslant 10^{-9}$ mol (bolus injection). For abbreviations see text.

a contraction equal to that induced by bolus injection of 100 μmol ATP the following order of potency was α,β-MeATP $\geqslant$ Ap_5A $\geqslant$ Ap_6A $\geqslant$ Ap_4A $\geqslant$ ATP $\geqslant$ Ap_3A = Ap_2A $\geqslant$ **R**(−)-PIA (Figure 1a and b). In Table 1 ED_{50} and maximal responses to 100 μmol doses are shown.

In the presence of suramin (100 μM) responses to Ap_5A (Figure 2a) and α,β-MeATP (Figure 2b) up to 100 μmol were almost completely abolished (each $P<0.05$ vs control). The vasoconstriction induced by Ap_4A (Figure 2b) and Ap_6A (Figure 2c) were partially inhibited by 100 μM suramin (each $P<0.05$ vs control). The dose-response curve was shifted to the right ($-\log ED_{50}$: Ap_4A in Tyrode 8.75±0.01; Ap_4A+-suramin 8.0±0.06; Ap_6A in Tyrode 8.63±0.01 and Ap_6A+-suramin 7.84±0.06). Dose-ratios for Ap_4A and Ap_6A could not be calculated or estimated. Maximal responses elicited by Ap_4A and Ap_6A under inhibition with suramin (100 μM) were reduced to 30±3.3% for Ap_4A and 39±4.9% for Ap_6A compared to the control responses. Ap_3A (Figure 2d), Ap_2A (Figure 2d) and **R**(−)-PIA (results not shown) were not significantly inhibited by suramin (100 μM) whereas ATP (results not shown) was completely inhibited.

Following incubation with PPADS (10 μM) (Figure 3) responses to α,β-MeATP and Ap_5A were completely abolished (each $P<0.05$ vs control). Responses to Ap_4A, Ap_6A and ATP were markedly attenuated to 35±4.7% (Ap_4A), 16.5±2.5% (Ap_6A) and 25.0±4.0% (ATP) compared to initial response without blockade by PPADS (each $P<0.05$ vs control). Reactions to Ap_3A, Ap_2A and **R**(−)-PIA were not significantly affected.

In the presence of DPCPX (10 μM) the response to **R**(−)-PIA (Figure 4a) was completely abolished ($P<0.05$ vs control). Contractions induced by Ap_2A (Figure 4b) and Ap_3A (Figure 4c) were inhibited by 10 μM DPCPX (each $P<0.05$ vs control), which caused a shift to the right in the dose-response curve, but because of the lack of a clearly defined maximal response under inhibition with DPCPX dose-ratios could not be calculated accurately. The potency of the compounds was compared by determining the dose that would cause an increase in perfusion pressure of 5 mmHg (pD_5). The pD_5 ($-\log$ mol) for Ap_3A was 8.48±0.12 without inhibition and 7.1±0.11 with inhibition by DPCPX. The pD_5 ($-\log$ mol) for Ap_2A was 8.34±0.12 without and 6.9±0.10 with inhibition by DPCPX. Estimated dose-ratios of Ap_2A and Ap_3A are about 27.5 and 24. Ap_4A (Figure 4d) is inhibited by DPCPX (10 μM), which caused a shift to the right in the dose-response curve.

Table 1 Vasoconstrictor ED_{50} values and maximal responses to 100 μmol doses of adenine- and dinucleotides in basal-tone preparations

Compound	*ED_{50}* ($-\log$ mol)	*Maximal response to 100 μmol of agonist* (mmHg)
α,β-meATP	8.9±0.05	87.0±7.3
Ap_5A	8.8±0.10	78.5±8.2
Ap_6A	8.6±0.01	53.5±4.5
Ap_4A	8.7±0.04	49.0±5.0
ATP	Not calculated	33.0±3.9
Ap_3A	8.1±0.10	16.0±1.7
Ap_2A	8.0±0.10	15.0±2.1
R(−)-PIA	7.9±0.02	11.0±3.5

Values are given as means±s.e.mean ($n=5$).

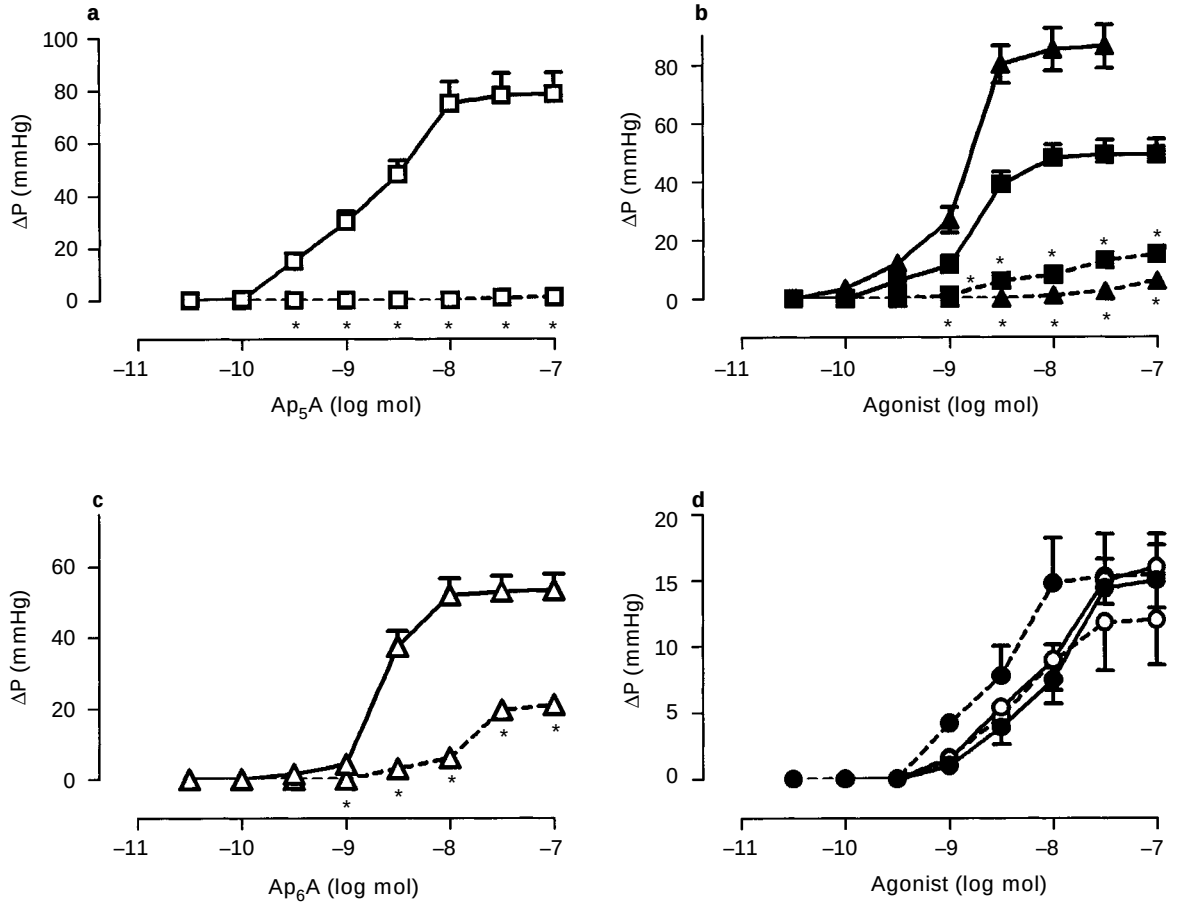

Figure 2 Changes in perfusion pressure in the rat isolated perfused kidney induced by (a) Ap_5A (□), (b) Ap_4A (■), α,β-MeATP (▲), (c) Ap_6A (△), and (d) Ap_2A (●), Ap_3A (○) in the absence (solid line) and presence (dotted line) of suramin (100 μM) in the perfusate. Each point is the mean of at least five determinations and the vertical lines show the s.e.mean. *$P<0.05$ suramin vs control. For abbreviations see text.

The ED_{50} (−log mol) was 8.78±0.04 without and 7.84±0.06 with inhibition by DPCPX – the calculated dose-ratio was 8.7. The dose-response curves of Ap_5A, Ap_6A (Figure 4a), ATP (results not shown) and α,β-MeATP (results not shown) were not significantly affected by inhibitory effects of DPCPX (10 μM).

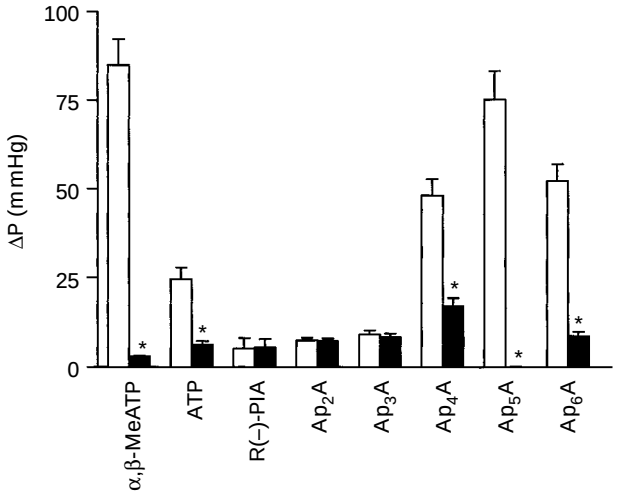

Figure 3 Changes in perfusion pressure in the rat isolated perfused kidney induced by bolus injection of 10 μmol of each agonist in the absence (open columns) and presence (solid columns) of PPADS (30 μM) in the perfusate. Each column is the mean of at least five determinations and the vertical lines show the s.e.mean. *$P<0.05$ PPADS vs control. For abbreviations see text.

Figure 5 shows a typical trace of the blockade of Ap_4A by two different receptor antagonists. Table 2 gives the means ±s.e.mean of all agonist responses. Permanent perfusion with suramin (100 μM) completely inhibited the vasoconstriction caused by α,β-MeATP (10 μmol). The effect of **R**(−)-PIA was not significantly affected by permanent perfusion with suramin. The vasoconstriction of Ap_4A was significantly attenuated in the presence of the P_{2X}-receptor antagonist suramin to about 36% with a 10 μmol and 40% with a 100 μmol bolus injection compared to the initial response with no receptor antagonist in the perfusate. Additional perfusion with DPCPX significantly blocked the remaining responses of Ap_4A and **R**(−)-PIA. During the whole experiment the vasoactive responses to the noradrenaline doses given (100 nmol) were not significantly affected.

Vasodilator responses in raised-tone preparations

When the tone of the rat isolated perfused kidney was raised by including angiotensin II (200 nM) in the perfusate, the perfusion pressure increased by 46.2±4.2 mmHg ($n=19$). Addition of suramin (100 μM final concentration) to the perfusate caused a decrease of perfusion pressure of 8±3 mmHg, whereas the addition of DMPX (10 μM) and L-NAME (50 μM) to the perfusate elicited no significant change.

At raised tone some nucleotides caused dose-dependent vasodilatation. The dose-response curves were not parallel, and the maximal dilatation induced varied considerably (Figure 6a and b), so it was not possible to calculate ED_{50} values. Therefore potency of compounds was compared by determining the dose that would cause a decrease in perfusion pressure of 7 mmHg (pD_{-7}, Table 3). The order of potency was 2-meSATP ≥ Ap_3A ≥ Ap_2A ≥ NECA (Figure 6a and b,

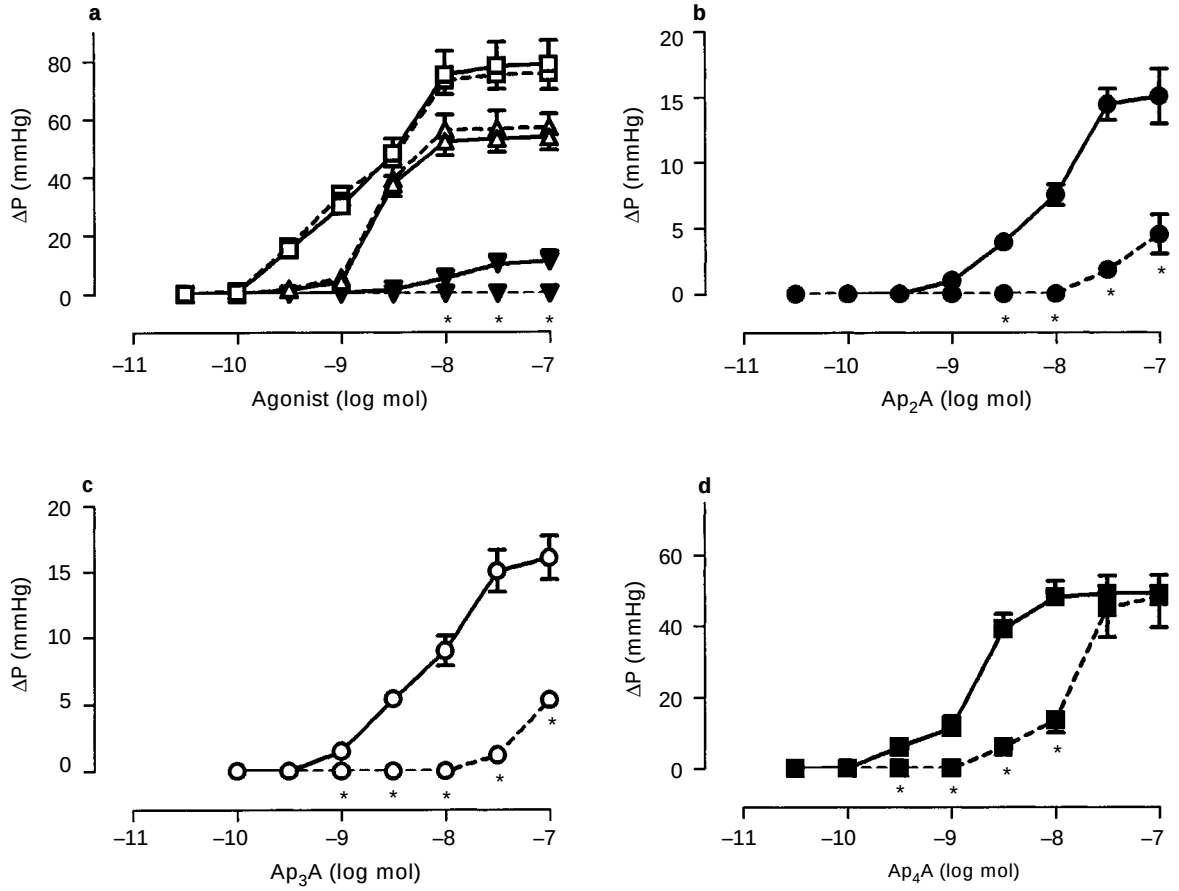

Figure 4 Changes in perfusion pressure in the rat isolated perfused kidney induced by (a) Ap_5A (□), Ap_6A (△) and **R**(−)-PIA (▼), (b) Ap_2A (●), (c) Ap_3A (○), (d) Ap_4A (■) in the absence (solid line) and presence (dotted line) of DPCPX (10 μM) in the perfusate. Each point is the mean of at least five determinations and the vertical lines show the s.e.mean. *$P<0.05$ DPCPX vs control. For abbreviations see text.

Table 3). The remaining compounds, α,β-MeATP (results not shown), Ap_4A, Ap_5A, Ap_6A (Figure 6a) and **R**(−)-PIA (results not shown), did not evoke dilatation. In some preparations, both Ap_2A and Ap_3A (Figure 6b), caused biphasic responses with a phase of constriction preceeding the vasodilatation (data not shown).

In the presence of suramin (100 μM) vasoconstrictor responses to Ap_5A and Ap_6A (Figure 7a) were completely abolished. The vasodilator response to 2-meSATP (Figure 7a) was almost completely abolished (all $P<0.05$ vs control). The vasodilator responses to Ap_2A (Figure 7b), Ap_3A (Figure 7c) and NECA (Figure 7c) were not significantly affected. Under constant perfusion with suramin (100 μM) the vasoconstriction of Ap_4A (Figure 7d) was completely blocked ($P<0.05$ vs control) and a vasodilator dose-response curve occurred. Inhibition of the P_{2X}-purinoceptor by suramin decreased the maximal response from 31.0 ± 0.8 mmHg to -18.0 ± 3.8 mmHg.

In the presence of DMPX (10 μM) the vasodilator response to Ap_2A (Figure 8a) and NECA (Figure 8b) were completely abolished (both $P<0.05$ vs control), resulting in a vasoconstrictor response. The vasodilator response of Ap_3A (Figure 8c) was almost completely and significantly blocked ($P<0.05$ vs control). Maximal vasodilatation induced by bolus injection of 100 μmol Ap_3A changed from -16.5 ± 3.3 mmHg to -2.0 ± 0.2 mmHg in the presence of DMPX. The vasodilator response to 2-meSATP (Figure 8b) was not affected by permanent perfusion with DMPX (10 μM).

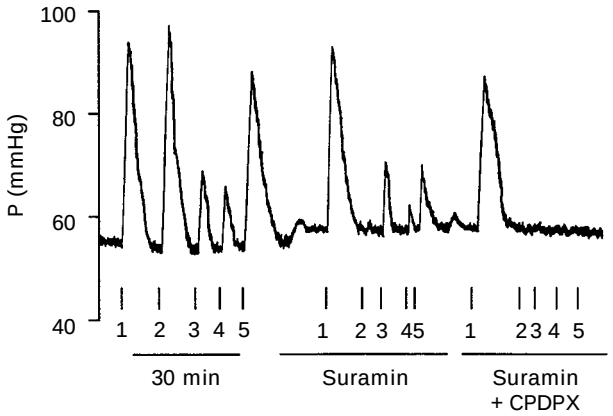

Figure 5 Representative trace out of 6 similar experiments showing changes in perfusion pressure in the rat isolated perfused kidney induced by bolus injections of Ap_4A in the absence of any A_1- or P_{2X}-receptor antagonist, in the presence of suramin (100 μM), and in the presence of suramin (100 μM) and CPDPX (10 μM) in the perfusate. (1) Noradrenaline 100 nmol, (2) α,β-MeATP 10 μmol, (3) **R**(−)-PIA 10 μmol, (4) Ap_4A 10 μmol and (5) Ap_4A 100 μmol ($n=6$). For abbreviations see text.

Figure 9 shows a typical trace showing the effect of NO-synthase inhibition by L-NAME (50 μM). Table 4 gives the means ± s.e.mean of all agonist responses. Permanent perfusion with L-NAME (50 mM) showed complete inhibition of the vasodilatation induced by bolus injection of acetylcholine (10 pmol), which is typically mediated by NO release. The

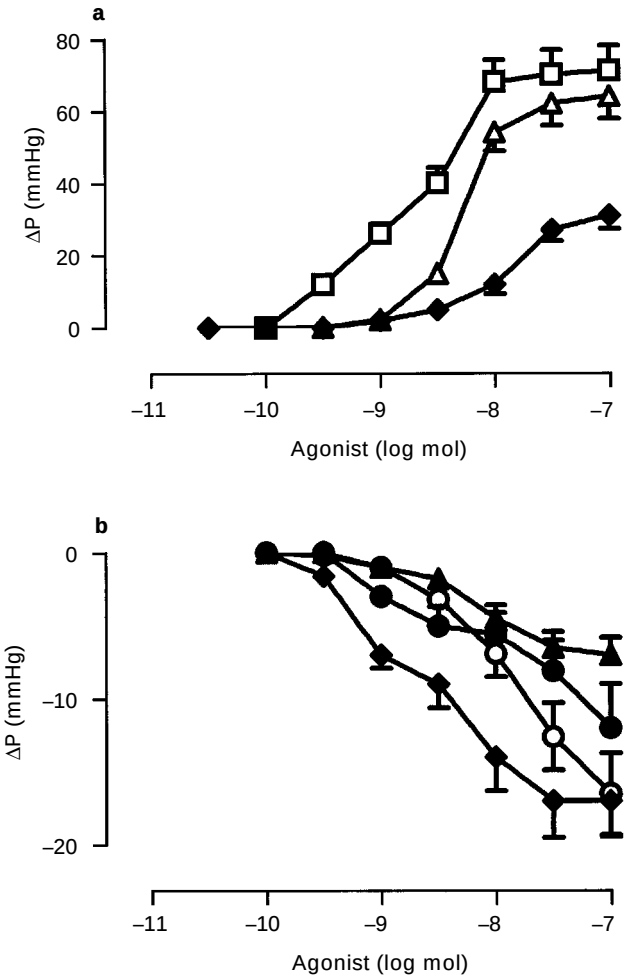

Figure 6 Changes in perfusion pressure in the rat isolated perfused kidney, during raised tone with angiotensin II (200 nM) in the perfusate, induced by (a) Ap_4A (■), Ap_5A (□), Ap_6A (△), (b) Ap_2A (●), Ap_3A (○), 2-meSATP (◆) and NECA (▲). Each point is the mean of at least five determinations and the vertical lines show the s.e.mean. Ap_5A and 2-meSATP significantly increased perfusion pressure ($P<0.05$) in doses $\geqslant 10^{-9.5}$ mol, Ap_2A, Ap_3A, Ap_4A, Ap_6A and NECA in doses $\geqslant 10^{-9}$ mol (bolus injections). For abbreviations see text.

Table 2 Agonist response to perfusion pressure in the rat isolated perfused kidney in the absence and presence of A_1- and/or P_{2X}-receptor antagonists

	Bolus injections of agonists				
Perfusate	*NA* (mmHg)	*α,β-MeATP* (mmHg)	***R*(−)-PIA** (mmHg)	*Ap_4A (10)* (mmHg)	*Ap_4A (100)* (mmHg)
Tyrode	40.13 ± 1.12	44.67 ± 0.80	17.33 ± 0.76	14.67 ± 0.72	35.00 ± 1.91
Tyrode + suramin (100 μM)	37.17 ± 1.01	0.00 ± 0.00*	15.67 ± 0.84	5.30 ± 0.46*	14.00 ± 0.82*
Tyrode + CPDPX (10 μM) + suramin (100 μM)	37.83 ± 0.96	0.00 ± 0.00§	$0.00\pm0.00^{\#}$	$0.00\pm0.00^{\#}$	$0.00\pm0.00^{\#}$

Changes in perfusion pressure (mmHg) in the rat isolated perfused kidney induced by bolus injections of noradrenaline (NA, 100 nmol), α,β-meATP (10 μmol), **R**(−)-PIA (10 μmol), Ap_4A (10 μmol) and Ap_4A (100 μmol) in the absence of any A_1- and P_{2X}-receptor antagonist, in the presence of suramin (100 μM), and in the presence of suramin (100 μM) and CPDPX (10 μM). Values are given as means and s.e.mean ($n=6$). *$P<0.05$ suramin vs control. §$P<0.05$ suramin + CPDPX vs control, $^{\#}P<0.05$ suramin + CPDPX vs suramin.

response to 2-meSATP (1 μmol and 10 μmol) changed from vasodilatation to vasoconstriction, whereas the vasodilatations evoked by NECA, Ap_2A and Ap_3A (10 μmol and 100 μmol) were not significantly affected by inhibition of the NO-synthases.

Discussion

Our results demonstrate that diadenosine phosphates activate different receptors depending on the number of phosphate groups. Furthermore, diadenosine phosphates simultaneously activate several different vascular purinoceptors. The present study in the rat isolated perfused kidney further shows important differences in the actions of diadenosine phosphates compared to other vessels such as the mesenteric arteries.

The vasoconstrictor action was most pronounced with Ap_5A and least with Ap_2A. The experiments with the P_2-purinoceptor antagonist, suramin, and the specific P_{2X}-purinoceptor antagonist, PPADS, showed that the vasoconstrictor actions of Ap_3A were due to activation of P_{2X}-purinoceptors. This finding is in accordance with observations by Ralevic *et al*. (1995) in mesenteric arteries; they also found that the Ap_5A-induced vasoconstriction was abolished by PPADS. The transient vasoconstriction induced by suramin in this preparation may be due to the unspecific inhibition of both vasoconstrictor and vasodilator P_2-purinoceptors. If the vasodilator purinoceptors are partially activated by endogenous nucleotides, the addition of suramin may result in a transient vasodilatation. In contrast to these observations in mesenteric vessels, the vasoconstriction induced by Ap_4A and Ap_6A was not completely inhibited by suramin or PPADS. This points to the involvement of other purinoceptor subtypes in the vasoconstrictor response. Another important difference to the effects of diadenosine polyphosphates in mesenteric arteries is that both Ap_2A and Ap_3A elicit vasoconstrictor effects, but to a much lesser extent than Ap_4A, Ap_5A and Ap_6A. Ap_2A and Ap_3A exert vasoconstrictor effects by stimulation of A_1 receptors, as evidenced by the inhibitory effect of DPCPX. In vascular smooth muscle, stimulation of A_1-receptors leads to vasoconstriction (Olsson & Pearson, 1990). Ap_4A appears to activate both, A_1- and P_{2X}-receptors.

Table 3 Vasodilator pD_{-7} values and maximal responses to 100 μmol doses of adenine- and dinucleotides in elevated tone preparations

Compound	pD_{-7} ($-$log mol)	*Maximal response to 100 μmol of agonist* (mmHg)
Ap_2A	7.75±0.13	−12.0±3.0
Ap_3A	7.98±0.12	−16.5±2.8
2-meSATP	9.0±0.14	−17.0±2.0
NECA	7.0±0.12	−7.0±1.2

Values are given as means±s.e.mean ($n=7$).

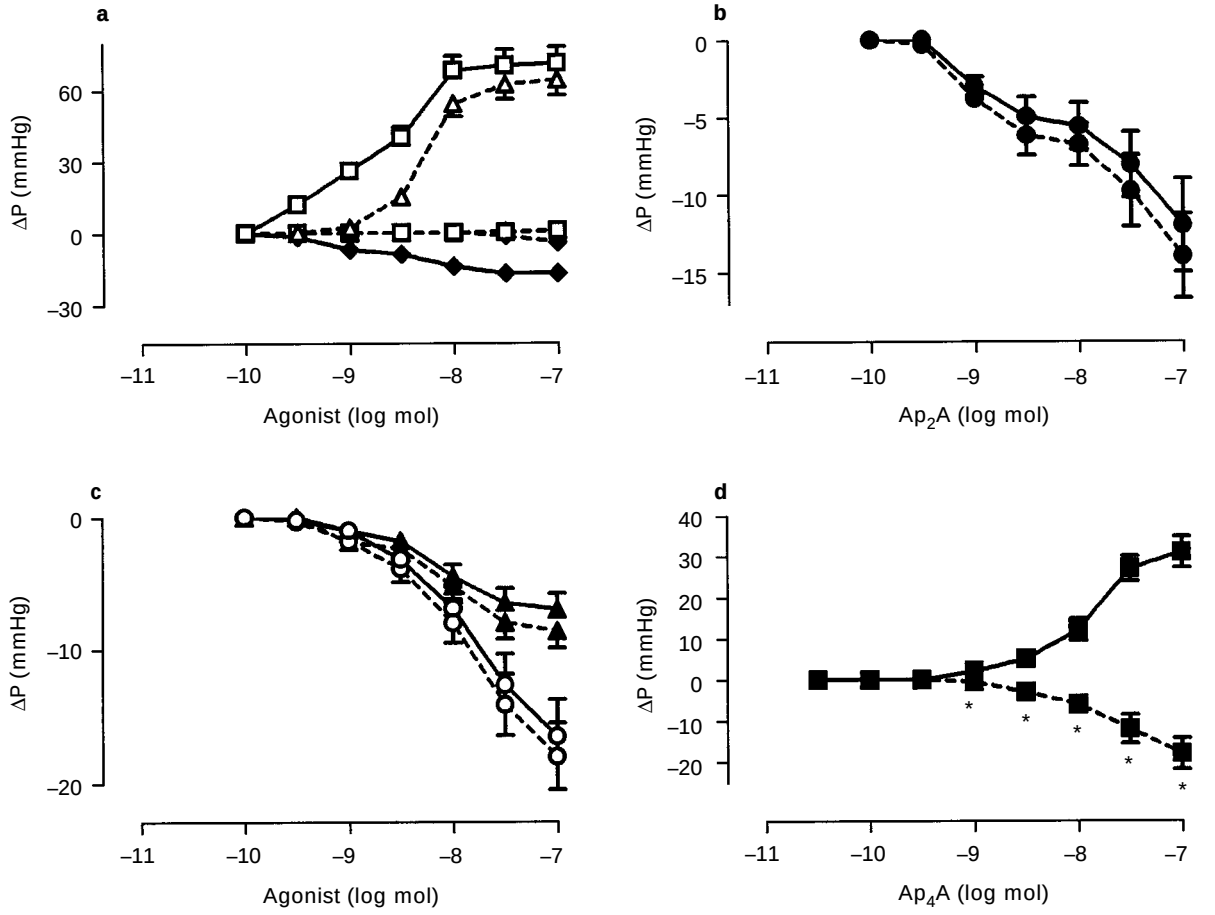

Figure 7 Changes in perfusion pressure in the rat isolated perfused kidney, during raised tone with angiotensin II (200 nM) in the perfusate, induced by (a) Ap_5A (□), Ap_6A (△) and 2-meSATP (◆), (b) Ap_2A (●), (c) Ap_3A (○), NECA (▲) and (d) Ap_4A (■) in the absence (solid line) and presence (dotted line) of suramin (100 μM) in the perfusate. The symbols indicating the effects of Ap_6A with suramin are partially superimposed by the symbols indicating the effects of Ap_5A with suramin. Each point is the mean of at least five determinations and the vertical lines show the s.e.mean. *$P<0.05$ suramin vs control. The asterisks were omitted in (a) for reasons of clarity, all differences with Ap_5A doses $\geqslant 10^{-9.5}$ mol and with Ap_6A and 2-meSATP doses $\geqslant 10^{-9}$ mol being significant ($P<0.05$). For abbreviations see text.

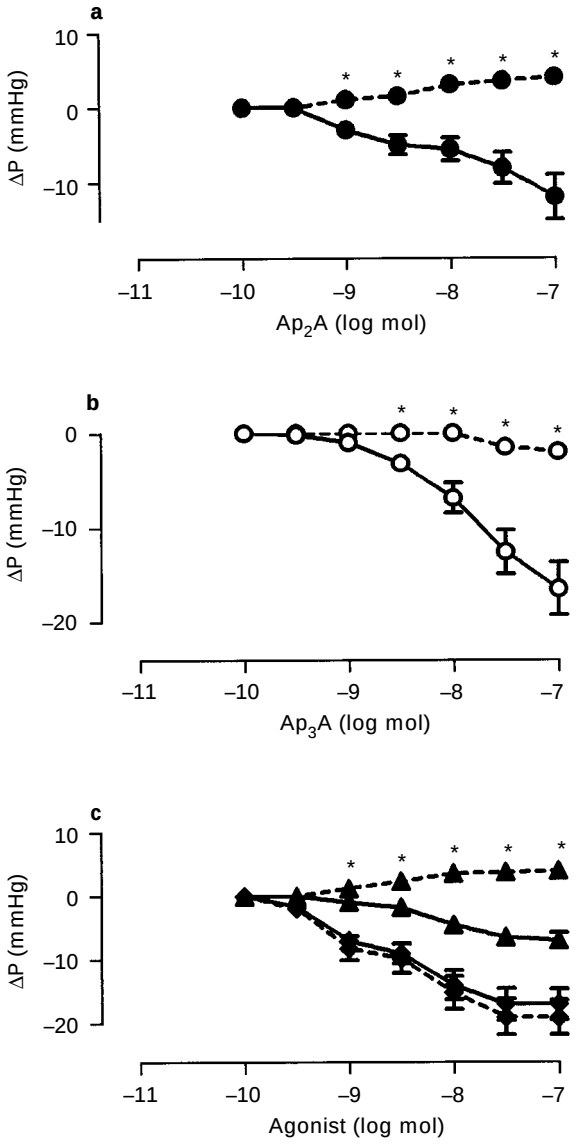

Figure 8 Changes in perfusion pressure in the rat isolated perfused kidney, during raised tone with angiotensin II (200 nM) in the perfusate, induced by (a) Ap_2A (●), (b) Ap_3A (○), (c) 2-meSATP (◆) and NECA (▲) in the absence (solid line) and presence (dotted line) of DMPX (10 μM) in the perfusate. Each point is the mean of at least five determinations and the vertical lines show the s.e.mean. $^*P<0.05$ DMPX vs control. For abbreviations see text.

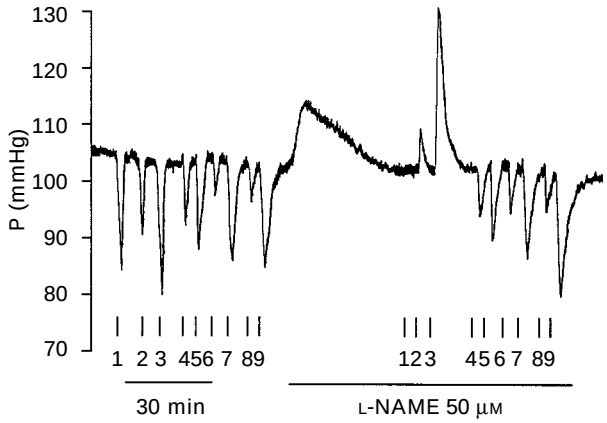

Figure 9 Representative trace showing out of 7 similar experiments changes in the perfusion pressure in the rat isolated perfused kidney in raised tone preparation with angiotensin II (200 nM) in the perfusate at constant flow and under permanent perfusion with L-NAME (50 μM) in the perfusate. Bolus injections: (1) Acetylcholine (10 pmol), (2) 2-meSATP (1 μmol), (3) 2-meSATP (10 μmol), (4) NECA (1 μmol), (5) NECA (10 μmol), (6) Ap_2A (10 μmol), (7) Ap_2A (100 μmol), (8) Ap_3A (10 μmol) and (9) Ap_3A (100 μmol). For abbreviations see text.

Table 4 Agonist response to perfusion pressure in the rat isolated perfused kidney in the absence and presence of L-NAME (50 μM)

	Tyrode (mmHg)	*Tyrode + L-NAME* (mmHg)
Acetylcholine (10 pmol)	−22.36±1.46	0.53±0.22
2-meSATP (1 μmol)	−15.14±0.71	8.6±0.33
2-meSATP (10 μmol)	−25.57±0.81	24.94±1.01
NECA (1 μmol)	−14.47±0.77	−11.43±0.92
NECA (10 μmol)	−18.54±0.60	−17.80±0.37
Ap_2A (10 μmol)	−8.64±0.39	−9.86±0.60
Ap_2A (100 μmol)	−19.29±0.89	−18.29±0.89
Ap_3A (10 μmol)	−7.17±0.75	−8.80±0.43
Ap_3A (100 μmol)	−20.64±0.71	−22.14±0.96

Changes in perfusion pressure (mmHg) in the rat isolated perfused kidney induced by bolus injection of acetylcholine (10 μmol), 2-meSATP (1 μmol), 2-meSATP (10 μmol), NECA (1 μmol), NECA (10 μmol), Ap_2A (10 μmol), Ap_2A (100 μmol), Ap_3A (10 μmol) and Ap_3A (100 μmol) in the absence and presence of the NO-synthase blocker L-NAME (50 μM). Values are given as means and s.e.mean ($n=7$).

With respect to the vasodilator effect, important differences from the findings in mesenteric arteries were also observed. Comparable to their effects in mesenteric arteries, Ap_4A, Ap_5A and Ap_6A had no vasodilator effect in raised tone preparations, and Ap_2A and Ap_3A caused vasodilatation. Ap_4A caused dose-dependent dilatation in raised tone preparations after the P_{2X}-receptor had been blocked. However, the mechanism of vasodilatation induced by Ap_2A and Ap_3A was different from that proposed in mesenteric arteries (Ralevic *et al*., 1995). The vasodilator action was unaffected by suramin, indicating that the P_{2Y}-purinoceptor may not be involved, though the P_{2Y}-receptor is present in the rat isolated perfused kidney. However, inhibition of A_2 receptors by DMPX completely abolished the vasodilator effects of Ap_2A and Ap_3A. The A_2-purinoceptor is known to induce vasodilatation in vascular smooth muscle cells (Biaggioni, 1992). An additional important difference is that the vasodilator effect is not NO-dependent. Thus the concept of diadenosine polyphosphate-induced vasodilatation being dependent on endothelial P_{2Y} purinoceptors and hence on NO release (Ralevic & Burnstock, 1991) does not explain the vasodilator effects in rat renal vasculature. The vasodilatation of Ap_4A induced in the isolated perfused kidney seems to be always covered by the more potent vasoconstrictor properties of this substance.

The present study leads to the following concept of vascular effects of diadenosine polyphosphates: Ap_5A activates P_{2X} purinoceptors to induce vasoconstriction, whereas the vasoconstrictor effects of Ap_2A and Ap_3A are mediated by A_1 receptors. Ap_4A appears to activate both types of receptors. Ap_6A activates P_{2X}-purinoceptors and another not identified receptor. Ap_3A activates, at high concentrations, the P_{2X} receptor. Ap_2A and Ap_3A have vasodilator properties and A_2 receptors mediate this vasodilatation. Ap_4A has vasodilator properties, too, which can be identified after blockade of the vasoconstrictor component. A role of NO release secondary to stimulation of endothelial P_{2Y}-purinoceptors was not apparent in this preparation. Although the P_{2Y}-receptor is present in the endothelial cells of the kidney, it is not activated by Ap_3A and Ap_2A as proposed by Ralevic *et al*. (1995). This indicates that the P_{2Y}-receptor in the kidney is different from the endothelial

mesenteric P_{2Y}-receptor (Burnstock *et al*., 1994) or also intestinal P_{2Y}-receptor (Hoyle & Burnstock, 1992). There might be various organ specific P_{2Y}-receptor subtypes (Abbracchio & Burnstock, 1994) with different activation by various diadenosine polyphosphates. Besides the activation of the known subtypes of purinoceptors, other yet unidentified components of the diadenosine polyphosphate-induced effects have been observed in this study. The vasoconstriction induced by Ap_6A could not completely be abolished by suramin, suggesting that components other than P_{2X}-purinoceptor stimulation may be involved. Suramin is also known to block the P_{2U}- and P_{2Y}-purinoceptors (Nakaoka & Yamashita, 1995), but the P_{2D} subtype may not be suramin-sensitive. Therefore, a part of the Ap_6A-induced vasoconstriction could be due to P_{2D}-purinoceptor activation (Pintor & Miras-Portugal, 1995), which is known to elicit a transmembrane Ca^{2+} influx (Castro *et al*., 1995). Recently Lazarowski *et al*. (1995) described Ap_4A as a potent agonist of a cloned human P_{2U}-receptor. Alternatively, a further yet unidentified receptor has to be taken into account.

In summary, our experiments showed that in rat renal vasculature various diadenosine polyphosphates induce differential effects on vascular tone. The receptors involved in these actions partly differ from those involved in mesenteric arteries and may include an as yet unknown receptor mediating vasoconstriction.

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