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The critical role of adenosine and guanosine in the affinity of dinucleoside polyphosphates to P_{2X}-receptors in the isolated perfused rat kidney

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1 The activation of P_{2x}-receptors in the rat renal vasculature by dinucleoside polyphosphates with variable phosphate group chain length (Xp_nX; X = Adenin (A) /Guanin (G), $n = 4-6$) was studied by measuring their effects on perfusion pressure of the isolated perfused rat kidney at constant flow in an open circuit.

2 Like Ap_4A, Ap_5A and Ap_6A the dinucleoside polyphosphates Ap_4G, Ap_5G and Ap_6G exerted a vasoconstriction which could be blocked by suramin and pyridoxal-phosphate-6-azophenyl-2; 4-disulphonic acid (PPADS).

3 Gp_4G, Gp_5G and Gp_6G showed only very weak vasoconstriction at high doses.

4 Ap_6A and α, β-meATP could not be blocked by the selective P_{2x1}-receptor antagonisten NF023 (30 μM), whereas Ap_4A, Ap_4G, Ap_5A, Ap_5G and Ap_6G were partially blocked by NF023.

5 Inhibition of endothelial NO-synthase by N^{ω}-nitro-L-arginine methyl ester (L-NAME) did not affect vasoconstrictions induced by dinucleosidepolyphosphates.

6 P_{2x}-receptor can only be activated if at least one adenosine moiety is present in the molecule.

7 Ap_nG show a weaker vasoconstrictive action than corresponding Ap_nA, concluding that two adenosine moieties enhance the P_{2x}-receptor binding and activation.

8 Xp_nX containing five phosphate groups show the most pronounced vasoconstrictive effect whereas four phosphate groups show the less effect, therefore the number of phosphate groups critically changes receptor affinity.

9 Additional experiments using permanent perfusion with α, β-methylene ATP (α,β-meATP) and the selective P_{2x1}-receptor antagonist NF023 showed that the newly discovered human dinucleoside polyphosphates activated the vascular P_{2x1}-receptor and an recently identified new P_{2x}-receptor subtype.

10 The differential effects of dinucleoside polyphosphates allow a fine tuning of local perfusion *via* composition of Xp_nXs.

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Abbreviations: ACH, acetylcholine; α, β-meATP, α, β-methylene adenosine 5′-triphosphate; Ap_nA, diadenosine polyphosphates; Ap_nG, adenosine (5′) oligophospho- (5′) guanosine; Ap_4A, P^1, P^4-diadenosine tetraphosphate; Ap_4G, adenosine (5′) tetraphospo-(5′) guanosine; Ap_5A, P^1, P^5-diadenosine pentaphosphate; Ap_5G, adenosine (5′) pentaphospo-(5′) guanosine; Ap_6A, P^1, P^6-diadenosine hexaphosphate; Ap_6G, adenosine (5′) hexaphospo-(5′) guanosine; CPDPX, 8-cylcopentyl-1, 3-dipropylxanthine; Gp_nG, diguanosine polyphosphates; Gp_4G, P^1, P^4-diguanosine tetraphosphate; Gp_5G, P^1, P^5-diguanosine pentaphosphate; Gp_5G, P^1, P^6-diguanosine hexaphosphate; GTP, guanosine 5′-triphosphate; L-NAME, N^{ω}-nitro-L-arginine methyl ester; PPADS, pyridoxal-phosphate-6-azophenyl-2;4-disulphonic acid; R (−) - PIA, R (−) N^6 - (2-phenylisopropyl) adenosine; Xp_nX, dinucleosides polyphosphates

Introduction

Recently, novel purinergic agonists have been identified in human platelets (Schluter *et al*., 1994; 1998; Jankowski *et al*., 1999). It was shown that platelets contain a number of dinucleoside polyphosphates, which vary with respect to the number of phosphate groups and the nucleoside moieties. Thus, in human platelets not only diadenosine polyphosphates (Ap_nA; $n = 3-6$) were found (Schluter *et al*., 1994), but also diguanosine polyphosphates (Gp_nG) and mixed dinucleoside polyphosphates containing one adenosine and one guanosine moiety (Ap_nG) (Schluter *et al*., 1998). The activation of platelets leads to the release

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of a variety of vasoactive nucleotides. Ap_nAs and Ap_nGs have been estimated to be present in concentrations of 0.5–3 μM in the supernatant following platelet aggregation (Schlüter *et al*., 1998). Recently Beigi *et al*. (1999) showed in platelets that the nucleotide concentration at the local microenvironment of the cell surface may be at least an order of magnitude higher than that measured in the supernatant. As expected from these findings, dinucleoside polyphosphates can be detected in human plasma (Jankowski *et al*., personal communication). These vasoactive nucleotides may contain 2–6 phosphate groups. The group of Ap_nAs has been characterized with respect to their vascular actions and the underlying receptors in earlier studies. These experiments revealed that Ap_nAs ($n=4-6$) are vasoconstrictors activating P_{2x}-receptors. Additionally Ap_4A also induces vasoconstriction *via* Al-receptors (Ralevic *et al*., 1995; van der Giet *et al*., 1997). In contrast to Ap_nAs, the Ap_nGs and Gp_nGs have only been investigated in a few studies. In one study recently published by Lewis *et al*. (2000), the receptor-mediated vascular actions of Ap_nGs have been investigated in mesenteric arteries. However Ap_nGs and Gp_nGs have not been characterized so far with respect to their effects on kidney vasculature. Therefore, in the present study we compared the vasoconstrictive actions of the various Xp_nXs at concentrations between 10 nM and 100 μM, which are likely to occur locally after release from platelets, and we identified the underlying purinergic receptors in the kidney. To test the activation of P_{2x}-receptor subtypes by different dinucleoside polyphosphates, the isolated perfused rat kidney was chosen for several reasons: Firstly, much previous work on diadenosine polyphosphates has been done in this model. For better comparison of the guanine containing dinucleoside polyphosphates with those compounds tested earlier, the vasculature of the isolated perfused kidney appeared to be suited best. Secondly, receptor expression studies by Nori *et al*. (1998) revealed that in renal vasculature, essentially the same P_{2x} purinoceptors are expressed as in other arteries such as aorta.

Methods

Preparation of the rat isolated perfused kidney

The following procedures were performed in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Science recommended by the German Physiological Society. Adult male Wistar-Kyoto rats (4–6-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.

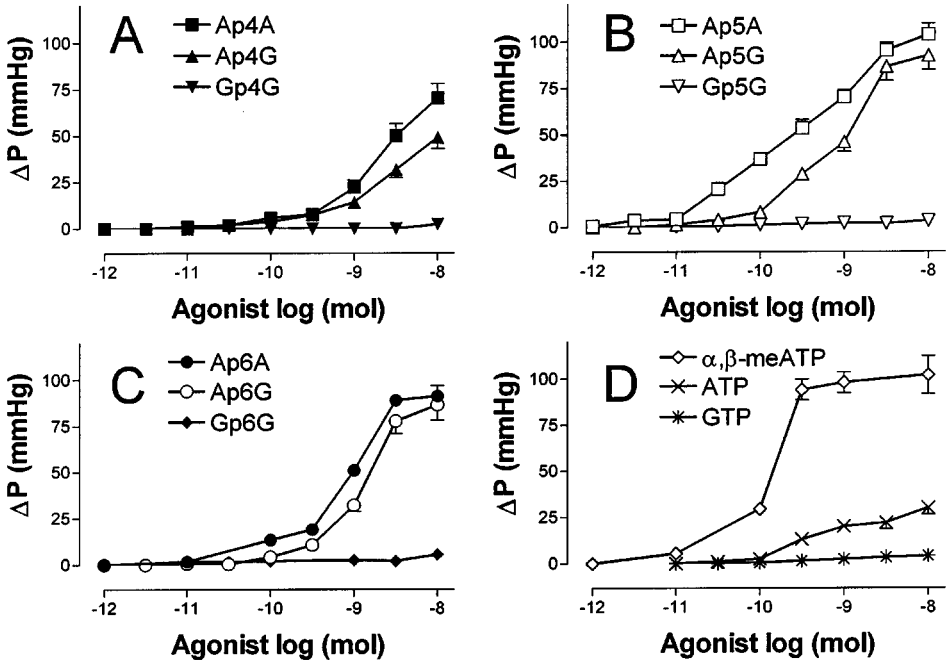

Figure 1 Changes in perfusion pressure in the isolated perfused rat kidney induced by (A) Ap_4A, Ap_4G, Gp_4G, (B) Ap_5A, Ap_5G, Gp_5G, (C) Ap_6A, Ap_6G, Gp_6G, (D) α, β-meATP, ATP and GTP. Each point is the mean of at least five determinations and vertical lines show s.e.mean. Significant difference ($^*P<0.05$) from baseline perfusion pressure for Ap_5A and α, β-meATP doses $\geqslant$5 pmol, for Ap_4A and Ap_6A doses $\geqslant$10 pmol, for Ap_4G and Ap_5G doses $\geqslant$50 pmol, for Gp_5G, Ap_6G, Gp_6G and ATP doses $\geqslant$100 pmol and for Gp_4G and GTP doses $\geqslant$1 nmol (bolus application). For abbreviations see text. Where error bars do not appear in figures, errors are within the symbol size.

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}, using a peristaltic pump as described previously (Bachmann *et al.*, 1991). The perfusate was Tyrode's 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, Germany), and recorded on a polygraph. Preparations were allowed to equilibrate for 30 min prior to experimentation.

Assessment of oedema during perfusion experiments

To assess the development of oedema during the perfusion experiments, rat kidneys were weighed before and after the experiments. After perfusion the weight was 156 ± 18% of the initial weight, indicating that a slight oedema of the kidneys developed. The response to 10 nM AngII at the end of the experiments was 94 ± 12% at the initial response.

Basal tone preparation for dose–response curves of dinucleoside polyphosphates

Vasoconstrictor responses to dinucleoside phosphates, α,β-meATP, ATP and GTP were assessed at basal tone. For each substance dose–response curves were constructed, with 15 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 cross-desensitization or auto-desensitization was not detected.

Permanent perfusion with P_{2X}-receptor antagonists and A1-receptor antagonist

The unspecific P_2-receptor antagonist, suramin (50 μM), the P_{2X}-receptor antagonist, pyridoxal-phosphate-6-azophenyl-2;4-disulphonic acid (PPADS, 10 μM), the selective P_{2X1}-receptorantagonist NF023 (30 μM), the selective A1-receptor antagonist 8-Cyclopentyl-1,3-dipropylxanthine (CPDPX, 10 μM) and N^{ω}-Nitro-L-arginine methyl ester (L-NAME, 50 μM) were added to the perfusate 30 min before challenge with dinucleoside polyphosphates, α,β-meATP, ATP and GTP. In an additional experiment, the P_{2X}-receptor agonist α,β-meATP (10 μM) was also continuously added to the perfusate before challenge with all agonists.

Desensitization experiments

The effect of desensitization of the P_{2X}-receptors due to dinucleoside polyphosphates was determined as follows. Control responses to bolus (100 μl) applications of agonists were obtained. Then the agonists were given as bolus applications every minute until bolus applications caused reproducible vasoconstrictions. To test recovery from desensitization, the period between bolus applications was increased from 30 s to 1, 2, 4, 8 and 16 min until maximal vasoconstrictions for agonists were achieved again.

Materials

All mono- and dinucleoside phosphates, acetylcholine (ACH) and angiotensin II (AngII) were applied as 100 μl bolus into a sample loop proximal to the preparation. For permanent perfusion with suramin, PPADS, CPDPX, NF023, L-NAME or α,β-meATP, substances were given into the perfusate. Drug dilutions were daily performed from stock solutions of 10 mM (concentrates stored frozen) in bidistilled water unless indicated otherwise. Heparin (sodium salt), suramin (hexasodium salt), α,β-meATP, ACH, CPDPX, L-NAME and PPADS came from Research Biochemicals Inc., U.S.A. NF023 was from Calbiochem, Schwalbach, Germany. Ap_nG and Gp_nG ($n = 4–6$) were synthesized and purified according to a procedure described by Schluter *et al.* (1998). Ap_4A, Ap_5A and Ap_6A and all other drugs were from Sigma Chemical Corporation (St. Louis, MO, U.S.A.). Before use Ap_4A, Ap_5A and Ap_6A were purified according to a procedure described previously (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 appropiate. All P values presented are 2-tailed. P values < 0.05 were considered significant.

Results

Results for diadenosinepolyphosphates (Ap_nA; $n = 4–6$) have been included in this study for reasons of comparison, though the results have been extensively published earlier (van der Giet *et al.*, 1997).

Table 1 Vasoconstrictor ED_{50} values and responses to 10 nmol doses of mono- and dinucleotides in basal tone preparation

Compound	*ED_{50}* (−log mol)	*Maximal response to 10 nmol of agonist* (mmHg)
α,β-meATP	−9.8 ± 0.03	102.8 ± 1.4
Ap_5A	−9.4 ± 0.16	103.8 ± 1.8
Ap_5G	−9.0 ± 0.11	92.6 ± 3.7
Ap_6A	−9.0 ± 0.09	91.2 ± 5.7
Ap_6G	−8.9 ± 0.04	86.6 ± 5.6
Ap_4A	−8.6 ± 0.06	70.5 ± 5.0
Ap_4G	−8.3 ± 0.16	49.3 ± 6.1
ATP	Not calculated	31.0 ± 4.1
Gp_5G	Not calculated	6.1 ± 1.2
Gp_6G	Not calculated	9.7 ± 4.7
Gp_4G	Not calculated	3.4 ± 0.9
GTP	Not calculated	4.8 ± 0.2

Values for ED_{50} and maximal responses to 10 nmol of agonist are given as mean ± s.e.mean.

Vasoconstrictor responses in basal tone preparation

The baseline perfusion pressure of the rat isolated perfused kidneys decreased by 10–15 mmHg during the first hour and by 5–7 mmHg during the second hour of perfusion. Vascular reactivity to vasoactive agents did not diminish during this time. After the equilibration period, the baseline perfusion pressure was 62 ± 6 mmHg ($n=98$). Addition of suramin (50 μM) or α,β-meATP (1 μM) to the perfusate caused an increase in perfusion pressure of 9 ± 3 mmHg ($P<0.01$) or 27 ± 3 mmHg ($P<0.01$) respectively. The addition of PPADS (10 μM), NF023 (30 μM), L-NAME (50 μM) or CPDPX (10 μM) to the perfusate did not significantly change the baseline pressure.

At basal tone the nucleotides caused dose-dependent vasoconstriction (Figure 1A–D). 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 a contraction equal to that induced by bolus application of 10 nmol ATP, the following order of potency was α,β-meATP $\geqslant$ Ap_5A $\geqslant$ Ap_5G = Ap_6A $\geqslant$ Ap_6G $\geqslant$

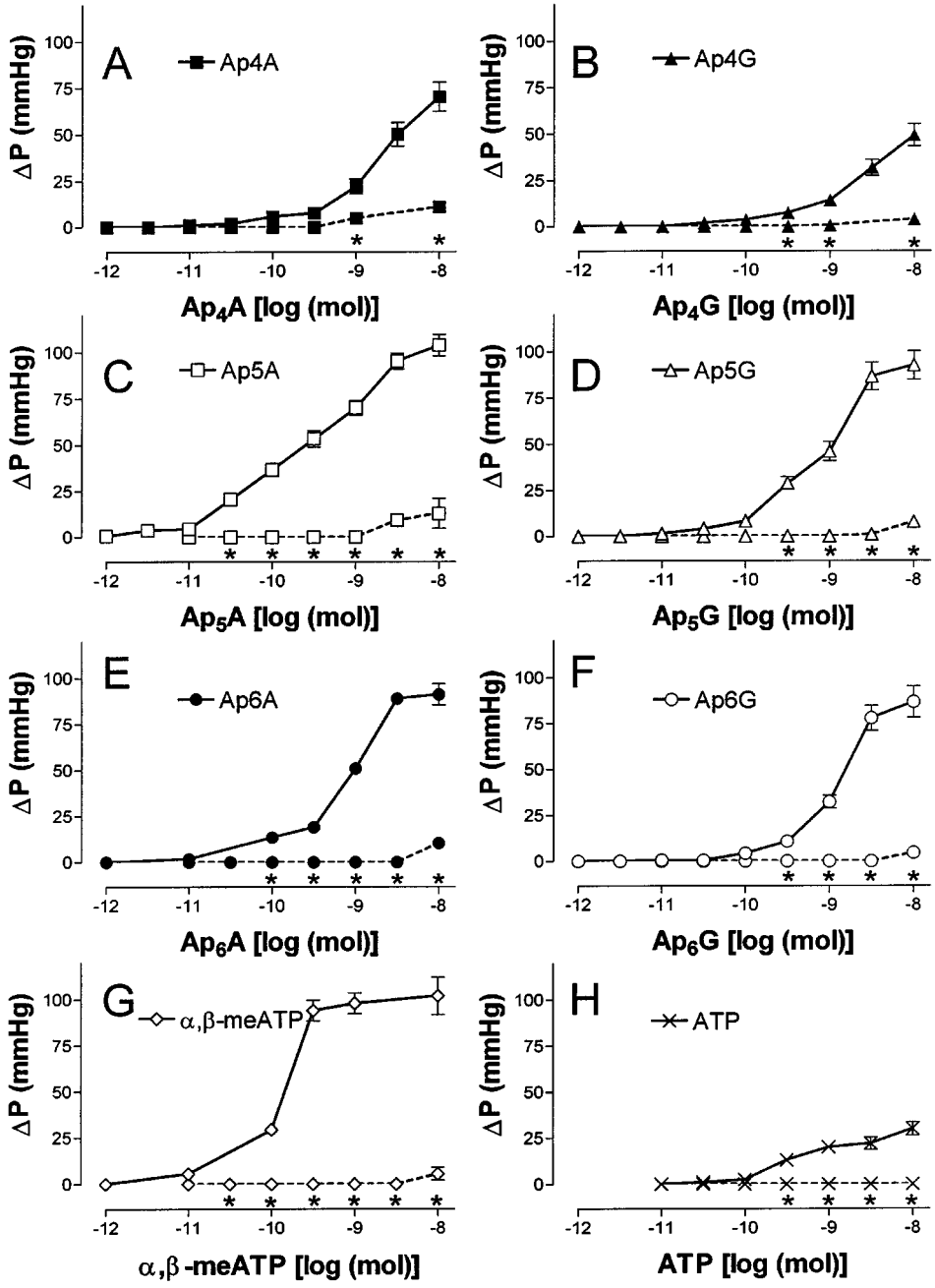

Figure 2 Changes in perfusion pressure in the rat isolated perfused kidney induced by (A) Ap_4A, (B) Ap_4G, (C) Ap_5A, (D) Ap_5G, (E) Ap_6A, (F) Ap_6G, (G) α,β-meATP and (H) ATP, in the absence (control) and presence of PPADS (10 μM) in the perfusate. Each point is the mean of at least five determinations and the vertical lines show the s.e.mean. $^*P<0.05$ PPADS vs control. For abbreviations see text. Where error bars do not appear in figures, errors are within the symbol size.

$Ap_4A \geqslant Ap_4G \geqslant ATP > > Gp_5G \approx Gp_6G \approx GP_4G \approx GTP$. In Table 1 ED_{50} and maximal responses to 10 nmol doses are shown.

Vasoconstrictor responses under P_{2X}-receptor antagonists

In the presence of PPADS (10 μM) responses to Ap_4A (Figure 2A), Ap_4G (Figure 2B), Ap_5A (Figure 2C), Ap_5G (Figure 2D), Ap_6A (Figure 2E) and Ap_6G (Figure 2F), α,β-meATP (Figure 2G) and ATP (Figure 2H) were almost completely abolished (each $P<0.05$ vs control). The control responses to AngII (results not shown) were not significantly affected. Responses to Gp_4G, Gp_5G and Gp_6G (results not shown) were not affected by inhibition with PPADS (10 μM).

Following incubation with suramin (50 μM) (Figure 3) responses to α, β-meATP, Ap_5A, Ap_5G, Ap_6A, Ap_6G and ATP were almost completely abolished (each $P<0.05$ vs control). After suramin application the responses to Ap_4A, Ap_4G were markedly attenuated to $15.9\pm2.9\%$ (Ap_4A) and $22.9\pm8.3\%$ (Ap_4G). Responses to Gp_4G, Gp_5G, Gp_6G and AngII were not significantly affected by suramin.

In the presence of NF023 (30 μM) (Figure 4) responses to Ap_4A, Ap_4G, Ap_5A, Ap_5G and Ap_6G were significantly but not completely blocked (each $P<0.05$ vs control). Vasoconstrictive responses Ap_4A in the presence of NF023 (30 μM) were reduced to about $28.8\pm8.7\%$, of Ap_4G to $54.0\pm13.8\%$, of Ap_5A to $53.8\pm10.0\%$, of Ap_5G to $36.9\pm3.1\%$ and of Ap_6G to $44.0\pm7.2\%$ compared with the initial vasoconstrictive response without presence of NF023. Responses to Gp_4A, Gp_5G, Ap_6A, Gp_6G, α, β-meATP and AngII were not significantly affected by permanent perfusion with NF023 (30 μM).

Repetitive application of P_{2x}-receptor agonists

To further characterize the receptors of Ap_5G and Ap_6G the desensitization on the P_{2x}-receptor by repetitive bolus application of Ap_5G and Ap_6G was tested. Bolus application (100 μl) of Ap_5G (10 nmol) and Ap_6G (10 nmol) in 1 min or 30 s intervals caused a rapid but not complete desensitization of vasoconstriction induced by both substances (Figure 5). Maximal responses of Ap_5G bolus applications were significantly ($P<0.05$) reduced from 94 ± 7 mmHg to repetitive responses of 17 ± 2 mmHg and for Ap_6G from 86 ± 3 mmHg to 14 ± 4 mmHg. Increasing the time between bolus applications of both substances from 1 min to 2, 4, 8 and 16 min showed a recovery from the desensitization. With 16 min-intervals a complete recovery from the desensitization was achieved. The transient vasoconstriction by Ap_5G and Ap_6G was after 16 min without activation of any P_{2x}-receptor was not significantly different from the control response induced by Ap_5G and Ap_6G.

Vasoconstrictor responses under permanent perfusion with P_{2x}-receptor agonist α, β-meATP

Permanent perfusion with α, β-meATP 10 μM caused an initial transient (158.0 ± 7.0 mmHg) and a sustained (27.0 ± 2.8 mmHg) vasoconstriction. In the presence of α, β-meATP reactions to α, β-meATP, Ap_4G, Ap_5A, Ap_5G, Ap_6A, Ap_6G and ATP were significantly and nearly completely blocked (Figure 6). Reactions to Ap_4A were significantly by only partly inhibited (Ap_4A with Tyrode: 70.5 ± 5.4 mmHg; Ap_4A with α, β-meATP: 12.0 ± 2.3 mmHg). AngII, Gp_4G, Gp_5G and Gp_6G were not significantly affected by permanent perfusion with α, β-meATP.

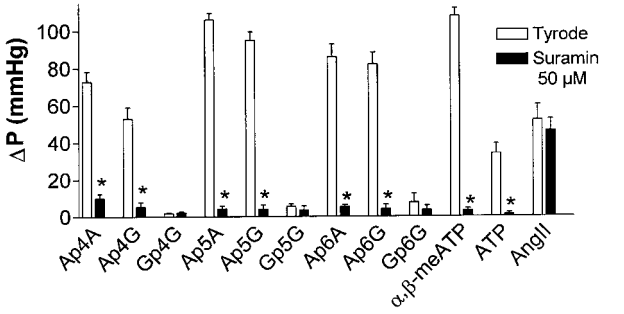

Figure 3 Changes in perfusion pressure (mmHg) in the rat isolated perfused kidney induced by bolus application of 10 nmol of each agonist in the absence (control) and presence of suramin [50 μM] in the perfusate. Each column is the mean of at least five determinations and the vertical lines show the s.e.mean. $^*P<0.05$ suramin vs control. For abbreviations see text.

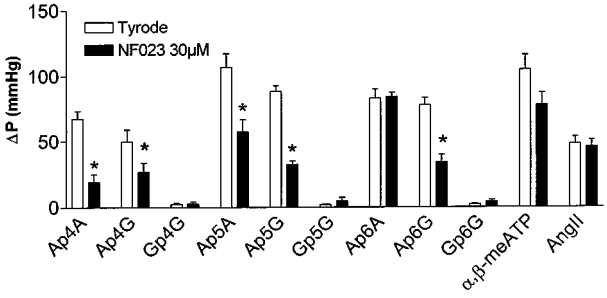

Figure 4 Changes in perfusion pressure (mmHg) in the rat isolated perfused kidney induced by bolus application of 10 nmol of each agonist in the absence (control) and presence of NF023 [30 μM] in the perfusate. Each column is the mean of at least five determinations and the vertical lines show the s.e.mean. $^*P<0.05$ NF023 vs control. For abbreviations see text.

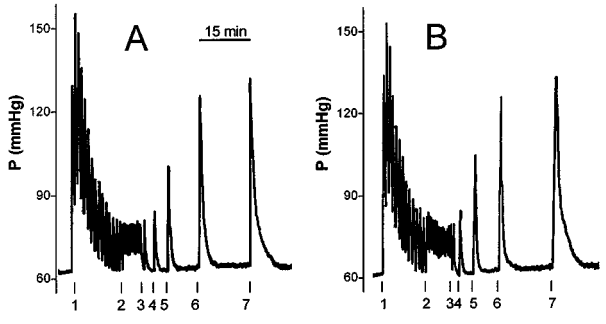

Figure 5 Original tracing showing desensitization of agonist-induced vasoconstriction. Representative trace out of five similar experiments showing changes in perfusion pressure in the rat isolated perfused kidney induced by repetitive bolus applications of Ap_5G (10 nmol) (A) and Ap_6G (10 nmol) (B) to show desensitization. Bolus was applied every (1) 1 min, (2) 30 s, (3) 1 min, (4) 2 min, (5) 4 min, (6) 8 min and (7) 16 min after the end of the repetitive bolus application.

Vasoconstrictor responses under A1-receptor antagonist

In the presence of CPDPX (10 μM) the response to R(−)-PIA was completely abolished ($P<0.05$ vs control; Figure 7). Contractions induced by Ap_4A were significantly but only partly inhibited. The response of Ap_4A given as a dose of 10 nmol was reduced from 70.5 ± 7.8 mmHg (100% as control response) to 50.4 ± 3.9 mmHg (71.5% of control). Responses to Ap_4G, Gp_4G, ATP, α, β-meATP and $Xp_{5/6}X$ (X = A/G; results not shown) were not significantly affected.

Vasoconstrictor responses under permanent perfusion with L-NAME

In the presence of L-NAME (50 μM) the vasodilator response to ACH was completely abolished ($P<0.05$ vs control; Figure 8) and reversed to a vasoconstriction. Contractions induced by Xp_nX (X = A/G; $n=4-6$), α, β-meATP and AngII were not affected by permanent perfusion with L-NAME (50 μM).

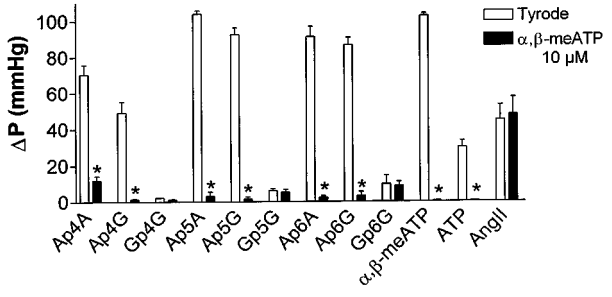

Figure 6 Changes in perfusion pressure (mmHg) in the rat isolated perfused kidney induced by permanent perfusion with α, β-meATP. Bolus application (10 nmol) of each agonist in the absence and presence of α, β-meATP (10 μM). Each column is the mean of at least five determinations and the vertical lines show the s.e.mean. $*P<0.05$ α, β-meATP vs control. For abbreviations see text.

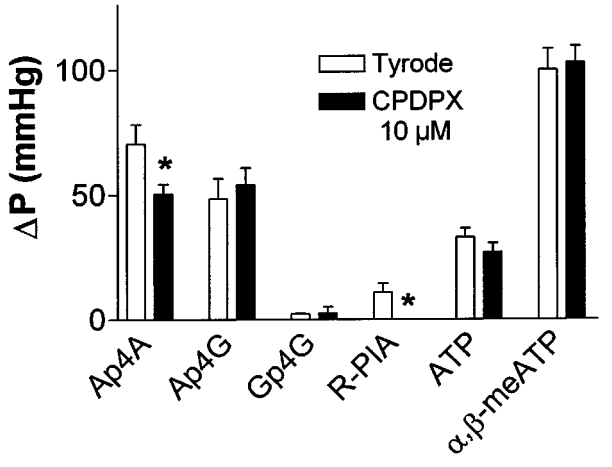

Figure 7 Changes in perfusion pressure in the rat isolated perfused rat kidney induced by bolus application of 10 nmol of each agonist in the absence (control) and presence of CPDPX (10 μM) in the perfusate. Each column is the mean of at least five determinations and the vertical lines show the s.e.mean. $*P<0.05$ CPDPX vs control. For abbreviations see text.

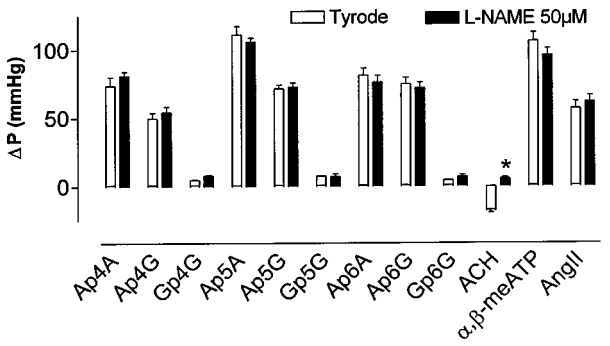

Figure 8 Changes in perfusion pressure in the rat isolated perfused rat kidney induced by bolus application of 10 nmol of each agonist in the absence (control) and presence of L-NAME (50 μM) in the perfusate. Each column is the mean of at least five determinations and the vertical lines show the s.e.mean. $*P<0.05$ L-NAME vs control. For abbreviations see text.

Discussion

The experiments showed that the vasoconstrictive action of Xp_nXs depends on the presence of at least one adenosine moiety in the molecule. It has been shown earlier (van der Giet *et al.*, 1997) that Ap_4A, Ap_5A and Ap_6A exert their vasoconstriction in the kidney *via* P_{2X}-receptors. Furthermore, most of the vasoconstrictive effects of Ap_4G, Ap_5G and Ap_6G, which have never been investigated before with respect to their receptor activation in the rat kidney, appear to be mediated by P_{2X}-receptors as evidenced by the inhibition by both suramin and PPADS. It has been shown recently that Ap_nG activate P_{2X}-receptors in isolated mesenteric arteries (Lewis *et al.*, 2000). Gp_4G, Gp_5G and Gp_6G only show very weak vasoconstriction at high dose applications. In the case of contraction of Gp_5G and Gp_6G the small size of the response make a physiological role of the vascular effects of these nucleotides unlikely. It may be concluded that the vascular P_{2X}-receptor can only be activated if at least one adenosine moiety is present in the molecule. However, a difference in degradation rate of the various dinucleoside phosphates by endogenous phosphorylases and phosphodiesterases during renal perfusion can not be excluded.

The vasoconstrictive effects of Xp_nX can be compared to the vasoconstrictive effects of the mononucleotides ATP and GTP. ATP has been shown to be a potent P_{2X}-receptor agonist (Eltze & Ullrich, 1996) in the kidney, whereas GTP exerts only weak vasoconstrictive properties which are not PPADS or suramin inhibitable (Bultmann *et al.*, 1997), as shown in the rat aorta. It has been shown earlier that the effects of Xp_nX cannot be mimicked by potential degradation products like, for example, ATP, GTP, ADP, GDP, AMP or GMP (Schluter *et al.*, 1998).

Ap_nGs show a weaker vasoconstrictive action than the corresponding Ap_nAs. Since both activate the same receptor, it may be concluded that the presence of two adenosine moeities enhances the chance of receptor binding and activation. Moreover, the number of phosphate groups determines the vasoconstrictive action, and hence, the apparent receptor affinity: In all three groups of Xp_nXs, the compound containing five phosphate groups shows the most

pronounced vasoconstrictive effect. This has already been shown for Ap_nA earlier (Ralevic *et al*., 1995; van der Giet *et al*., 1997). Ap_nG shows a similar pattern like Ap_nA, therefore it can be concluded that not only the adenosine moeity is important for P_{2X}-receptoractivation but also the number of phosphate groups critically changes the apparent receptor affinity.

Only Ap_4A activated A1-receptor besides the P_{2X}-receptors, as shown in earlier studies (van der Giet *et al*., 1998). At present it cannot be explained why the exchange of both adenosines for guanosine shifts the apparent affinity of the molecule from A1 to P_{2X}-receptors.

Inhibition of NO-synthase by L-NAME did not affect the renovascular response to Xp_nX, ruling out that the apparent low affinity of some compounds may be due to simultaneously occurring NO-dependent vasodilator responses as suggested by some previous studies (Churchill *et al*., 1993; Eltze & Ullrich, 1996).

In the present experiments the underlying subtype of the P_{2X}-receptors has not been determined. However, the pertinent literature reveals that in vascular smooth muscle cells only the P_{2X1}-, P_{2X2}- and P_{2X4}-subtypes occur (Chan *et al*., 1998; Nori *et al*., 1998). Desensitization experiments with α,β-meATP reveal further, that this synthetic P_{2X}-agonist can block the receptor which is activated by all tested Xp_nXs. Since the P_{2X2} is not activated by α,β-meATP (Evans *et al*., 1995), and the P_{2X4} cannot be inhibited by PPADS or suramin (Bo *et al*., 1995), it can be speculated that the P_{2X1}-receptor subtype underlies the vasoconstrictive actions of the Xp_nXs. The role of P_{2X1}-receptors for the Xp_nX response was further analysed using the specific P_{2X1}-receptor antagonist NF023 at usual pA_2 (Ziyal *et al*., 1997). Indeed another P_{2x}-receptor subtype other than the P_{2X1}-receptor subtype contributes to the vasoconstrictive effect of the Xp_nX. α,β-meATP and Ap_6A could not be blocked by NF023, whereas the other vasoactive dinucleosides were reduced by 40–60% of their initial vasoconstriction. From these findings it can be estimated that the vasoconstrictive Xp_nX effect is mediated by another P_{2X}-receptor subtype than P_{2X1}.

In renal tissue the P_{2X1}-, P_{2X2}- and P_{2X4}-receptor subtypes have been found (Nori *et al*., 1998). Due to the limitations of the isolated perfused kidney, only the sum of the vasoconstrictions mediated by those purine receptor subtypes can be shown in this model.

Recently an unknown P_{2X}-receptor has been pharmacologically characterized in the rat kidney (van der Giet *et al*., 1999). This receptor is suramin and PPADS inhibitable and can be activated by α,β-meATP, but in contrast to the P_{2X1}-receptor, the newly identified receptor is not completely desensitizable. Like Ap_5A and Ap_6A, which activate this receptor shown by repetitive application, Ap_5G and Ap_6G show the same pattern, concluding that the mixed dinucleoside polyphosphates can activate the yet identified P_{2X}-receptor subtype.

The results show that the dinucleoside polyphosphates affect vascular tone *via* different P_{2X}-receptor subtypes depending on the type of the nucleoside moieties and on the number of phosphate groups present in the molecule. Given the great variability of the compounds and their vascular effects, it may be speculated that the physiological role of the dinucleoside polyphosphates be the fine tuning of local perfusion especially in situations characterized either by platelet aggregation or by dinucleoside polyphosphate release from other cells. Although presently it is open, how exactly local perfusion can be regulated by the release of various dinucleoside polyphosphates, this issue may also be relevant under pathological conditions, especially in thrombotic processes and tissue ischaemia.

In summary, the findings document that the newly discovered human dinucleoside polyphosphates, Ap_nGs and Gp_nGs, can activate the vascular P_{2X1}-receptor subtype and the recently identified new P_{2X}-receptor subtype, provided that one adenosine moeity is present. Potentially, these compounds may play a role in the regulation of local perfusion. The differential effects of these compounds allows a fine tuning of local perfusion *via* composition of Xp_nX_s released from platelets or other tissues.

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