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Evidence for two different P_{2X}-receptors mediating vasoconstriction of Ap_5A and Ap_6A in the isolated perfused rat kidney

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1 The activation of various P_2-receptor subtypes in rat renal vasculature by P^1, P^5-diadenosine pentaphosphate (Ap_5A) and P^1, P^6-diadenosine hexaphosphate (Ap_6A) were studied by measuring their effects on perfusion pressure during continuous perfusion in a rat isolated perfused kidney.

2 Permanent perfusion with Ap_5A and Ap_6A elicited both a transient and sustained vasoconstriction with both vasoconstrictions to be different: the transient vasoconstriction can be elicited with concentrations $\geqslant$10 nM, whereas the sustained vasoconstriction is observed with concentrations $\geqslant$1 nM.

3 Ap_5A and Ap_6A act *via* the same receptors as α,β-methylene ATP (α,β-meATP).

4 The rank order of potency for transient vasconstriction was α,β-meATP = Ap_5A > Ap_6A > β,γ-meATP, and for sustained vasoconstriction α,β-meATP = Ap_5A > β,γ-meATP $\geqslant$ Ap_6A.

5 Suramin, a non-selective P_2-receptor antagonist, and pyridoxal-phosphate-6-azophenyl-2;4-disulphonic acid (PPADS) a highly selective P_{2X}-receptor antagonist antagonized both the transient and the sustained vasoconstriction.

6 Taken together the results of the agonist profile of Ap_5A and Ap_6A and comparing its findings to literature it can be demonstrated that the transient but not the sustained vasoconstriction is mediated *via* the P_{2X1}-receptor which is present in rat renal vasculature.

7 It is demonstrated that the agonist profile of the sustained vasoconstriction induced by Ap_5A and Ap_6A does not fit to any currently known P_{2X}- or P_{2Y}-receptor subtype.

8 We conclude a yet unidentified P_{2X}-receptor or chimeric P_{2X}-receptor may contribute to the effects on rat renal vasculature produced by Ap_5A and Ap_6A and which may play an important role in glomerular perfusion pressure and blood pressure control.

Keywords: Ap_5A; Ap_6A; P_{2X}; P_{2Y}; purinoceptor; isolated perfused rat kidney; vasoconstriction; perfusion pressure; hypertension

Abbreviations: Ap_5A, P^1, P^5-diadenosine pentaphosphate; Ap_6A, P^1, P^6-diadenosine hexaphosphate; α,β-meATP, α,β-methylene ATP; β,γ-meATP, β,γ-methylene ATP; ANGII, Angiotensin II; PPADS, pyridoxal-phosphate-6-azophenyl-2;4-disulphonic acid

Introduction

Recently, diadenosine polyphosphates have been identified as potent vasoconstrictors in human platelets (Schlüter *et al*., 1994). Especially the renal vasculature appeared to be affected by these agents (van der Giet *et al*., 1997). Furthermore it has been shown that the diadenosine polyphosphates act on vascular purinoceptors (Davies *et al*., 1995; Ralevic *et al*., 1995; van der Giet *et al*., 1997) especially diadenosinepentaphosphate (Ap_5A) and diadenosinehexaphosphate (Ap_6A) which are acting *via* a P_{2X}-receptor.

From these findings the question arises which purinoceptor subtypes are activated in renal vessels by diadenosine polyphosphates. In literature there are several reports on renal vascular P_2-receptors. Various studies have shown that the vasoconstrictive effects of ATP on the renal microvasculature are mainly observed in arcuate and interlobular arteries and glomerular afferent arterioles (Inscho *et al*., 1992; 1994; 1995). Recently Inscho *et al*. (1998) presented evidence that the vasoconstriction induced in the juxtamedullary afferent arterioles induced by various nucleotid derivates are mediated *via* P_{2X} and P_{2Y2} (P_{2U})-receptor subtypes in the kidney. In addition Chan *et al*. (1998) have shown by autoradiography and immunohistochemistry that the P_{2X1}-receptor subtype is present on the afferent vasculature of the kidney. Inscho *et al*. (1996) postulated that P_2-receptors participate in the autoregulation, renal blood flow and glomerular filtration rate.

It is generally known that in essential hypertension total peripheral resistance is increased, indicating a permanently elevated arterial or arteriolar tone. Considering a role of vasoconstrictor diadenosine polyphosphates in essential hypertension, a permanent elevation of vascular tone has to be reconciled with the current knowledge on vasoconstrictor purinoceptor subtypes (Burnstock 1996), by which diadenosine polyphosphates could affect vascular tone especially in the kidney. The P_{2X1}-receptor shows rapid and complete desensitization upon stimulation, and therefore can only mediate a transient vasoconstriction (Evans *et al*., 1998). Therefore it was examined in the present study, whether Ap_5A and Ap_6A can induce a sustained vasoconstriction in renal vasculature, potentially activating an already known P_2-receptor subtype or a different one from those found in vascular tissue in recent studies.

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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 Physiological Society in Germany. 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.

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'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 maintained at 37°C and a pH of 7.4. Responses were measured as changes in perfusion pressure (mmHg) with a pressure transducer (Statham Tansducer 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 before experimentation. The baseline perfusion pressure of the rat isolated perfused kidneys decreased by 10–15 mmHg during the first hour and by 6 mmHg during the second hour of perfusion. Vascular reactivity to vasoactive agents did not diminish during this time.

Permanent perfusion with P_{2X}-receptor agonists

Vasoconstrictor responses to permanent perfusion with P^1,P^5-diadenosine-(5′,5′)-pentaphosphate (Ap_5A), P^1,P^6-diadenosine-(5′,5′)-hexaphosphate (Ap_6A), α,β-methylene adenosine 5′-triphosphate (α,β-meATP) and β,γ-methylene adenosine 5′-triphosphate (β,γ-meATP) were assessed at basal tone. For each substance dose-response curves were constructed, with 20 min being allowed to elapse between consecutive permanent perfusions. This procedure allowed dose-response curves for at least two agonists to be constructed for the same preparation. A significant cross-desensitization or auto-desensitization was not detected when substances were being given in intervals of at least 20 min.

Permanent perfusion with P_{2X}-receptor antagonists

The unspecific P_2-receptor antagonist suramin (100 μM) and the P_{2X}-receptor antagonist pyridoxal-phosphate-6-azophenyl-2;4-disulphonic acid (PPADS, 30 μM) were added to the perfusate 30 min before challenge with Ap_5A, Ap_6A, α,β-meATP and β,γ-meATP. In an additional experiment, the P_{2X}-receptor agonist α,β-meATP (1 μM) was also perfused before challenge with Ap_5A.

Desensitization experiments

The effect of desensitization of the P_{2X}-receptor due to α,β-meATP and Ap_5A was determined as follows. Control responses to bolus (100 μl) injections of α,β-meATP (100 nmol) and Ap_5A (100 nmol) were obtained. Then α,β-meATP and Ap_5A were given as bolus injections every minute and, after achieving a steady state, every 30 s. The test lasted until bolus injections 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 α,β-meATP and Ap_5A were achieved again.

Materials

All mono- and diadenosine phosphates and angiotensin II (ANGII) were applied as 100 μl bolus into a sample loop proximal to the preparation. For permanent perfusion with Ap_6A, Ap_5A, α,β-meATP and β,γ-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, β,γ-meATP, PPADS came from Research Biochemicals Inc., U.S.A. Ap_5A and Ap_6A and all other drugs were from Sigma Chemical Corporation (St. Louis, MO, U.S.A.). Before use 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

Equilibration and permanent perfusion with suramin and PPADS

Vasoconstrictor responses to agonists were measured in the rat isolated perfused kidney. After the equilibration period (30 min), the baseline pressure was 58 ± 3 mmHg ($n=45$). Addition of suramin (100 μM) to the perfusate significantly increased perfusion pressure by 9 ± 2.3 mmHg ($P<0.01$), whereas, after addition of PPADS (30 μM) to the perfusate the baseline pressure did not change significantly.

Permanent perfusion with Ap_5A, Ap_6A, α,β-meATP and β, γ-meATP

At basal tone, permanent perfusion with Ap_5A, Ap_6A, α,β-meATP and β, γ-meATP caused dose-dependent vasoconstriction. The dose-response curves for α,β-meATP, β, γ-meATP, Ap_5A and Ap_6A as shown in Figure 1A–D induce a transient and a sustained vasoconstriction. The sustained response was already observed at lower concentrations than the transient response.

The maximum of the sustained vasoconstriction (sustained) was lower compared to the transient vasoconstriction (tran-

sient) (Figure 1A–D) [Maximum vasoconstrictions at 1 μM permanent perfusion of (1) α,β-meATP (Figure 1A) transient: 158.0 ± 7.0 mmHg and sustained: 27 ± 2.8 mmHg ($17.0 \pm 2.7\%$ of transient), (2) β, γ-meATP (Figure 1B) transient: 48.8 ± 6.2 mmHg and sustained: 9.8 ± 1.5 mmHg ($20.0 \pm 6.5\%$ of transient), (3) Ap_5A (Figure 1C) transient: 160.0 ± 14.0 mmHg and sustained: 30.0 ± 6.2 mmHg ($18.8 \pm 6.0\%$ of transient) and (4) Ap_6A (Figure 1D) transient: 106.5 ± 12.2 mmHg and sustained: 7.2 ± 1.8 mmHg ($6.7 \pm 1.8.0\%$ of transient)]. Ap_6A and β, γ-meATP were effective in higher concentrations according to transient and sustained vasoconstrictions than Ap_5A and α,β-meATP, and their maximum permanent pressor effect was lower than that of Ap_5A and α,β-meATP. The concentration-response curves were not parallel, and the maximal vasoconstriction, especially for the permanent vasoconstriction, varied considerably (Figure 1A–D). So it was not possible to calculate EC_{50} values neither for transient vasoconstriction nor for sustained vasoconstriction. Therefore the potency of compounds was compared by determining the concentration of agonist that would cause an increase in perfusion pressure of 50 mmHg (pC_{50} Table 1) for transient vasoconstriction and an increase of 15 mmHg (pC_{15} Table 1) for sustained vasoconstriction. The order of potency was α,β-meATP = Ap_5A > Ap_6A > β,γ-meATP (Table 1) for transient vasoconstriction and α,β-meATP = Ap_5A > β,γ-meATP $\geqslant Ap_6A$ for sustained vasoconstriction. In Table 1 pC_{50} for transient, pC_{15} for permanent vasoconstriction, respectively, and maximal responses to 1 μM agonist perfusion are shown.

Figure 2 shows an original tracing showing transient and sustained vasoconstriction induced by Ap_5A. As shown, Ap_5A in concentrations lower than 10 nM caused only a sustained vasoconstriction. In concentrations $\geqslant 10$ nM, Ap_5A additionally elicited a transient vasoconstriction. Principally similar the same findings were obtained with Ap_6A, α,β-meATP and β, γ-

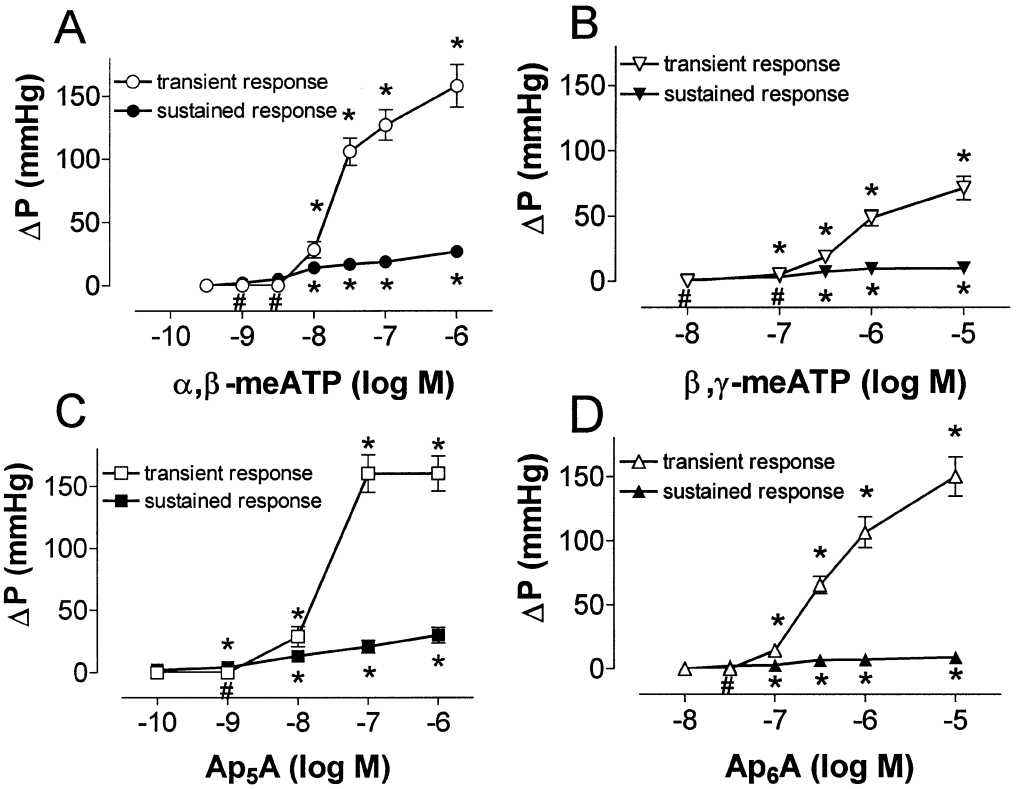

Figure 1 Concentration response curves of agonist induced vasoconstriction by Ap_5A, Ap_6A, α,β-meATP and β,γ-meATP. Changes in perfusion pressure (mmHg) in the rat isolated perfused kidney induced by (A) α,β-meATP, (B) β,γ-meATP, (C) Ap_5A and Ap_6A. Each point is the mean of at least five separate determinations and the vertical lines show the s.e.mean. Symbols (*, #) above line give significance for transient vasoconstriction and, below the line, for sustained vasoconstriction. $^*P<0.05$ significant difference from baseline perfusion and $\#P<0.05$ significant difference from baseline perfusion and from transient response. For abbreviations, see text.

Table 1 Transient vasoconstrictor pC_{50} values/maximal responses and sustained vasoconstrictor pC_{15} values/maximal responses to 1 μM permanent perfusion with adenine- and diadenosinephosphates

	Transient vasoconstriction		*Sustained vasoconstriction*	
Compound	*pC_{50}* ($-\log$ M)	*Maximal response to 1 μM of permanent agonist perfusion* (mmHg)	*pC_{15}* ($-\log$ M)	*Maximal response to 1 μM of permanent agonist perfusion* (mmHg)
α,β-meATP	7.85 ± 0.10	158.0 ± 17.0	7.93 ± 0.09	27.0 ± 2.8
Ap_5A	7.82 ± 0.08	160.0 ± 14.0	7.82 ± 0.12	30.0 ± 6.2
Ap_6A	6.64 ± 0.09	106.5 ± 12.2	not calculated	7.2 ± 1.8
β,γ-meATP	5.97 ± 0.10	48.8 ± 6.2	not calculated	9.8 ± 1.5

For abbreviations see text.

meATP. For permanent perfusion with α,β-meATP at 1 and 5 nM, β, γ-meATP at 10 nM, Ap_5A at 1 nM, and Ap_6A at 50 nM sustained vasoconstriction was significantly ($P<0.05$) higher than transient vasoconstriction.

Blockade of P_2-receptors

In the presence of PPADS (30 μM) transient and sustained vasoconstrictions induced by Ap_5A, Ap_6A, α,β-meATP and β, γ-meATP (Figure 3, results for Ap_6A and β, γ-meATP only shown in Table 2) were completely abolished (each $P<0.05$ vs control) (Table 2). After washout of PPADS for 20 min responses to Ap_5A, Ap_6A, α,β-meATP and β,γ-meATP recovered completely. Responses to ANGII were not affected by permanent perfusion with PPADS (30 μM).

Following incubation with suramin (50 μM) transient and sustained vasoconstrictions induced by Ap_5A, Ap_6A, α,β-meATP and β, γ-meATP (Figure 4) were completely blocked (each $P<0.05$ vs control). The responses to ANGII (Figure 4) were not affected by inhibition with suramin.

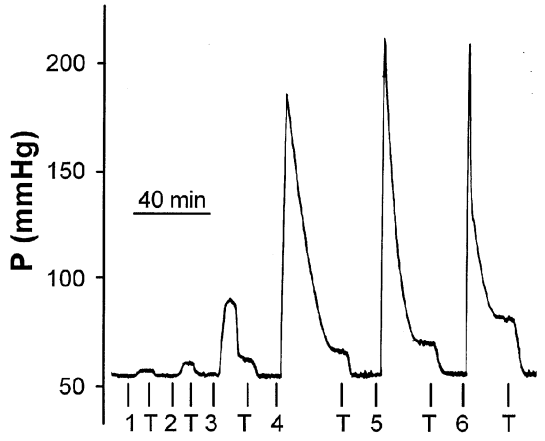

Figure 2 Original tracing showing Ap_5A induced vasoconstriction. Representative trace out of six similar experiments showing changes in perfusion pressure in the rat isolated perfused kidney induced by permanent perfusion with tyrode's solution (T: start of perfusion with Tyrode's solution without Ap_5A) or tyrode's solution + Ap_5A at various concentrations: (1) 1 nM, (2) 5 nM, (3) 10 nM, (4) 50 nM, (5) 100 nM and (6) 1 μM ($n=6$). For abbreviations, see text.

Desensitization experiments

To further characterize the receptor action of Ap_5A and α,β-meATP the desensitization on the p_{2x}-receptor by repetitive bolus application of α,β-meATP and Ap_5A was tested. Bolus application (100 μl) of Ap_5A [100 nmol] (Figure 5A) or α,β-meATP [100 nmol] (Figure 5B) in 1 min or 30 s intervals caused a rapid but not complete desensitization of vasoconstriction induced by both substances. Maximal responses of α,β-meATP bolus injections were significantly ($P<0.05$) reduced from 114±5 mmHg to repetitive responses of 30±4 mmHg and for Ap_5A from 128±12 to 27±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. After 16 min a complete recovery from the desensitization was achieved. The transient vasoconstriction by Ap_5A and α,β-meATP was after 16 min without activation of any P_2-receptor was not significantly ($P>0.05$) different from the control response induced by Ap_5A and α,β-meATP.

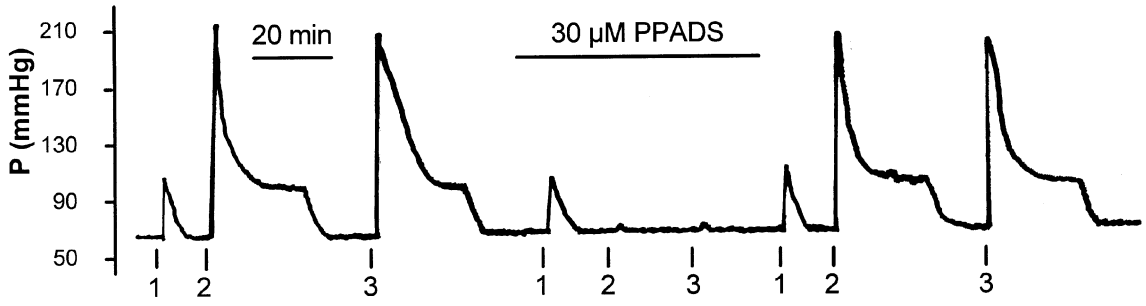

Figure 3 Effect of PPADS on agonist induced vasoconstriction. Representative trace out of six similar experiments showing changes in perfusion pressure in the rat isolated perfused kidney induced by bolus injection (100 μl) of ANGII and by permanent perfusion with Ap_5A and α,β-meATP in the absence and presence of PPADS (30 μM). (1) ANGII 10 nM (100 μl bolus), (2) Ap_5A 1 μM, (3) α,β-meATP 1 μM ($n=6$). For abbreviations, see text.

Table 2 Transient vasoconstrictor and sustained vasoconstrictor responses in the presence and absence of PPADS 30 μM to 1 μM permanent perfusion with adenine- and diadenosinephosphates

	Tyrode (ΔmmHg)		*Tyrode + 30 μM PPADS* (ΔmmHg)		*Tyrode washout PPADS* (ΔmmHg)	
Compound	*Transient**	*Sustained**	*Transient**	*Sustained**	*Transient**	*Sustained**
Ap_5A 1 μM	160.0±14	30.0±6.2	3.2±0.8†	0.0±0.0†	152.0±10.2#	28.2±3.2#
Ap_6A 1 μM	106.5±12.2	7.2±1.8	0.0±0.0†	0.0±0.0†	108.3±12.1#	7.9±2.1#
α,β-meATP 1 μM	158.0±7.0	27.0±2.8	3.5±1.2†	0.0±0.0†	149.0±18.0#	28.2±4.5#
β,γ-meATP 1 μM	48.8±6.2	9.8±1.5	0.0±0.0†	0.0±0.0†	54.2±4.6#	9.7±2.1#
ANGII 10 nM	46.6±5.3	–	43.5±4.8	–	46.3±6.3	–

Changes in perfusion pressure (mmHg) in the rat isolated perfused kidney induced by permanent perfusion with Ap_5A, Ap_6A, α,β-meATP and β,γ-meATP each 1 μM and induced by bolus injection (100 μl) of ANGII in the absence and presence of the P_{2X}-receptor antagonist PPADS (30 μM). Values are given as means±s.e.mean ($n=6$). †$P<0.05$ PPADS vs control and #$P<0.05$ washout of PPADS vs PPADS. *Transient = transient vasoconstriction and sustained = sustained vasoconstriction.

Experiments with permanent perfusion with α,β-meATP

Permanent perfusion with α,β-meATP 10 μM caused an initial transient vasoconstriction and a sustained vasconstriction. Addition of Ap_5A (10 μM) (Figure 6) to a perfusate containing α,β-meATP (10 μM) in Tyrode's solution did not significantly increase permanent perfusion pressure (sustained vasoconstriction induced by Ap_5A 30.0±6.2 mmHg and by Ap_5A + α,β-meATP 30.5±6.5 mmHg). Responses to ANGII were not affected by permanent perfusion with (62.3±4.7 mmHg) or without (59.5±3.5 mmHg) α,β-meATP (10 μM).

Discussion

In addition to the transient vasoconstriction, which has been shown earlier (van der Giet *et al*., 1997), our present study demonstrates a sustained vasoconstriction induced by Ap_5A and Ap_6A in the rat isolated perfused rat kidney. Similar results have been reported for α,β-meATP in rat renal vessels (Inscho *et al*., 1998). Until now it is not clear which receptor is responsible for the sustained vasoconstriction induced by α,β-meATP and the two diadenosine polyphosphates, Ap_5A and Ap_6A, which according to our data activate the same receptors as α,β-meATP.

From the data in literature, the responses of the P_{2X1}-receptor to various nucleotides especially α,β-meATP and β,γ-meATP appear to be very similar to the responses obtained in our study (Evans *et al*., 1995). Indeed, the P_{2X1}-receptor subtype has been repeatedly demonstrated in vascular smooth muscle cells and especially in renal vasculature (Bo & Burnstock, 1993; Valera *et al*., 1994; Chan *et al*., 1998). However, one finding argues against a crucial role of the P_{2X1} subtype for the sustained vasoconstriction. Whereas the P_{2X1}-receptor is known to show desensitization independent of the agonist concentration (Evans *et al*., 1998), our findings show desensitization only with higher agonist concentrations, indicating that lower agonist concentrations activate a

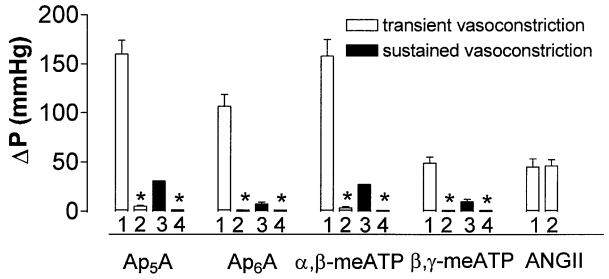

Figure 4 Effect of suramin on agonist induced vasoconstriction. Changes in perfusion pressure (mmHg) in the rat isolated perfused kidney induced by permanent perfusion with 1 μM of each agonist in the absence (open columns for transient vasoconstriction (1), filled columns for sustained vasoconstriction (3)) and presence of suramin (50 μM) (open columns for transient vasoconstriction (2), filled columns for sustained vasoconstriction (4)) 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.

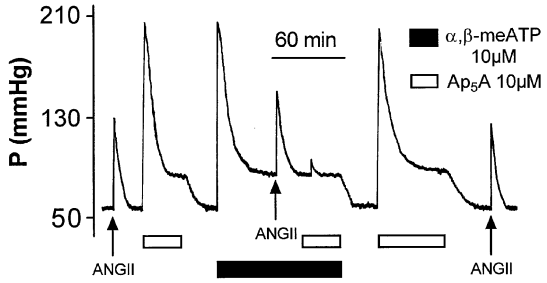

Figure 6 Original tracing showing the effect of α,β-meATP on Ap_5A induced vasoconstriction. Representative trace out of five similar experiments showing changes in perfusion pressure in the rat isolated perfused kidney induced by permanent perfusion with Ap_5A (10 μM) without (open column) and with (filled column) permanent perfusion with α,β-meATP (10 μM). Bolus of ANG II was 20 nM. For abbreviations see text.

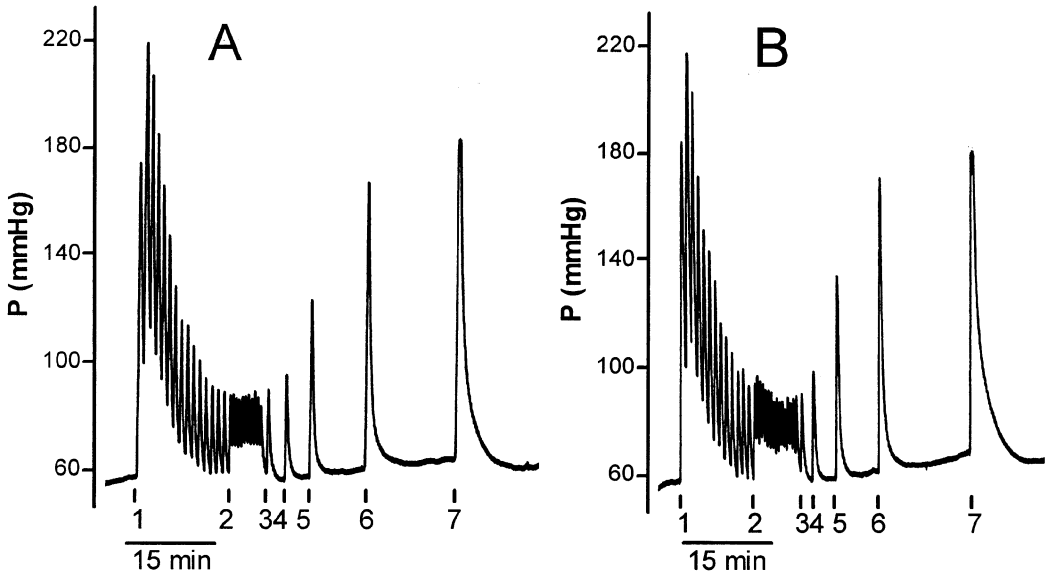

Figure 5 Original tracing showing desensitization of agonist induced vasoconstriction. Representative trace out of five similar experiments showing changes in perfusion pressure in the rat isolated perfused kidney induced by repetitive bolus applications of Ap_5A (10 nmol) (A) and α,β-meATP (10 nmol) (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.

different purinoceptor subtype and that higher agonist concentrations activate both the P_{2X1} and an additional purinoceptor subtype.

Given the P_{2X1}-receptor subtype may not mediate the sustained vasoconstriction the question arises whether one of the other known P_{2X}-receptor subtypes or a P_{2Y}-receptor may account for the sustained vasoconstriction. The P_{2X2}-receptor can be excluded because this receptor is insensitive for activation by α,β-meATP (Evans *et al*., 1995) and the vasoconstrictive effects for Ap_5A and Ap_6A can be mimicked by α,β-meATP. In addition the P_{2X2}-receptor has not been shown to be expressed in kidneys. Likewise the P_{2X3}-receptor which is activated by α,β-meATP and which shows a strong but concentration-dependent desensitization to α,β-meATP is not reported to be expressed in kidneys (Lewis *et al*., 1995; Chen *et al*., 1995). P_{2X4} has been shown to be activated by high agonist concentrations of α,β-meATP but its activation is not blocked by PPADS or suramin (Bo *et al*., 1995). P_{2X5} and P_{2X6} have been shown not to be activated *via* α,β-meATP (Collo *et al*., 1996) and therefore not *via* Ap_5A or Ap_6A either. The P_{2X7} is a cytolytic pore forming channel and is expected to play a role in apoptosis and cell death (Surprenant *et al*., 1996). This receptor subtype has not been shown to play a role in inducing vasoconstriction.

Given the P_{2X1}-receptor as the only subtype inducing the transient vasoconstriction as observed so far, none of the other P_{2X}-receptor subtypes fits our findings in renal vasculature. Ap_5A and Ap_6A induce an α,β-meATP like sustained vasoconstriction which is antagonized by suramin and PPADS. Interestingly an α,β-meATP sensitive, non-desensitizing phentoype of P_{2X}-receptor has also been observed in neuronal tissue (Khakh *et al*., 1995), but not cloned and classified up to now. To explain the functional properties different from those of P_{2X}-receptor subtypes cloned and expressed in oocytes or mammalian cells, Surprenant (1996) proposed the association of heteromeric subunits to form a receptor with new functional properties. Such a receptor phenotype may be generated from a new gene product, or, alternatively by heteropolymerization of subunits of several P_{2X}-receptor subtypes. Indeed, it was demonstrated that coexpression of two P_{2X}-receptor subtypes in the same cells resulted in a P_{2X}-receptor phenotype exhibiting functional properties of both subtypes (Radford *et al*., 1997; Lewis *et al*., 1995). Recently Nori *et al*. (1998) could show a coexpression mRNA for P_{2X1}-, P_{2X2}- and P_{2X4}-receptors in rat vascular smooth muscle cells. Additionally the genes expressing the various P_{2X}-receptors were shown to be different in a splice variant ($P_{2X2\text{-}2}$) carrying a 207 bp deletion in the intracellular C-terminus (Brandle *et al*., 1997). This isoform of the P_{2X2}-receptors was detected in rat tissue using RT–PCR. Furthermore, the desensitization of the P_{2X1}-receptor could be removed by introducing segments from the P_{2X2}-receptors (Werner *et al*., 1996).

Furthermore a P_{2Y}-receptor subtype may underly the sustained vasoconstriction in our study. The P_{2Y2}-subtype, which is involved in vasoconstriction in rat juxtamedullary afferent arterioles, cannot be blocked by PPADS (Charlton *et al*., 1996) and hence is unlikely to underly the sustained vasoconstriction in our experiments (Inscho *et al*., 1998). The P_{2Y4}-receptor which is expressed in rat kidneys (Harden *et al*., 1998) has been identified and pharmacologically characterized in vascular smooth muscle cells (Harper *et al*., 1998). The P_{2Y4}-receptor subtype is insensitive to inhibition to suramin and PPADS and therefore not a candidate for a sustained vasoconstriction. The P_{2Y6}-receptor can be antagonized by PPADS and suramin as shown by Chang *et al*. (1995), but it is activated by uridinenucleotides mainly and not by α,β-meATP. The remaining P_{2Y}-receptor subtypes P_{2Y3}, P_{2Y5}, P_{2Y7} and P_{2Y8} have not been identified in rat tissue at all. As a consequence it is unlikely that one of the known P_{2Y}-receptor subtypes account for the observed sustained vasoconstriction by Ap_5A and Ap_6A.

Due to the fact that α,β-meATP is only a weak agonist at the P_{2Y1}-receptor which is expected to induce vasodilation in vascular smooth muscle cells and that α,β-meATP is not reported to activate the remaining P_{2Y}-receptor subtypes P_{2Y2-8} we assume that a P_{2Y}-receptor is not responsible for the sustained vasoconstriction observed for Ap_5A and Ap_6A.

These results suggest the possibility that a naturally occurring chimeric receptor may exhibit properties from several subtypes or it cannot be dismissed that a further yet unidentified P_{2X}-receptor subtype may contribute to the effects on renal vasculature produced by Ap_5A and Ap_6A. The pattern of vasoconstriction observed in response to Ap_5A and Ap_6A lends support to the hypothesis that a permanently elevated vascular tone in man might be caused by vasoconstrictor diadenosine polyphosphates stimulating a P_{2X}-receptor subtype.

Whether a subgroup of essential hypertensive suffers from an increased vascular tone due to purinergic mechanisms, cannot be clarified yet. Either the determination of plasma levels of diadenosine polyphosphates or of purinoceptor density on vascular smooth muscle cells could help to answer this question.

In summary, the experiments showed that in renal vasculature Ap_5A, Ap_6A, α,β-meATP and β,γ-meATP elicited a vasoconstrictor response consisting of a transient and a permanent component. This pattern suggests the presence of a P_{2X}-receptor known to exist in vascular smooth muscle and different from already known P_{2X}-receptor subtypes. This receptor might play an important role in glomerular perfusion pressure, blood pressure and consequently hypertension.

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