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Characterization of vascular P_2 purinoceptors in the rat isolated perfused kidney

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Abstract

In isolated, constant-pressure perfused rat kidneys at basal vascular tone, injected P_2 purinoceptor agonists evoked vasoconstriction (α,β-methylene ATP > β,γ-methylene ATP > ATP-γ-S > 2-methylthio ATP > ATP > ADP = UTP). In kidneys with raised tone, the nucleotides produced vasodilatation at low doses (2-methylthio ATP > ADP = ATP = ATP-γ-S > UTP; α,β-methylene ATP and β,γ-methylene ATP, inactive), and constriction at high doses (α,β-methylene ATP > β,γ-methylene ATP > ATP-γ-S > 2-methylthio ATP > ADP = ATP > UTP). Removal of the endothelium abolished the dilator responses to the agonists. N^G-Nitro-L-arginine methylester (L-NAME, 5×10^{-5} M) abolished vasorelaxation in response to 2-methylthio ATP, a response which could be restored by additional L-arginine (3×10^{-3} M). Both vasodilatation and constriction due to the nucleotides remained unaffected by indomethacin (3×10^{-6} M), *S*-(*p*-nitrobenzyl)-6-thioinosine (3×10^{-5} M) and 8-phenyltheophylline (3×10^{-6} M). Pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS, $1-3 \times 10^{-6}$ M), inhibited vasoconstriction caused by α,β-methylene ATP, 2-methylthio ATP and UTP, but not by ATP. Suramin (3×10^{-5} M) caused a rightward shift of the dose-response curves for constriction caused by α,β-methylene ATP (27-fold) and 2-methylthio ATP (5-fold), whereas the ATP curve was shifted to the left (20-fold). With Evans blue (10^{-5} M), vasodilatation due to the nucleotides was abolished and the dose-response curves for vasoconstriction caused by ATP and UTP were shifted left more than 100-fold, the effect to both could not be antagonized by PPADS (3×10^{-6} M). These results suggest: (1) the different rank orders of P_2 purinoceptor agonist potencies for constrictor and dilator responses in perfused rat kidney are consistent with mediation via P_{2X} and P_{2Y} purinoceptors, respectively; (2) P_{2X} purinoceptors, selectively sensitive to blockade by PPADS, are located on vascular smooth muscle; (3) endothelial P_{2Y} purinoceptor stimulation results in vasodilatation involving NO synthesis but not release of prostanoids; (4) Evans blue, which appears to combine selective P_{2Y} purinoceptor blockade and strong inhibition of ecto-nucleotidases, potentiates vasoconstriction in response to the degradable nucleotides, ATP, 2-methylthio ATP and UTP; (5) additionally, Evans blue unmasks a PPADS-insensitive P_{2U} purinoceptor where the nearly equipotent nucleotides, ATP and UTP, can produce vasoconstriction.

Keywords: Kidney, perfused, rat; Purinoceptor P_{2X}; Purinoceptor P_{2Y}; Purinoceptor P_{2U}; Purinoceptor agonist; Purinoceptor antagonist

1. Introduction

On the basis of different rank orders of agonist potencies, cell surface P_2 purinoceptors, which mediate the effects of extracellular ATP, have been divided into at least five subtypes (Abbracchio et al., 1993). The original classification of the P_2 purinoceptors into P_{2X} and P_{2Y} subtypes was based largely upon the selective agonism by α,β-methylene ATP at P_{2X}, and 2-methylthio ATP at P_{2Y} purinoceptors to induce functional excitatory and inhibitory responses, respectively, in various tissues (Burnstock and Kennedy, 1985). Further members of the P_2 purinoceptor family comprise platelet P_{2T}, mast cell P_{2Z} (Gordon, 1986), and P_{2U} purinoceptors (O'Connor, 1992). Generally, sensitivity of the response to nucleotide agonists in the order α,β-methylene ATP = β,γ-methylene ATP > ATP = 2-methylthio ATP > UTP is characteristic of the P_{2X} purinoceptor, whilst at the P_{2Y} subtype, agonist potency is in the order 2-methylthio ATP > ATP $\gg$ α,β-methylene ATP = β,γ-methylene ATP (for recent reviews, see Dalziel and Westfall, 1994; Fredholm et al., 1994). However, it is now well established that the potencies of some hydrolysable triphosphate agonists, e.g. ATP, 2-methylthio ATP and UTP, are greatly underestimated due to their rapid breakdown by ecto-nucleotidases, to which α,β-methylene ATP is resistant (Welford et al., 1987), particularly in isolated tissue experiments, demonstrating

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that the two broad patterns of agonist potencies originally used to designate subtypes of P_2 purinoceptors (Burnstock and Kennedy, 1985) need to be reassessed under conditions when nucleotide breakdown is prevented (Kennedy and Leff, 1995; McKechnie et al., 1995).

Generally, vascular P_{2X} purinoceptors are located on smooth muscle to mediate contraction, while P_{2Y} purinoceptors, present either on endothelial cells or on smooth muscle, are involved in vasodilatation (Burnstock, 1988; Ralevic and Burnstock, 1991a). Furthermore, in some tissues non-classical, so-called 'pyrimidine' or P_{2U} purinoceptors exist at which UTP and ATP cause endothelium-dependent vasodilatation, e.g. rat mesenterial bed and rabbit aorta (Ralevic and Burnstock, 1991b; Chinellato et al., 1992), rat aorta and hamster mesenteric arterial bed (Dainty et al., 1991; Ralevic and Burnstock, 1995) or vasoconstriction, e.g. in rabbit basilar artery (Von Kügelgen and Starke, 1990). In the case of the rat renal vasculature, exogenous administration of P_2 purinoceptor agonists can either increase or decrease vascular resistance, depending upon which subtype of P_2 purinoceptor, P_{2X} or P_{2Y}, is activated (Churchill and Ellis, 1993). Furthermore, it has been shown that ATP or a related nucleotide is co-released with (−)-noradrenaline during periarterial nerve stimulation in rat kidney and activate postjunctional P_{2X} purinoceptors and α_1-adrenoceptors, respectively, to evoke renal vasoconstriction (Schwartz and Malik, 1989; Rump et al., 1990).

One purpose of this study was to further characterize these P_2 purinoceptors in the isolated, constant-pressure perfused rat kidney by determining the different rank orders of agonist potencies for vasodilatation and/or vasoconstriction in preparations at basal or raised vascular tone after bolus injections of ATP, its analogues α,β-methylene ATP, β,γ-methylene ATP, ATP-γ-S and 2-methylthio ATP as well as ADP and UTP. In kidneys with tone raised by (−)-noradrenaline, the responses to the nucleotides were also investigated after endothelium removal by means of detergent and after inhibition of either nitric oxide (NO) synthesis by N^G-nitro-L-arginine methyl ester (L-NAME) or of cyclooxygenase by indomethacin in order to determine the vascular location of the different P_2 purinoceptors and the mediator(s) involved in vasodilatation. In order to investigate the contribution of adenosine to the responses to ATP, experiments were conducted with the adenosine receptor antagonist, 8-phenyltheophylline, and the adenosine uptake inhibitor, *S*-(*p*-nitrobenzyl)-6-thioinosine, known to block and enhance, respectively, the effects of adenosine possibly formed from ATP degradation by ecto-nucleotidases. The P_2 purinoceptor blocking drugs, pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) (Lambrecht et al., 1992; Ziganshin et al., 1994; Windscheif et al., 1994; Bültmann et al., 1995) and reactive blue 2 (Burnstock and Warland, 1987), were tested for their ability to selectively attenuate the responses to either P_{2X} or P_{2Y} purinoceptor stimulation in the rat renal vasculature. Additionally, we investigated the functional effects elicited by suramin (Hoyle et al., 1990) and Evans blue (Bültmann and Starke, 1993), two drugs known to combine both P_2 purinoceptor blockade and inhibition of ecto-nucleotidases (Hourani and Chown, 1989; Crack et al., 1994; Bültmann et al., 1995), on the renovascular responses to injected agonists.

2. Materials and methods

2.1. Rat isolated perfused kidney

The experiments were performed on kidneys taken from normotensive rats (Sprague-Dawley, male, 400–450 g, Wiga, Sulzfeld, Germany), similarly to the method described previously (Eltze et al., 1993). Briefly, after the aorta adjacent to the left renal artery had been cannulated and the abdominal vena cava cut, the kidney was removed and perfused in a single-pass, non-recirculating system at a constant pressure of 100 cm H_2O with prewarmed (37°C) Tyrode solution of the following composition (mM): NaCl 137.0, KCl 2.7, $CaCl_2$ 1.25, $MgCl_2$ 1.1, $NaHCO_3$ 12.0, NaH_2PO_4 0.42, Ca-EDTA 0.026 and glucose 5.6, gassed with a mixture of 95% O_2-5% CO_2. The prerenal perfusate flow was measured continuously using an electromagnetic flowmeter.

2.1.1. Effect of P_2 purinoceptor agonists

Each experiment consisted of a 40 min equilibrium period during which renal perfusate flow at basal vascular tone stabilized at 17.9 ± 1.9 ml/min; mean ± S.D., $n = 51$). To avoid possible receptor desensitization, the purinoceptor agonists (100 μl aqueous bolus) were injected within 2 s at intervals of 6–8 min into the renal inflow tract and the resulting decrease in perfusion flow was recorded. For practical reasons, bolus injections were used instead of time and drug consuming continuous infusions to quantify the responses to P_2 purinoceptor agonists. In raised vascular tone experiments, a second reservoir containing the vasoconstrictor agent, (−)-noradrenaline (2.5×10^{-7} M, additionally containing 10^{-4} M ascorbic acid), which was at a concentration appropriate to reduce renal perfusate flow between 40–60% (resulting flow = 9.3 ± 1.3 ml/min; mean ± S.D., $n = 51$), was connected via a three-way stopcock to continuously perfuse the kidney. Once the resulting constriction had stabilized, increasing doses of the purinoceptor agonists were injected and the resulting change in perfusion flow was recorded. Prior to and at the end of each series of test drug administration, endothelial integrity was validated by a maximally effective bolus injection of the muscarinic receptor agonist, arecaidine propargyl ester (3×10^{-7} mol), which caused a nearly complete reversal of renovascular flow to basal values (Eltze et al., 1993). The kidneys were repeatedly constricted by (−)-noradrenaline in cycles of 1.5 h. Each

preparation was used to evaluate the responses of the renal vasculature to maximally four different agonists in random order, provided that (a) the vasoconstriction due to (−)-noradrenaline could be exactly reproduced and (b) the vasodilator response to bolus injections of 3×10^{-7} mol arecaidine propargyl ester to verify endothelial integrity remained stable. No significant time-dependent changes in the vascular responses either to continuous perfusion with (−)-noradrenaline or to injected APE and nucleotides could be detected.

2.1.2. Removal of endothelium

Vascular endothelium was removed by perfusion of the kidney for 5 min with 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS, 5–10 mg/ml) via an alternative perfusion line into the main cannula. The degree of endothelial damage was verified functionally by the inability of 3×10^{-7} mol arecaidine propargyl ester to elicit a vasodilatation of more than 7% in kidneys with raised tone (Eltze et al., 1993). Functional integrity of the vascular smooth muscle after detergent treatment was verified with nitroprusside (10^{-7} mol) which evoked a nearly maximal vasodilatation in raised-tone kidneys.

2.1.3. Experiments with L-NAME, indomethacin, 8-phenyltheophylline and S-(p-nitrobenzyl)-6-thioinosine

The effects of injected purinoceptor agonists were also investigated in kidneys with raised tone (2.5×10^{-7} M (−)-noradrenaline, unless otherwise stated) in the continuous presence of the NO synthase inhibitor, L-NAME (5×10^{-5} M), L-NAME (5×10^{-5} M) plus L-arginine (3×10^{-3} M), the cyclooxygenase inhibitor, indomethacin (3×10^{-6} M), the adenosine receptor antagonist, 8-phenyltheophylline (3×10^{-6} M), and the adenosine uptake inhibitor, *S*-(*p*-nitrobenzyl)-6-thioinosine (3×10^{-5} M). L-NAME (5×10^{-5} M) enhanced the vasoconstriction caused by 2.5×10^{-7} M (−)-noradrenaline to 87 ± 6% (mean ± S.D., $n = 8$), suggesting an involvement of NO in the maintenance of rat renovascular tone (Churchill and Ellis, 1993). Thus, in the experiments with L-NAME (5×10^{-5} M), a reduction in the concentration of (−)-noradrenaline to 5×10^{-8} M was necessary which decreased the perfusion flow by 42 ± 8% (mean ± S.D., $n = 15$). However, in combination experiments with L-NAME (5×10^{-5} M) plus L-arginine (3×10^{-3} M), it was necessary to increase the concentration of (−)-noradrenaline to that previously used (2.5×10^{-7} M).

2.1.4. Effect of P_2 purinoceptor antagonists

In antagonist experiments, dose-response curves for purinoceptor agonists were performed in raised-tone kidneys perfused with 2.5×10^{-7} M (−)-noradrenaline together with pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS, 10^{-6}, 3×10^{-6} and 10^{-5} M), suramin (3×10^{-5} M), Evans blue (10^{-5} M) or reactive blue 2 (5×10^{-5} M).

2.1.5. Data analysis

At basal tone, the decrease in perfusion flow in response to successive injections of increasing doses of the purinoceptor agonists was calculated from each kidney and expressed as percent vasoconstriction. Agonist doses (mol) producing half-maximal vasoconstriction ($-\log ED_{50}$ values) were determined graphically from semi-logarithmic plots derived from 5–6 kidneys. For making dose-response curves for the agonists when tone was raised, the amplitudes for both reversal of vasoconstriction and further vasoconstriction were set at 100%, allowing effects in both directions in response to injection of purinoceptor agonists, i.e. vasodilatation or vasoconstriction, to be quantified as percent values. Under these conditions, vasorelaxation in response to the agonists investigated did not reach more than 50%, whereas vasoconstriction greatly exceeded this value. For assessment of the relative rank order of agonist potencies, these responses were calculated for different levels: $-\log (ED_{20})$ mol values relate to a 20% vasodilatation and $-\log (ED_{50})$ mol values to a 50% vasoconstriction. For all vasoconstrictor effects, the potency difference of the agonists indicated by (>) was based on a statistical analysis ($P < 0.05$). In case of vasodilator effects, where shallow dose-response curves were obtained for the agonists, inevitably only the numerical values could be used for defining their potency rank order. However, this should be regarded as a purely qualitative measure.

2.2. Drugs

ATP disodium salt (Serva); 2-methylthio ATP tetrasodium salt (RBI, Cologne, Germany); α,β-methylene ATP lithium salt, β,γ-methylene ATP sodium salt, ADP sodium salt, UTP sodium salt and adenosine 5′-*O*-(3-thiotriphosphate) tetralithium salt (ATP-γ-S), adenosine, N^G-nitro-L-arginine methylester (L-NAME), Evans blue, reactive blue 2, 8-phenyltheophylline, *S*-(*p*-nitrobenzyl)-6-thioinosine (Sigma, Munich, Germany); suramin (Bayer); pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) and arecaidine propargyl ester hydrobromide were gifts from Professor G. Lambrecht (Frankfurt/M, Germany).

3. Results

3.1. Effect of agonists on kidneys with basal vascular tone

In rat kidneys perfused at basal tone without vasoconstrictor, the average perfusion flow amounted to 17.9 ± 1.9 ml/min (mean ± S.D., $n = 51$) which remained constant over the 6 h duration of the experiment. The P_2 purinoceptor agonists, α,β-methylene ATP, β,γ-methylene ATP, ATP-γ-S, 2-methylthio ATP, ATP, ADP and UTP caused an instantaneously developing, dose-dependent and reversible reduction in perfusion flow when injected at doses

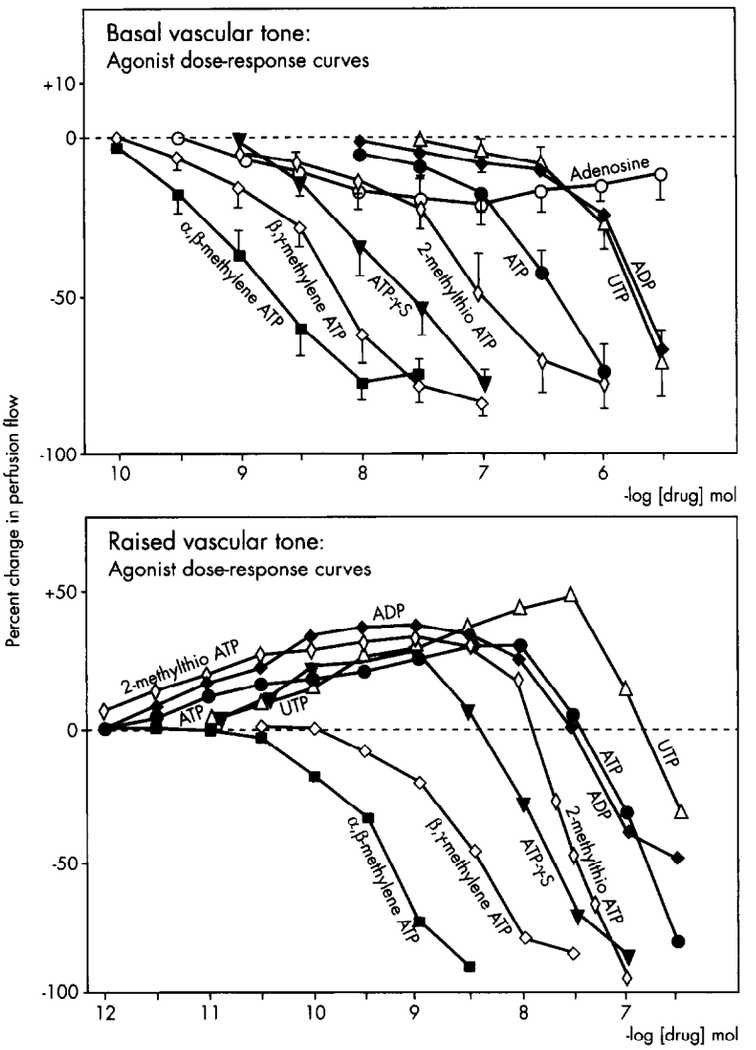

Fig. 1. Dose-response curves for the change in perfusion flow in response to nucleotides and adenosine in the isolated perfused rat kidney at basal vascular tone (*top*), and to nucleotides with vascular tone raised by 2.5×10^{-7} M (−)-noradrenaline (*bottom*). Means ± S.D. are given (for clarity, not depected at bottom, S.D. < 12%) for $n = 5$–6 kidneys for each agonist.

Table 1
Vasoconstrictor and vasodilator potencies of P_2 purinoceptor agonists in the isolated perfused rat kidney at basal and with vascular tone raised by 2.5×10^{-7} M (−)-noradrenaline

Agonist	Basal tone		Raised tone			
	Vasoconstriction ($-\log ED_{50}$ mol)		Vasodilatation ($-\log ED_{20}$ mol)		Vasoconstriction ($-\log ED_{50}$ mol)	
α,β-Methylene ATP	8.72 ± 0.13	(78 ± 6)	No effect		9.20 ± 0.12	(90 ± 4)
β,γ-Methylene ATP	8.20 ± 0.11	(84 ± 3)	No effect		8.42 ± 0.15	(83 ± 5)
ATP-γ-S	7.62 ± 0.19	(78 ± 5)	10.04 ± 0.23	(28 ± 5)	7.76 ± 0.10	(85 ± 5)
2-Methylthio ATP	7.00 ± 0.18	(78 ± 9)	10.92 ± 0.25	(34 ± 3)	7.48 ± 0.09	(94 ± 4)
ATP	6.42 ± 0.20	(75 ± 9)	9.75 ± 0.31	(31 ± 7)	6.84 ± 0.10	(80 ± 7)
UTP	5.75 ± 0.23	(69 ± 8)	9.75 ± 0.27	(48 ± 9)	< 6.50	(32 ± 17)
ADP	5.70 ± 0.22	(68 ± 7)	10.70 ± 0.22	(38 ± 4)	< 6.50	(48 ± 21)

$-\log ED_{50}$ mol values for vasoconstriction and $-\log ED_{20}$ mol values for vasodilatation were obtained by non-linear regression analysis of each individual tissue response. Percent maximal responses is shown in parentheses. Means ± S.E.M. are given for $n = 5$–6 kidneys for each experimental series.

of 10^{-10}–3×10^{-6} mol into the renal inflow tract at intervals of 6–8 min. In general, the peak effect of vasoconstriction was achieved within 10 s after drug injection and then declined within 1–2 min. Desensitization of the responses, even to α,β-methylene ATP at high doses, was not observed because of the short injection time, the rapid stabilization of the response to the injected agonists and their rapid washout during continuous perfusion of the kidney with drug-free solution. No difference in response was observed on testing α,β-methylene ATP at gradually increasing or decreasing doses. The maximal vasoconstriction obtained with the agonists amounted to 70–80%, whereas the less potent agonists, ADP and UTP, did not evoke a more than 69% reduction in perfusion flow. Adenosine did not elicit vasoconstriction of more than 20%, a response which diminished with even higher doses. The rank order of potency of the nucleotides was: α,β-methylene ATP > β,γ-methylene ATP > ATP-γ-S > 2-methylthio ATP > ATP > ADP = UTP. The dose-response curves obtained for the agonists are depicted in Fig. 1 (top) and the doses necessary to evoke a half-maximal vasoconstriction ($-\log ED_{50}$ mol values) are summarized in Table 1.

3.2. Effect of agonists on kidneys with raised tone

In kidneys with tone raised by 2.5×10^{-7} M (−)-noradrenaline, which reduced the renal perfusion flow by 40–60% (resulting flow = 9.3 ± 1.3 ml/min; mean ± S.D., $n = 51$), the nucleotides produced biphasic dose-response curves (Fig. 1, bottom): vasodilatation of 30%–50% of maximum at low doses (rank order of potency: 2-meth-

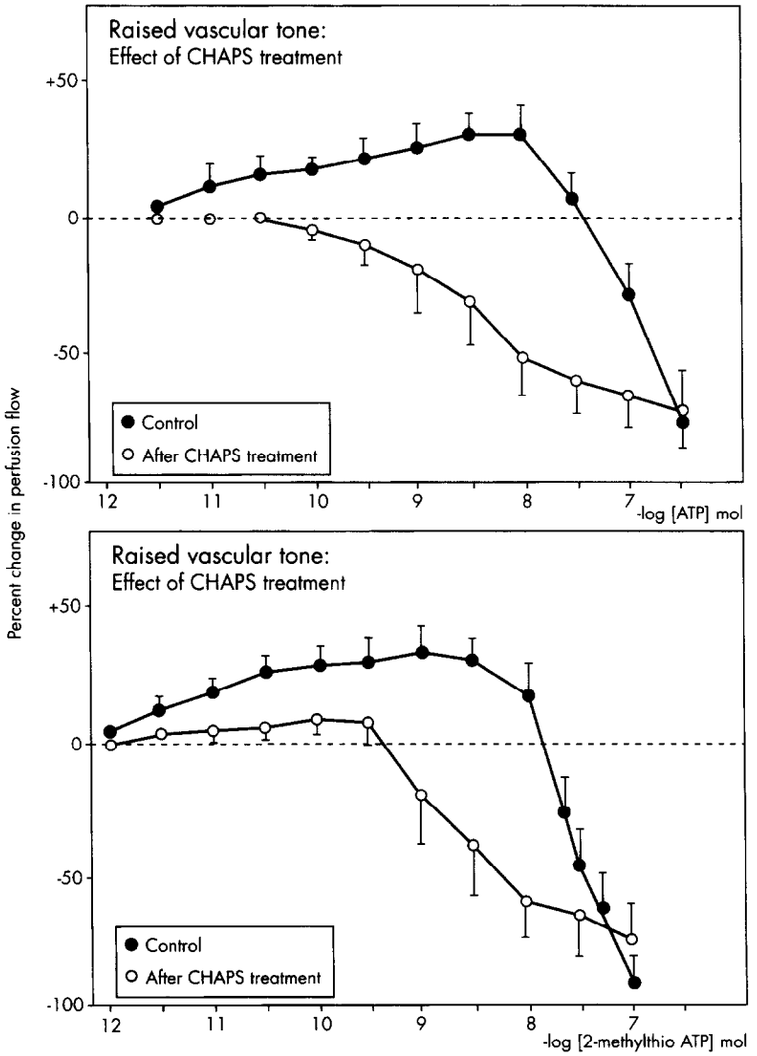

Fig. 2. Dose-response curves for the change in perfusion flow in response to ATP (*top*) and 2-methylthio ATP (*bottom*) in the isolated perfused rat kidney with vascular tone raised by 2.5×10^{-7} M (−)-noradrenaline (control), and after removal of the endothelium by detergent (CHAPS treatment). Means ± S.D. are given for $n = 5$–6 kidneys for the agonists.

ylthio ATP > ADP = ATP = ATP-γ-S > UTP; α,β-methylene ATP and β,γ-methylene ATP being inactive), followed by up to 90% vasoconstriction at high doses (rank order of potency: α,β-methylene ATP > β,γ-methylene ATP > ATP-γ-S > 2-methylthio ATP > ATP = ADP > UTP). Due to the increased drug removal during increased perfusion flow after injection of low doses, the dose-response curves of the nucleotides for vasodilatation were extremely shallow and spanned approximately three orders of magnitude thereby limiting their own maximal vasodilator effect. Conversely, steady state conditions for the injected agonists were apparently more efficiently reached during decreased perfusion flow after injection of high doses resulting in steep dose-response curves and greater maximal vasoconstrictor effects.

3.2.1. Effect of agonists on raised-tone kidneys without endothelium

The role of endothelium in the vasodilator and vasoconstrictor responses to the nucleotides, ATP, 2-methylthio ATP and UTP, was studied after treatment of the kidney with CHAPS (5–10 mg/ml infused during 5 min). The success of endothelium removal was confirmed by the failure of arecaidine propargyl ester (3×10^{-7} mol) to evoke a vasodilatation of more than 7% whereas that induced by nitroprusside (80% at 10^{-7} mol) remained unchanged as compared to the controls (not shown). The treatment with CHAPS slightly enhanced the subsequent vasoconstrictor effect of (−)-noradrenaline (2.5×10^{-7} M) from 50 ± 17% to 69 ± 18% (mean ± S.D., $n = 7$). In kidneys so treated, vasodilator responses to low doses of

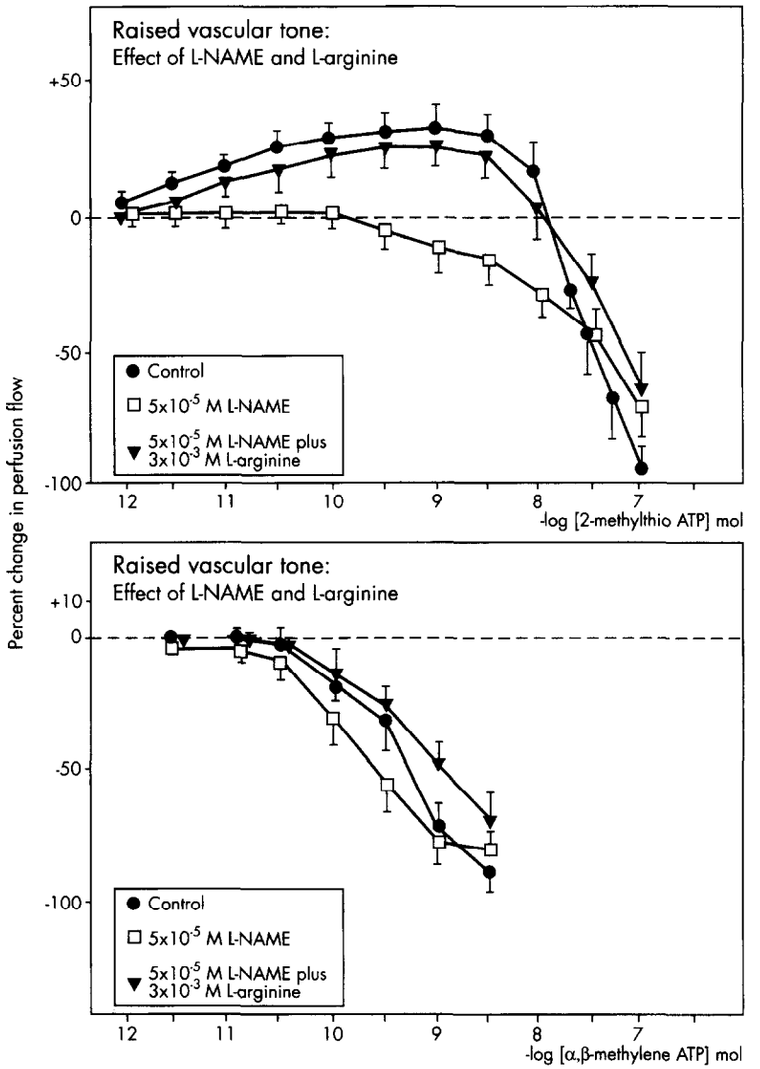

Fig. 3. Dose-response curves for the change in perfusion flow in response to 2-methylthio ATP (*top*) and α,β-methylene ATP (*bottom*) in the isolated perfused rat kidney with tone raised by 2.5×10^{-7} M (−)-noradrenaline (control), in the presence of 5×10^{-5} M L-NAME (in this case, the (−)-noradrenaline concentration was reduced to 5×10^{-8} M) and in the additional presence of 3×10^{-3} M L-arginine. Means ± S.D. are given for $n = 4$ kidneys for the agonists.

ATP, 2-methylthio ATP (Fig. 2, top and bottom) and UTP were abolished and their subsequent constrictor responses to higher, but not to maximal doses were slightly enhanced, whereas vasoconstriction by α,β-methylene ATP remained unaffected (not shown).

3.2.2. Effects of L-NAME, indomethacin, 8-phenyltheophylline and S-(p-nitrobenzyl)-6-thioinosine on raised-tone kidneys

Continuous perfusion of the kidney with 5×10^{-8} M (−)-noradrenaline plus L-NAME (5×10^{-5} M) produced a vasoconstriction of 42 ± 8% (mean ± S.D., $n = 15$). In the presence of L-NAME, a weak vasodilatation in response to injected arecaidine propargyl ester (3×10^{-7} mol) was still detectable, however, this effect amounted to less than 10% (not shown). L-NAME abolished the vasodilator effect of low doses of 2-methylthio ATP (10^{-12}–3×10^{-9} mol), but led to no significant change in vasoconstriction with high doses (3×10^{-8} and 10^{-7} mol). The inhibition by L-NAME of 2-methylthio ATP-induced vasodilatation could be nearly totally reversed by the additional presence of L-arginine (3×10^{-3} M) in the perfusion medium (Fig. 3, top). The vasoconstriction induced by α,β-methylene ATP was slightly enhanced by L-NAME, the effect of which could also be reversed by additional L-arginine (Fig. 3, bottom).

In the continuous presence of indomethacin (3×10^{-6} M) in the perfusion medium, the dose-response curves for the α,β-methylene ATP-, 2-methylthio ATP-, ATP- and UTP-evoked vasoconstriction and/or vasodilatation in kidneys with raised tone remained nearly unaffected. As an example, the lack of indomethacin effect on the dose-response curve of ATP is shown in Fig. 4.

Since ATP is known to be rapidly hydrolysed to adenosine, we investigated the possible contribution of this metabolite to the observed responses of ATP by adding two different modulators of adenosine effects, the adenosine receptor antagonist, 8-phenyltheophylline (3×10^{-6} M), or the adenosine uptake inhibitor, *S*-(*p*-nitrobenzyl)-6-thioinosine (3×10^{-5} M), into the perfusion medium. Neither agent significantly affected either the vasoconstriction elicited by 2.5×10^{-7} M (−)-noradrenaline or the ATP-induced vasorelaxation and vasoconstriction (Fig. 4), apparently ruling out any significant contribution of adenosine to the observed responses after ATP.

3.3. Effects of antagonists on P_{2X} and P_{2Y} purinoceptor-mediated responses in raised-tone kidneys

In kidneys with raised vascular tone, the presence of PPADS at 10^{-6}, 3×10^{-6} and 10^{-5} M did not significantly modify the vasoconstriction in response to (−)-noradrenaline. PPADS at 10^{-6} and 3×10^{-6} M, antagonized the vasoconstriction caused by α,β-methylene ATP; the resulting shifts of its dose-response curves were 2- and 5-fold, respectively (Fig. 5, top), yielding an apparent pK_B of 6.0. PPADS enhanced the vasodilatation in response to low doses of 2-methylthio ATP and caused a rightward displacement of the curve for vasoconstriction (Fig. 5, middle). When measured at the level of these parallel curves between 30 and 40% vasoconstriction, the shift was 4-fold at 10^{-6} M and 12-fold at 3×10^{-6} M of PPADS, giving an apparent pK_B of 6.5 for PPADS. Similarly, PPADS (10^{-6} M) caused a 3-fold shift of the dose-response curve for the vasoconstriction caused by UTP, resulting in an apparent pK_B of 6.3 (not shown). Interestingly, in the presence of up to 3×10^{-6} M PPADS, both vasodilator and constrictor responses to injected ATP re-

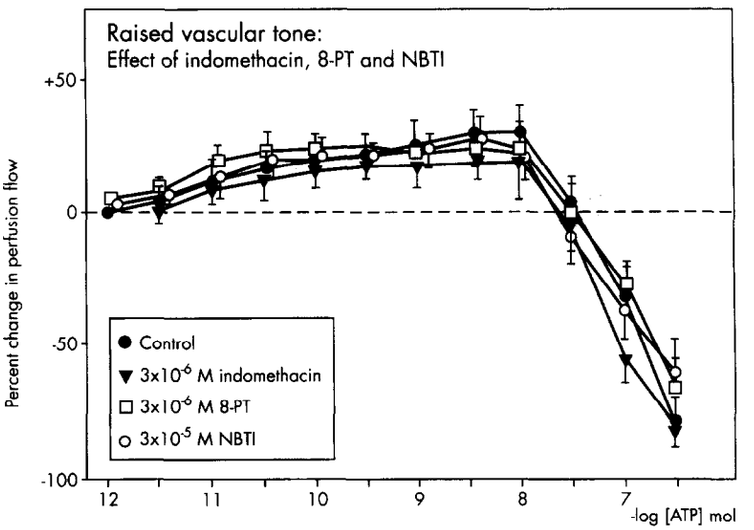

Fig. 4. Dose-response curves for the change in perfusion flow in response to ATP in the isolated perfused rat kidney with vascular tone raised by 2.5×10^{-7} M (−)-noradrenaline (control), and in the presence of 3×10^{-6} M indomethacin, 3×10^{-6} M 8-phenyltheophylline (8-PT) and 3×10^{-5} M *S*-(*p*-nitrobenzyl)-thioinosine (NBTI). Means ± S.D. are given for $n = 5$ kidneys for the agonist.

mained unchanged over its entire dose-response curve. However, in the presence of 10^{-5} M PPADS, the vasodilatation in response to ATP was significantly enhanced and, concomitantly, a 13-fold rightward shift of the dose-response curve for vasoconstriction was observed, resulting in an apparent pK_B of 6.1 (Fig. 5, bottom).

Suramin (3×10^{-5} M) did not change the vasoconstriction induced by 2.5×10^{-7} M (−)-noradrenaline but caused a parallel rightward shift of the dose-response curves of vasoconstriction in response to α,β-methylene

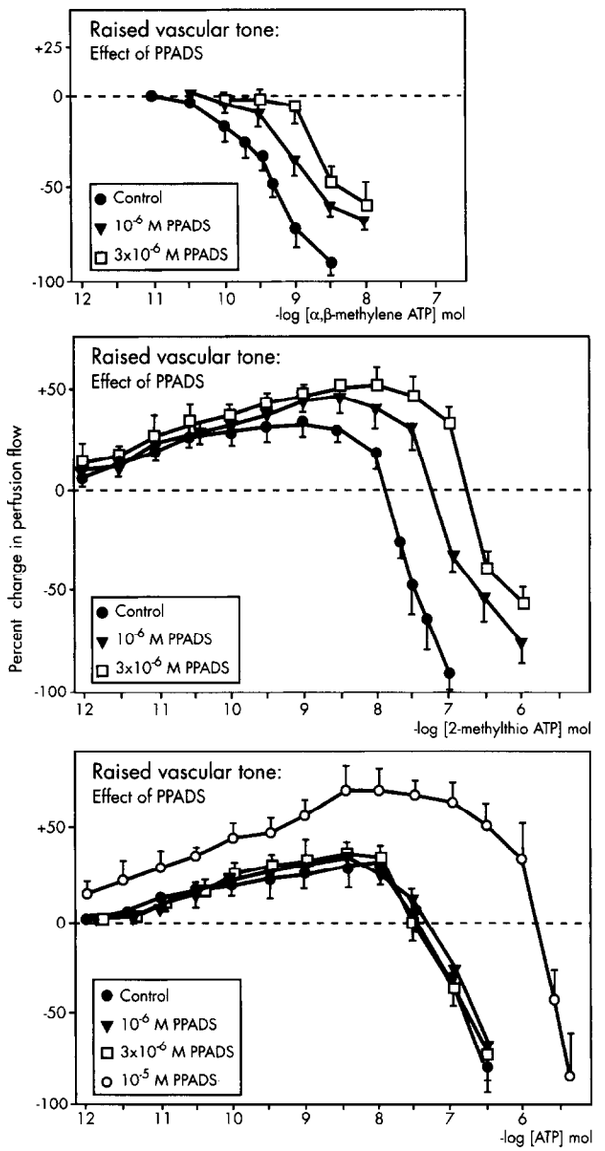

Fig. 5. Dose-response curves for the change in perfusion flow in response to α,β-methylene ATP (*top*), 2-methylthio ATP (*middle*) and ATP (*bottom*) in the isolated perfused rat kidney with vascular tone raised by 2.5×10^{-7} M (−)-noradrenaline (control), and in the presence of 10^{-6}, 3×10^{-6} and 10^{-5} M PPADS. Means ± S.D. are given for $n = 7$ kidneys for the agonists.

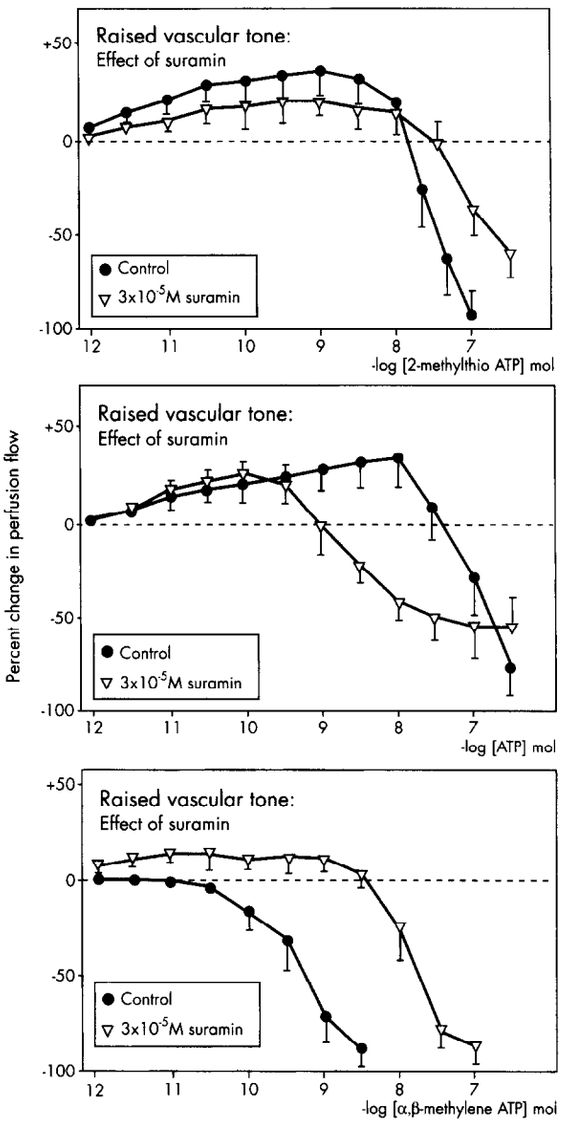

Fig. 6. Dose-response curves for the change in perfusion flow in response to 2-methylthio ATP (*top*), ATP (*middle*) and α,β-methylene ATP (*bottom*) in the isolated perfused rat kidney with vascular tone raised by 2.5×10^{-7} M (−)-noradrenaline (control), and in the presence of 3×10^{-5} M suramin. Means ± S.D. are given for $n = 5$–7 kidneys for the agonists.

ATP (27-fold) and 2-methylthio ATP (5-fold), whereas the curve for ATP was shifted to the left (20-fold). In contrast, the vasodilatation due to low doses of ATP remained unaffected and that with 2-methylthio ATP was partially inhibited (Fig. 6, top and middle). Interestingly, suramin caused low concentrations of α,β-methylene ATP to elicit a small, but significant vasorelaxation (Fig. 6, bottom).

When the perfusion fluid contained Evans blue (10^{-5} M), the relaxant responses to ATP, 2-methylthio ATP and UTP disappeared totally and their concentration-response curves for vasoconstriction were differentially shifted to

the left (Fig. 7, top). Particularly when measured at the level of 30% vasoconstriction, these leftward shifts were 210-fold for UTP, 150-fold for ATP and 25-fold for 2-methylthio ATP, whereas the dose-response curve of α,β-methylene ATP remained nearly unchanged. Thus, in the presence of 10^{-5} M Evans blue the potencies of ATP and UTP to cause vasoconstriction approached that of α,β-methylene ATP. However, in the presence of Evans blue (10^{-5} M), PPADS (3×10^{-6} M) failed to antagonize the vasoconstriction induced by these nucleotides (Fig. 7, bottom).

Reactive blue 2 (5×10^{-5} M) slightly diminished the vasoconstrictor effect at perfused (−)-noradrenaline (control: 9.3 ± 3.7 ml/min; mean ± S.D., $n = 12$; in the presence of 5×10^{-5} M reactive blue 2: 12.7 ± 2.0 ml/min; mean ± S.D., $n = 5$). In contrast to suramin, reactive blue 2 completely attenuated the vasodilator responses to the nucleotides, 2-methylthio ATP, ATP (Fig. 8, top and middle) and UTP (not shown). Similarly to suramin, reactive blue 2 caused differential rightward or even leftward shifts of the dose-response curves for vasoconstriction in response to injected α,β-methylene ATP (60-fold to the right) and 2-methylthio ATP (up to 10-fold to the right), whereas the curve for ATP only became less steep. The curve for UTP was shifted 3-fold to the left (not shown).

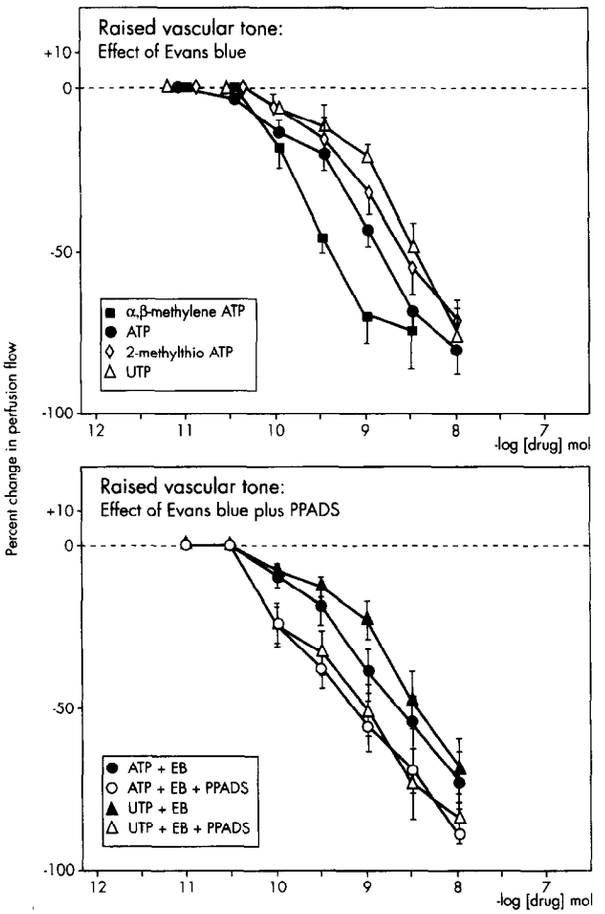

Fig. 7. Dose-response curves for the change in perfusion flow in response to α,β-methylene ATP, ATP, 2-methylthio ATP and UTP in the isolated perfused rat kidney with vascular tone raised by 2.5×10^{-7} M (−)-noradrenaline in the presence of 10^{-5} M Evans blue (*top*), and dose-response curves for ATP and UTP in the presence of 10^{-5} M Evans blue (EB) plus 3×10^{-6} M PPADS (*bottom*). Means ± S.D. are given for $n = 5$–6 kidneys (*top*) and $n = 4$ (*bottom*) for the agonists.

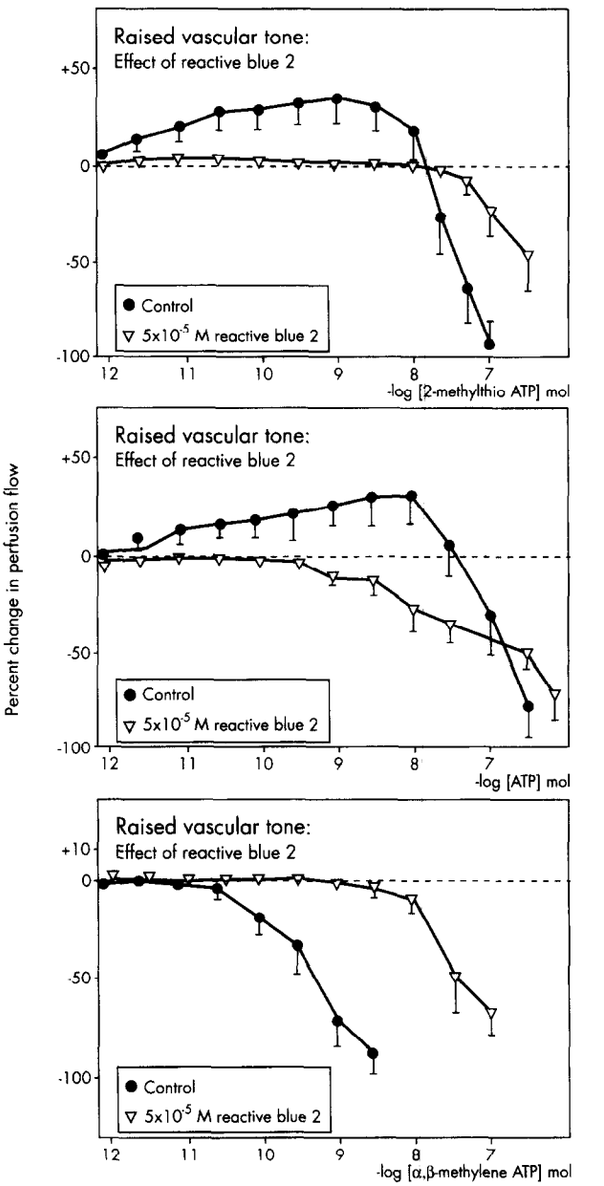

Fig. 8. Dose-response curves for the change in perfusion flow in response to 2-methylthio ATP (*top*), ATP (*middle*) and α,β-methylene ATP (*bottom*) in the isolated perfused rat kidney with vascular tone raised by 2.5×10^{-7} M (−)-noradrenaline (control), and in the presence of 5×10^{-5} M reactive blue 2. Means ± S.D. are given for $n = 5$–6 kidneys for the agonists.

4. Discussion

4.1. General considerations

In the past, much attention has been directed towards the purine nucleoside, adenosine, regarding its participation in effects on various renal functions. Recent studies, however, have provided considerable evidence that the purine nucleotide, ATP, may serve as a paracrine regulator of renal microvascular resistance, to modulate mesangial cell contraction, to alter epithelial ion transport and to influence the tubuloglomerular feedback mechanism (for a recent review, see Inscho et al., 1994).

Depending on the species investigated, ATP evokes different renovascular effects, e.g. increase in renal blood flow in the dog (Tagawa and Vander, 1970), but a decrease in rabbit (Needleman et al., 1970) and rat kidney (Sakai et al., 1979) that is not reduced by adenosine receptor blockade (Sakai et al., 1979; Inscho et al., 1994), demonstrating that the renal effects of ATP differ from those elicited by adenosine. Other than platelets, other non-neuronal sources of ATP within the kidney comprise endothelial and vascular smooth muscle cells, which can release ATP into the extracellular environment after appropriate stimuli. In addition, recent studies have provided evidence for the existence of a neuronal purinergic system in rat kidney, able to co-release different amounts of ATP and (−)-noradrenaline from separate vesicles, thereby causing renal vasoconstriction in response to low- and high-frequency sympathetic nerve stimulation, respectively (Schwartz and Malik, 1989; Rump et al., 1990). Finally, the renal vasculature of the rat shares with other vascular beds the ability to either constrict or dilate in response to exogenous administration of nucleotide agonists, depending upon which subtype of purinoceptor, P_{2X} or P_{2Y}, is activated (Churchill and Ellis, 1993).

4.2. Agonist studies

The two different orders of potencies of the nucleotides for constriction and dilatation in perfused rat kidney conform to the original classification of P_2 purinoceptors into two major subclasses, P_{2X} and P_{2Y} (Burnstock and Kennedy, 1985) and thus characterize the mediation of the responses via stimulation of P_{2X} and P_{2Y} purinoceptors, respectively: α,β-methylene ATP > β,γ-methylene ATP > ATP-γ-S > 2-methylthio ATP > ATP > UTP for constriction with both basal and raised vascular tone, with α,β-methylene ATP being nearly three orders of magnitude more potent than ATP, and 2-methylthio ATP > ADP = ATP = ATP-γ-S > UTP, α,β-methylene ATP and β,γ-methylene ATP being inactive, for vasodilatation in raised-tone kidneys. Nearly identical rank orders of potencies for the nucleotides have been found in other vascular preparations containing smooth muscle vasoconstrictor P_{2X} and endothelial dilator P_{2Y} purinoceptors, e.g. the rabbit ear artery (McKechnie et al., 1995), rabbit mesenteric artery (Burnstock and Warland, 1987) and rat mesenteric arterial bed (Ralevic and Burnstock, 1988; Windscheif et al., 1994).

4.3. Effect of endothelium removal and inhibition of nitric oxide or prostanoid synthesis

The vasorelaxation induced in rat kidney by the nucleotides was found to be completely abolished after removal of the endothelium by detergent treatment. This observation is consistent with results of studies on the location of vasodilator P_{2Y} purinoceptors in other vessels, e.g. rat aorta (White et al., 1985) and rat perfused mesenteric arterial bed (Ralevic and Burnstock, 1988), which have shown that endothelial P_{2Y} purinoceptor stimulation causes NO-mediated relaxation of vascular smooth muscle. In the present study, destruction of the endothelium enhanced P_{2X} purinoceptor-mediated vasoconstriction by 2-methylthio ATP and ATP, which is probably due to removal of the opposing relaxant response via P_{2Y} purinoceptors located on the endothelium.

In some vessels, e.g. guinea-pig and rabbit coronary artery (Keef et al., 1992), ATP has been found to evoke endothelium-independent relaxation directly by stimulation of P_{2Y} purinoceptors on smooth muscle. Additionally, it has been reported that there are, in some vascular preparations, other, non-classical types of P_2 purinoceptors, so-called 'pyrimidine' or 'nucleotide' receptors or P_{2U} purinoceptors (for a recent review, see O'Connor, 1992), at which the similarly potent nucleotides ATP and UTP mediate e.g. contraction of rabbit basilar artery (Von Kügelgen and Starke, 1990), endothelium-dependent dilatation of human pial vessels (Hardebo et al., 1987) or rat aorta (Dainty et al., 1991), and endothelium-independent dilatation of rat mesenteric arterial bed (Ralevic and Burnstock, 1991b) or rabbit aorta (Chinellato et al., 1992). Our findings exclude the possible existence of smooth muscle vasodilator P_{2Y} purinoceptors, otherwise the relaxation in response to the purine nucleotides and UTP should have been retained after endothelium destruction. Notably, based on the general rank order of agonists at P_{2U} purinoceptors, i.e. UTP = ATP > ATP-γ-S > 2-methylthio ATP > α,β-methylene ATP (O'Connor, 1992), which is quite the inverse of the profile of the agonists to cause dilatation in rat perfused kidney, participation of P_{2U} purinoceptors, at least in the vasodilator responses to the agonists in this organ, can be excluded.

Similarly, the 2-methylthio ATP-induced vasodilatation was abolished by L-NAME, an inhibition could be nearly totally reversed by the additional presence of L-arginine, supporting the hypothesis that NO, synthetized and released by endothelial cells, mediates P_{2Y} purinoceptor-induced vasodilatation of the rat kidney (Churchill and Ellis, 1993). The observation that L-NAME slightly potentiated vasoconstriction in response to low doses of α,β-methyl-

ene ATP may suggest that this agonist can also stimulate P_{2Y} purinoceptors and cause vasodilatation in rat renal vasculature, a phenomenon which was also detectable at low doses of α,β-methylene ATP when suramin was used to block vasoconstriction by this nucleotide (see below).

In contrast, both dilator and constrictor responses to 2-methylthio ATP, ATP and UTP as well as the vasoconstrictor response to α,β-methylene ATP remained unaffected by indomethacin, suggesting that the responses after P_{2Y} and P_{2X} purinoceptor stimulation were not mediated or modulated by products of cyclooxygenase metabolism. Such an involvement has recently been demonstrated in the P_{2Y} purinoceptor-mediated relaxation of bovine aortic collateral artery (Wilkinson et al., 1994).

4.4. Potential contribution of adenosine to the response to ATP

ATP is sequentially and rapidly hydrolyzed by a family of membrane-bound ecto-enzymes whose catalytic sites face the extracellular space, ultimately giving rise to adenosine. Within the kidney, these enzymes are found in abundance on endothelial and vascular smooth muscle cells (Gordon et al., 1989). Ecto-ATPase, which hydrolyzes extracellular ATP, has been identified on arteriolar and peritubular capillary endothelial cells located in rat renal cortex (Sabolic et al., 1992). Rat afferent and efferent arterioles but not arcuate arteries or mesangial cells possess ecto-5′-nucleotidase, which catalyzes the breakdown of AMP to adenosine (Le Hir and Kaissling, 1993). Thus, a breakdown product such as ADP, AMP or adenosine itself could theoretically contribute to the responses to ATP that we observed. Previously, it has been shown that adenosine, via activation of adenosine A_1 receptors, causes vasoconstriction of the smallest vessels of the rat renal vasculature, whereas the adenosine A_2 receptor-mediated vasodilatation occurs in the larger vessels (Holz and Steinhausen, 1987). In the present study, however, the involvement of adenosine can be ruled out. First, in accordance with similar experiments comparing the vasoconstrictor effect of adenosine and ATP in rat renal afferent arterioles (Inscho et al., 1994), adenosine did not elicit a vasoconstriction of more than 20%, a response which diminished with even higher doses. Thus, even if all the ATP injected into the rat kidney was hydrolyzed to adenosine, its resulting concentration would still be insufficient to explain the ATP-induced vasoconstriction of maximally 75% (this study). Second, it has been shown that the addition of the non-selective adenosine A_1/A_2 receptor antagonist, 1,3-dipropyl-8-*p*-sulfophenylxanthine, enhanced rather than decreased ATP-evoked vasoconstriction, suggesting some involvement of vasodilator adenosine receptors in modulating the vasoconstrictor ATP response via P_{2X} purinoceptors (Inscho et al., 1994). Third, any contribution of adenosine via stimulation of adenosine A_1 receptors to the vasoconstrictor effect evoked by ATP should have been blocked by 8-phenyltheophylline at a concentration of 3×10^{-6} M, that was sufficient for selective antagonism of adenosine A_1 receptor-mediated effects (pK_i at adenosine A_1 receptors = 6.7, pK_i at adenosine A_2 receptors = 5.4; Schwabe et al., 1985), an effect which, however, was not observed. Fourth, the relaxant and constrictor responses to ATP were not significantly influenced by the adenosine uptake inhibitor, *S*-(*p*-nitrobenzyl)-6-thioinosine. Thus, our present findings, together with those reported by Inscho et al. (1994), suggest that at least the vasoconstrictor response is mediated directly by ATP and is not a consequence of enzymatic breakdown to adenosine.

4.5. Antagonist studies and inhibition of nucleotide breakdown

Since purinoceptor classification based only on agonist rank order potencies is limited by potential differences in agonist efficacy, receptor reserve and, most decisively, by the extent of possible degradation by ecto-nucleotidases (Evans and Kennedy, 1994; Trezise et al., 1994; Kennedy and Leff, 1995), antagonist studies are indispensable for receptor classification. However, most of the currently available P_2 purinoceptor antagonists are unselective and their use is often hampered by the concomitant inhibition of ecto-nucleotidases, as shown with suramin and Evans blue (Welford et al., 1987; Crack et al., 1994). This additional property should not alter their antagonism against nucleotidase-resistant agonists, e.g. α,β-methylene ATP, but one would expect it to differentially modulate their observable inhibitory effects against degradable agonists, i.e. triphosphates such as ATP, 2-methylthio ATP and UTP.

Suramin has been described as an antagonist of P_{2X} (Dunn and Blakeley, 1988) and of P_{2Y} purinoceptors (Hoyle et al., 1990) and, in addition, it exhibits considerable inhibition of ecto-nucleotidase activity which potentially counteracts its antagonistic effect at both P_2 purinoceptor subtypes (Von Kügelgen and Starke, 1991; Beukers et al., 1995). Thus, suramin cannot be used to identify selectively responses mediated by specific P_2 purinoceptor subtypes (Fredholm et al., 1994). In the present study, suramin only weakly depressed P_{2Y} and P_{2X} purinoceptor-mediated vasorelaxation and vasoconstriction, respectively, in response to 2-methylthio ATP, while it caused a strong inhibition of the vasoconstrictor response to α,β-methylene ATP. Interestingly, suramin shifted the dose-response curve for vasoconstriction due to ATP to the left, which might reflect its ability to inhibit ecto-nucleotidases (Crack et al., 1994). It is plausible, that enhancement of the biophase concentration of ATP, with its functional consequence of counteracting P_{2X} and P_{2Y} purinoceptor blockade, could be of the cause for the unexpected failure of suramin to antagonize vasoconstrictor and vasodilator responses to ATP, respectively. The observation that, in the presence of suramin, low concentrations of α,β-methylene ATP evoked slight vaso-

dilatation could be taken to mean that this agonist is able to activate P_{2Y} purinoceptors, but with such low potency, that any tendency to evoke vasorelaxation is masked by the predominant P_{2X} purinoceptor-mediated constriction (Churchill and Ellis, 1993).

Evans blue has been shown to block P_{2X} purinoceptors in rat vas deferens (Bültmann and Starke, 1993) and differs from suramin by its more potent ecto-nucleotidase inhibition (Bültmann et al., 1995). However, it is as yet unknown whether it also exerts inhibitory effects on P_{2Y} purinoceptor-mediated responses. In the present study, Evans blue caused complete loss of the vasodilator response to the nucleotides. In accordance with earlier studies (Bültmann et al., 1995), Evans blue at 10^{-5} M caused strong leftward shifts of the dose-response curves for vasoconstriction in response to the degradable nucleotides, 2-methylthio ATP (24-fold), ATP (150-fold), and most prominently, for UTP (210-fold), while leaving the vasoconstrictor response to α,β-methylene ATP nearly unchanged. The different degrees of the shift possibly reflect different degradation liabilities of the nucleotides as substrates for ecto-nucleotidases in rat kidney. In the presence of Evans blue, α,β-methylene ATP, 2-methylthio ATP, ATP and UTP appeared nearly equipotent to evoke vasoconstriction, which resembled their equal potencies at P_{2X} purinoceptors in preparations under conditions where nucleotide breakdown is prevented (Kennedy and Leff, 1995). Theoretically, one part of the potentiation of the vasoconstrictor effect of ATP, 2-methylthio ATP and UTP in the presence of Evans blue could be due to concomitant blockade of the opposing relaxant response via P_{2Y} purinoceptors, similar to the block observed after endothelium removal. However, the parallel leftward shifts of the dose-response curves for ATP (150-fold) and 2-methylthio ATP (25-fold) evoked by Evans blue exceeded the weaker effect caused by endothelium removal with CHAPS (20- and 6-fold, respectively), which might indicate predominance of ecto-nucleotidase inhibition by Evans blue. Further experiments aimed at clear separation of these two components, i.e. inhibition of nucleotide breakdown and blockade of vasodilator P_{2Y} purinoceptors, both of which could contribute to the observable increase in vasoconstrictor potency of ATP, 2-methylthio ATP and UTP in the presence of Evans blue, were not performed.

Although reactive blue 2, within a narrow concentration range, has initially been proposed to be a relatively selective antagonist at P_{2Y} purinoceptors (Burnstock and Warland, 1987), it is now evident that it also antagonizes the effect of ATP and α,β-methylene ATP at P_{2X} purinoceptors in different tissues and, additionally, displays non-specific effects (Reilly et al., 1987; Bültmann and Starke, 1994; Connolly and Harrison, 1994). In view of such different findings with reactive blue 2, selective inhibition of either P_{2X} or P_{2Y} purinoceptor-mediated responses cannot be expected. In accordance with these observations, reactive blue 2 abolished P_{2Y} purinoceptor-mediated vasodilatation to the nucleotides. The reason why reactive blue 2 caused decreasing rightward and even leftward shifts of the dose-response curves for vasoconstriction in response to α,β-methylene ATP (60-fold to the right) > 2-methylthio ATP (10-fold to the right) > ATP (no observable shift) > UTP (3-fold to the left) could be interpreted as showing that reactive blue 2, in addition to P_{2Y} purinoceptor blockade, can inhibit differentially ecto-nucleotidase activity that counteracts P_{2X} purinoceptor blockade by the degradable nucleotides, although such an effect has not been described previously.

In contrast to the antagonists mentioned, PPADS has been described as a novel functionally selective antagonist at P_{2X} purinoceptors (Lambrecht et al., 1992; for a controversial finding see Brown et al., 1995) that differentiates P_{2X} from other P_2 purinoceptor subtype-mediated responses, e.g. at P_{2Y} and P_{2U} purinoceptors, at which the compound has an appreciably lower affinity (Ziganshin et al., 1994; Windscheif et al., 1994, 1995). In raised-tone kidney, PPADS at concentrations of 10^{-6} M and 3×10^{-6} M, inhibited the vasoconstriction caused by α,β-methylene ATP, 2-methylthio ATP and UTP in a nearly competitive manner, resulting in pA_2 values between 6.0 and 6.5, which is consistent with its affinity value of 6.34 at P_{2X} purinoceptors in rabbit vas deferens (Lambrecht et al., 1992). The observation that PPADS enhanced P_{2Y} purinoceptor-mediated vasodilatation by 2-methylthio ATP and ATP can easily be explained by the effective blockade of opposing vasoconstrictor P_{2X} purinoceptors present in the rat renal vasculature. Thus, this study confirmed earlier results regarding the ability of PPADS to selectively inhibit P_{2X} purinoceptor-mediated responses. However, an unexpected finding was that PPADS, at concentrations lower than 10^{-5} M, did not antagonize ATP-evoked vasoconstriction in raised-tone kidney. Two possible explanations can be considered. First, PPADS could fail to attenuate the vasoconstrictor effect of ATP because blockade of P_{2X} purinoceptors is balanced by concomitant inhibition of the rapid breakdown of this nucleotide by PPADS, which leads to enhancement of the biophase concentration of ATP. Combination of these two properties could theoretically result in 'self-cancellation' of the dose-response displacement (Crack et al., 1994). Second, ATP might have elicited its vasoconstrictor activity to a considerable extent via non-classical, so-called 'pyrimidine' or P_{2U} purinoceptors (O'Connor, 1992), which are not susceptible to antagonism by PPADS (Windscheif et al., 1994). However, PPADS has been shown to inhibit ecto-ATPase very weakly at 10^{-4} M concentrations (Windscheif et al., 1995). Therefore, the failure of PPADS at concentrations of $1–3 \times 10^{-6}$ M to antagonize the vasoconstrictor effect of ATP cannot be explained by ecto-nucleotidase inhibition. Otherwise, the same phenomenon should have been observed for 2-methylthio ATP and UTP, which did not happen under these conditions. Although it is difficult to interpret the failure of lower PPADS concentrations to antagonize the

vasoconstrictor response to ATP, an alternative explanation could be that ATP-evoked vasoconstriction in rat kidney has two components, i.e. predominantly via PPADS-insensitive P_{2U} and to a lesser extend via PPADS-sensitive P_{2X} purinoceptors, the latter component only being detectable when PPADS concentrations are increased up to 10^{-5} M. It may be consistent with this assumption that, when ecto-nucleotidase activity in rat kidney is inhibited by Evans blue, the nucleotides, ATP and UTP, nearly reach the potency of α,β-methylene ATP to cause vasoconstriction, thereby resembling the potency found in rabbit basilar artery, where the similarly potent ATP and UTP mediate contraction via activation of P_{2U} purinoceptors (Von Kügelgen and Starke, 1990). This hypothesis is further strengthened by the inability of the selective P_{2X} purinoceptor antagonist, PPADS (Lambrecht et al., 1992; Windscheif et al., 1994, 1995), to attenuate the vasoconstriction caused by ATP and UTP in the presence of Evans blue. The results obtained from the experiments in raised-tone kidneys suggest that ATP, with nucleotide breakdown both allowed and prevented, predominantly activates PPADS-insensitive P_{2U} purinoceptors. In contrast, the low or high potency of UTP to cause either PPADS-sensitive vasoconstriction or, in the presence of Evans blue, PPADS-insensitive vasoconstriction, respectively, might reflect its ability to activate both constrictor P_{2X} and P_{2U} purinoceptors in the rat renal vasculature.

4.6. Summary

In summary, the present study investigated the P_2 purinoceptors in resistance vessels of the rat isolated perfused kidney. The results indicate that the rat renal vasculature can either constrict or dilate in response to P_2 purinoceptor stimulation. The different rank orders of agonist potencies of the nucleotides for constriction and dilatation are typical of mediation by P_{2X} and P_{2Y} purinoceptors, respectively. Constrictor purinoceptors of the P_{2X} subtype and selectively sensitive to blockade by PPADS, are located on vascular smooth muscle. In addition, under conditions that prevent nucleotide breakdown by Evans blue, a P_{2U} purinoceptor is detectable in rat kidney, in which the highly potent ATP and UTP can produce PPADS-resistant vasoconstriction. The P_{2Y} subtype located on vascular endothelium mediates vasodilatation in rat kidney, which disappears after destruction of the endothelium by means of detergent. P_{2Y} purinoceptor stimulation involves NO synthesis but not the release of cyclooxygenase-derived mediators of vasodilatation. In endothelium-denuded rat kidney, P_2 purinoceptor agonists fail to evoke vasorelaxation at all.

Acknowledgements

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