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An *in vitro* analysis of purine-mediated renal vasoconstriction in rat isolated kidney

Terry P. Kenakin[1] & Nicholas B. Pike[2]

Department of Pharmacology, Burroughs Wellcome Company, 3030 Cornwallis Road, Research Triangle Park, North Carolina 27709, U.S.A.

1 In the rat isolated perfused kidney, 2-chloroadenosine and L-N^6-phenyl-isopropyl adenosine (L-PIA) produced a modest vasodilatation.

2 After kidneys had been pretreated with methoxamine (to elevate vascular tone) and forskolin (to activate adenyl cyclase and reduce vascular tone), both purine agonists produced vasoconstriction at low doses and vasodilatation at higher doses. This was consistent with the working hypothesis that vasoconstriction resulted from activation of A_1-purinoceptors mediating adenyl cyclase inhibition and vasodilatation from activation of A_2-purinoceptors stimulating adenyl cyclase.

3 These kidney preparations also demonstrated a marked potentiation of purine-mediated vasoconstriction in the presence of various concentrations of 8-*p*-sulpho-phenyltheophylline (8-SPT), a drug reported in the literature to be a competitive antagonist of A_1- and A_2-purinoceptors.

4 Maximal renal vasoconstriction to 2-chloroadenosine and L-PIA was observed in the presence of 10 mM 8-SPT; the fact that this vasoconstriction was sensitive to the selective A_1-receptor antagonist 8-(2-amino-4-chlorophenyl)-1,3-dipropylxanthine (PACPX) and that the order of potency of agonists for this effect was L-PIA > 2-chloroadenosine > D-PIA > N^6-ethylcarboxamide adenosine (NECA) was consistent with activation of vascular A_1-purinoceptors.

5 While these data are consistent with the hypothesis that purines activate vascular A_1- and A_2-receptors in the rat isolated kidney, the nature of the results did not allow definitive classification of the receptors mediating the purine effects.

Introduction

Adenosine has been implicated in the pathogenesis of renal ischaemia and acute renal failure (Churchill & Bidani, 1982; Churchill, 1982; Osswald, 1984; Bowmer *et al.*, 1986). The effect of purines on the renal vasculature may be relevant to this problem in view of the possible importance of decreased renal blood flow in the early stages of these conditions (Borner & Klinkman, 1980).

Previous studies on the renal effects of purines have utilized *in vivo* preparations in which the relative importance of modulation of neural vasomotor control, renin release and/or direct activation of purinoceptors could not easily be determined. The present experiments were designed to study the direct vascular effects of purines in the rat isolated kidney and to classify the receptors mediating these responses.

Methods

Kidney isolation

Male albino Wistar rats (300–350 g) were anaesthetized with sodium pentobarbitone (80 mg kg^{-1} i.p.); heparin (1200 U.S.P. units kg^{-1}) was injected via the jugular vein. From a midline and bilateral incision, the left kidney and renal artery were cleared of fat and connective tissue. The renal artery was cannulated with PE 50 tubing and immediately perfused with ice cold oxygenated (95% O_2/5% CO_2) Krebs-Henseleit solution of the following composition (in mM): Na^+ 143, K^+ 5.9, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 128, $H_2PO_4^-$ 2.2, SO_4^{2-} 1.2, HCO^- 25, D-glucose 10 at a rate of 4.3 ml min^{-1}. The kidney was removed and

[1] Present address: Department of Molecular Pharmacology, Glaxo Research, Labs, Five Morre Drive, Research Triangle Park, NC 27709, U.S.A.
[2] Present Address: School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY.

transferred to the perfusion apparatus; only animals with normal respiration at this stage were utilized.

Kidney perfusion

Kidneys were perfused (Harvard peristaltic pump single pass perfusion) for 30 min with warmed (37°C) oxygenated (95% O_2/5% CO_2) Krebs-Henseleit solution at a constant rate of flow to produce a basal perfusion pressure of 100 mmHg. Dose-response curves were obtained by perfusion of kidneys with Krebs-Henseleit solution containing a given concentration of agonist; perfusion solutions were changed after a steady-state response was obtained, or after 5 min in the absence of a response. For studies utilizing an antagonist, this was perfused for at least 15 min before perfusion with the agonist; the antagonist was present in all subsequent solutions containing the various concentrations of agonist. In kidneys pretreated with methoxamine (5 μM) and forskolin (1 μM), preparations were perfused with methoxamine until a maximal vasoconstriction was obtained; then, forskolin was added (methoxamine still present) until a steady-state reduction of the previous vasoconstriction was observed. Agonists were then tested on this preparation in the presence of both methoxamine and forskolin throughout the experiment when a steady-state had been attained.

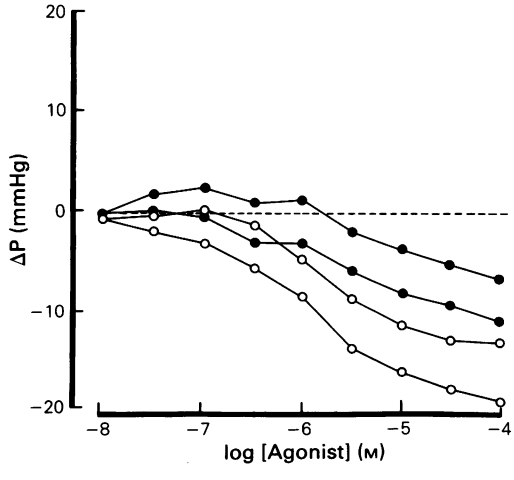

Figure 1 Responses of isolated perfused kidneys to 2-chloroadenosine and L-N^6-phenyl-isopropyl adenosine (L-PIA). Ordinates: changes in perfusion pressure (mmHg) from resting perfusion pressure of 100 mmHg. Abscissae: log molar concentrations of agonist in perfusate. Responses to 2-chloroadenosine (●) and L-PIA (○).

Drugs

Drugs used in this study were 2-chloroadenosine (Sigma Chemical Co., St. Louis, MO), L-N^6-phenyl-isopropyl adenosine (L-PIA), D-N^6-phenyl-isopropyl adenosine (D-PIA), 5-N-ethylcarboxamide adenosine (NECA), N^6-cyclohexyladenosine (CHA), 8-*p*-sulpho-phenyl-theophylline (8-SPT), 8-(2-amino-4-chlorophenyl)-1,3-dipropylxanthine (PACPX) (all from Research Biochemical, Inc., Wayland, MA), methoxamine HCl (Burroughs Wellcome Co., Research Triangle Park, N.C.) and forskolin (Behring Diagnostics, San Diego, CA).

Results

Purine-mediated renal vasoconstriction

As shown in Figure 1, 2-chloroadenosine and L-PIA

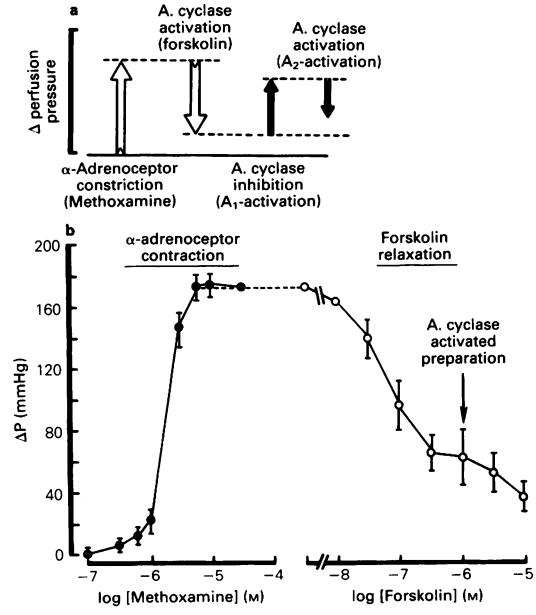

Figure 2 Adenyl cyclase activated preparation of isolated perfusion kidney. (a) Schematic representation of opposing effects of α-adrenoceptor plus A_1-purinoceptor activation vs adenyl cyclase (A. cyclase) A_2-purinoceptor activation on perfusion pressure in isolated kidneys. (b) Dose-response curves showing the vasoconstrictor effects of α-adrenoceptor activation by methoxamine ($n = 3$) and vasorelaxant effects of adenyl cyclase activation by forskolin ($n = 3$). The preparations used for all further studies were perfused with medium containing 5 μM methoxamine and 1 μM forskolin. Vertical lines represent s.e.mean.

produced a slight dose-dependent decrease in perfusion pressure with no obvious vasoconstriction. In view of data which indicate that purines inhibit adenyl cyclase (van Calker *et al.*, 1979; Londos *et al.*, 1980) and the fact that *in situ*, the kidney exists in an environment in which this enzyme is most likely partially activated (i.e. by circulating catecholamines), isolated kidneys with activated adenyl cyclase were prepared. In these kidneys, vascular tone was increased by α-adrenoceptor activation (methoxamine) and then subsequently decreased by adenyl cyclase activation with forskolin (Figure 2a). Under these conditions, purine inhibition of adenyl cyclase would cancel the forskolin effect thereby producing vasoconstriction, while activation of adenyl cyclase by purines would produce an effect additive to that of forskolin and induce further vasodilatation. Figure 2b shows concentration-response curves of kidneys to methoxamine and forskolin; to produce a preparation sensitive to purine inhibition or activation of adenyl cyclase, 5 μM methoxamine and 1 μM forskolin was infused into the vasculature.

Under these conditions, 2-chloradenosine (Figure 3a) and L-PIA (Figure 3b) produced vasoconstriction, as indicated by an increased perfusion pressure. The clearly biphasic, with respect to concentration, effects on perfusion pressure suggested possible purine-mediated adenyl cyclase inhibition and activation. Current receptor classifications indicate that separate purinoceptors may mediate adenyl cyclase inhibition (denoted A_1-receptor) and activation (A_2-receptor); this was assumed to be a working hypothesis in the subsequent attempts to classify the biphasic effects of purines on kidney perfusion pressure.

Effects of antagonists

On the assumption that concomitant A_1- and A_2-purinoceptor activation was produced in rat isolated kidneys, experiments were conducted in the presence of varying concentrations of the water soluble purine receptor antagonist 8-*p*-sulpho-phenyl-theophylline (8-SPT) in attempts to separate the two purine responses. Figure 4 (a–e) shows the effects of increasing

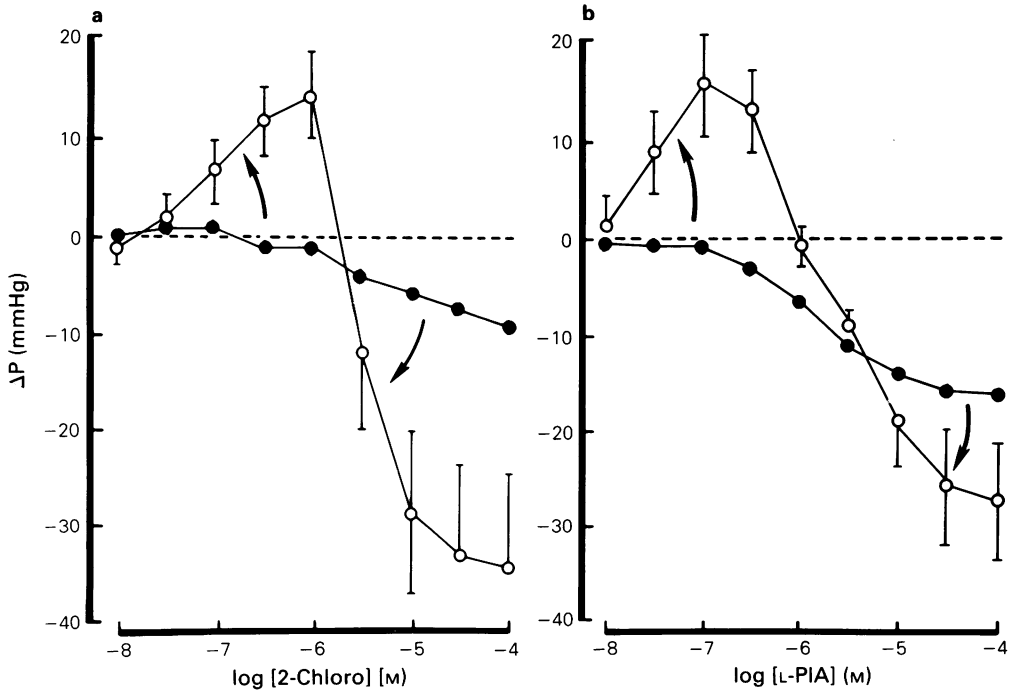

Figure 3 Differences between normal and adenyl cyclase activated kidneys. Ordinates: changes in perfusion pressure from basal in mmHg. Abscissae: log molar concentrations of agonist in perfusate. Responses to (a) 2-chloroadenosine (2-chloro; ●, $n = 2$; ○, $n = 6$) and (b) L-N^6-phenyl-isopropyl adenosine (L-PIA; ●, $n = 2$; ○, $n = 5$) are compared in normal kidneys (●) and those pretreated with methoxamine/forskolin (○). Vertical lines represent s.e.mean. It should be noted that the methoxamine/forskolin treated kidney preparations had a 30 to 60 mmHg higher basal perfusion pressure than untreated preparations.

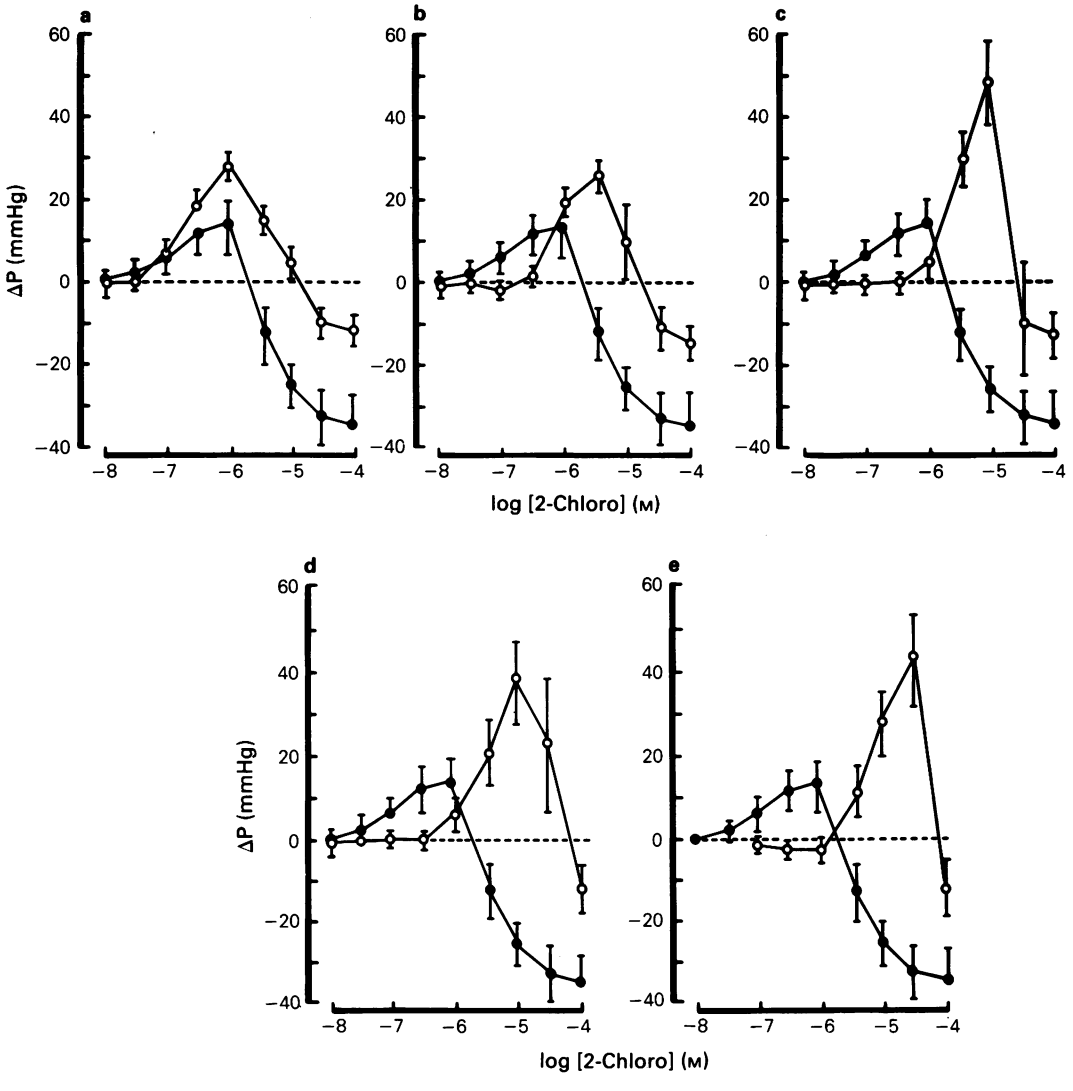

Figure 4 Effect of 8-*p*-sulpho-phenyltheophylline (8-SPT) on responses to 2-chloroadenosine (2-chloro) in adenyl cyclase activated perfused isolated kidneys. Ordinates: as for Figure 3. Abscissae: log molar concentrations of 2-chloroadenosine in the perfusate. Responses in the absence (●, $n = 4$ for all panels) and presence (○) of 8-SPT, 1 μM (a, $n = 6$), 3μM (b, $n = 6$), 10 μM (c, $n = 4$), 30 μM (d, $n = 5$), and 100 μM (e, $n = 4$). Vertical lines represent s.e.mean.

concentrations of 8-SPT on the dose-response curve to 2-chloroadenosine. Two general observations were noted: (1) the threshold for vasoconstriction was increased by 8-SPT (initial portion of the dose-response curve was shifted to the right) and (2) a striking increase in the maximal vasoconstriction produced by 2-chloroadenosine was observed. This latter effect was also associated with an inhibition of the vasodilator responses to 2-chloroadenosine. Qualitatively identical effects were observed when 8-SPT was utilized as an antagonist of the response to L-PIA (Figure 5 a–e). However, with this agonist, the increase in the maximal vasoconstriction was observed at concentrations which did not shift the threshold of the dose-response curve (i.e. see Figure 5b). Further studies on the nature of the purine agonist-mediated vasoconstriction were conducted in adenyl cyclase activated kidneys pretreated with 8-SPT (10 μM) because these

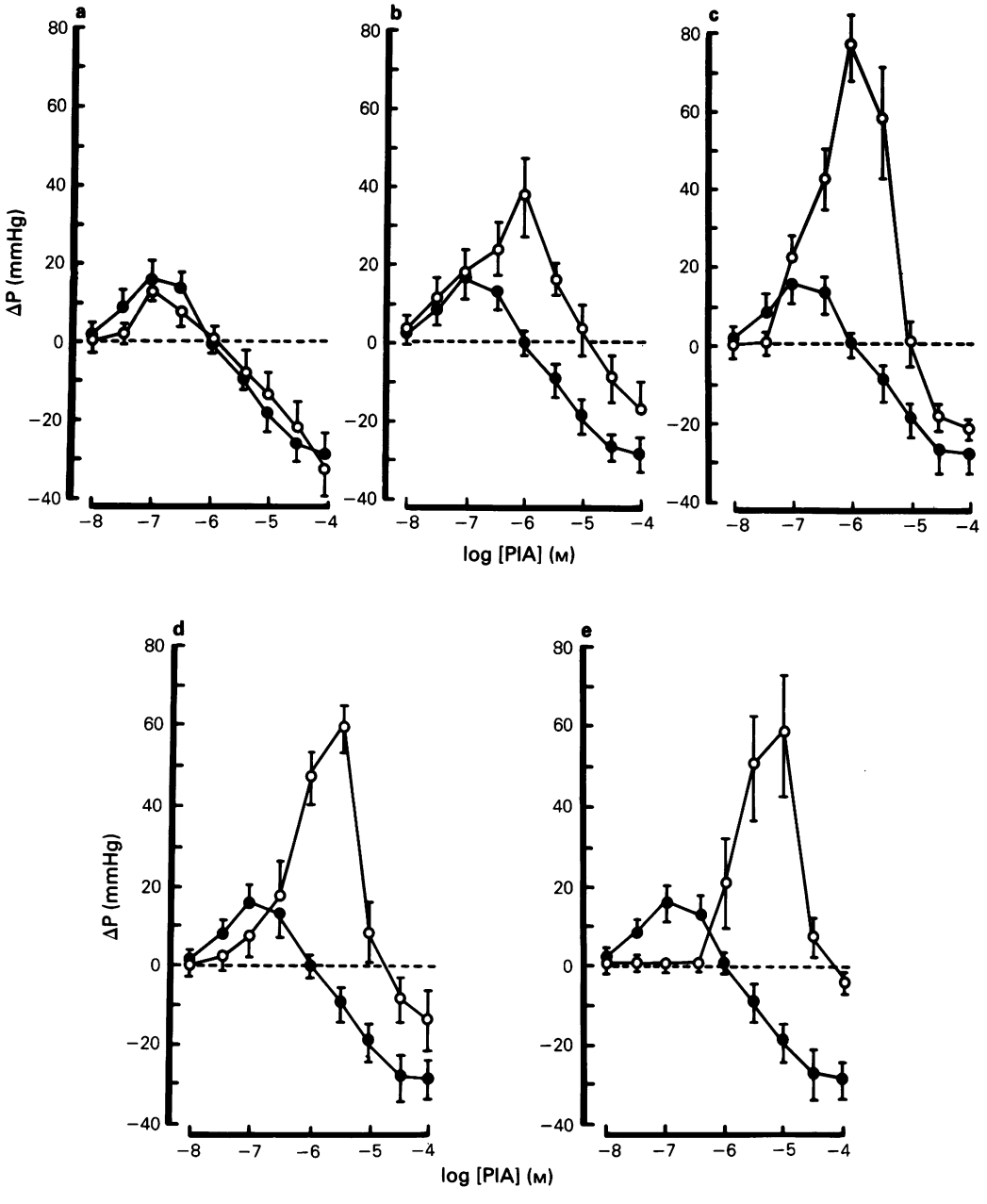

Figure 5 Effects of 8-*p*-sulpho-phenyltheophylline (8-SPT) on responses to L-N^6-phenyl-isopropyl adenosine (L-PIA) in adenyl cyclase activated perfused isolated kidneys. Ordinates: as for Figure 3. Abscissae: log molar concentrations of L-PIA in the perfusate. Responses in the absence (●, $n = 4$ for all panels) and presence (○) of 8-SPT, 1 μM (a, $n = 4$) 3 μM (b, $n = 5$), 10 μM (c, $n = 5$), 30 μM (d, $n = 4$) and 100 μM (e, $n = 4$). Vertical lines represent s.e.mean.

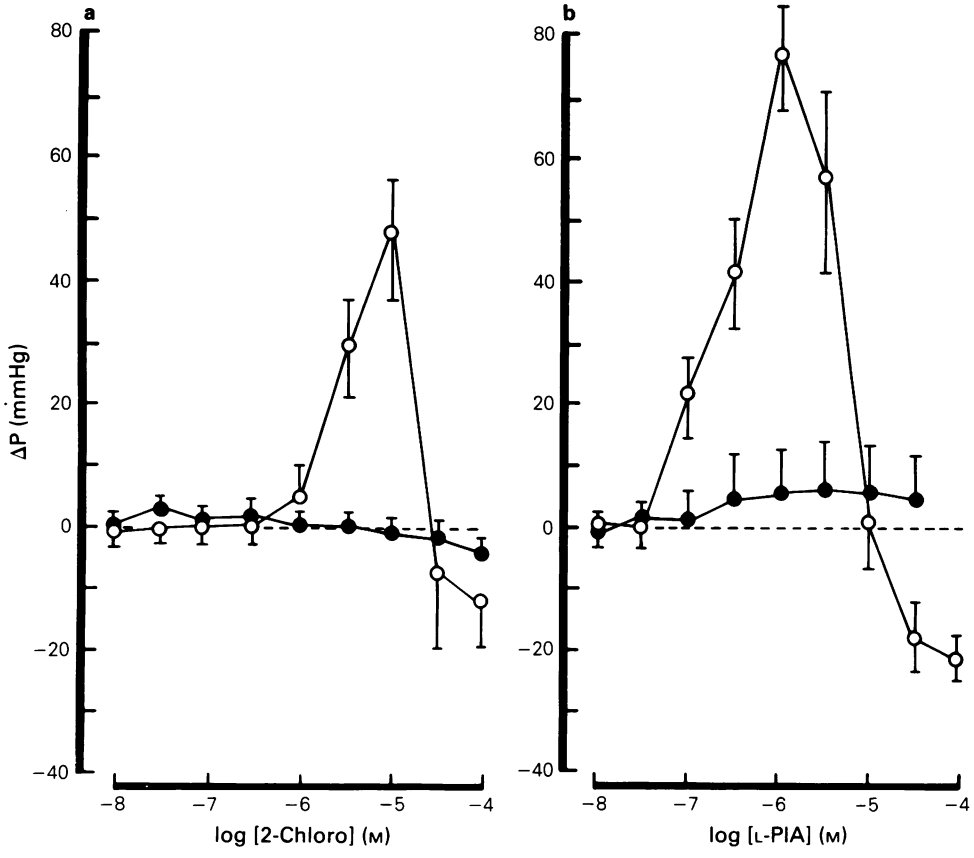

Figure 6 Effects of adenyl cyclase activation on responses of 8-*p*-sulpho-phenyltheophylline (8-SPT) pretreated kidneys to (a) 2-chloroadenosine (2-chloro) and (b) L-N^6-phenyl-isopropyl adenosine (L-PIA). Ordinates: as for Figure 3. Abscissae: log molar concentrations of 2-chloroadenosine (a) or L-PIA (b) in the perfusate. Responses in normal (●, $n = 3$ for (a); $n = 4$ for (b)) and methoxamine/forskolin pretreated (○, $n = 4$ for (a); $n = 5$ for (b)) kidneys. All responses were measured in the presence of 8-SPT (10 μM). Vertical lines represent s.e.mean.

preparations demonstrated the largest and most reproducible vasoconstriction.

Receptor classification of renal vasoconstriction

Figure 6 shows the effects of 2-chloroadenosine and L-PIA in adenyl cyclase activated and non-activated kidneys in the presence of 8-SPT (10 μM). The sensitivity of the potentiated vasoconstrictor-response to L-PIA to the reportedly A_1-receptor selective antagonist 8-(2-amino-4-chlorophenyl)-1,3-dipropylxanthine (PACPX) (Daly *et al.*, 1985) is shown in Figure 7. Accurate calculation of the potency of PACPX was not possible because of the interference of the vasodilator response to L-PIA and also because simple competitive kinetics were precluded by the prior partial A_1-receptor occupancy by 8-SPT. However, the sensitivity of the vasoconstrictor response to PACPX was consistent with A_1-receptor activation and blockade.

The relative potency of a series of purine agonists was determined on adenyl cyclase-activated kidneys pretreated with 8-SPT. Figure 8 shows the vasoconstriction produced by L-PIA, N^6-cyclohexyladenosine (CHA), 2-chloroadenosine, D-PIA and N^6-ethylcarboxamide adenosine (NECA). Two aspects of these data were consistent with the assumption that vasoconstriction in these preparations was due to A_1-receptor activation. The first was the relative potency of the agonists (as measured by the maximal vasoconstriction produced by each agonist); L-PIA > 2-chloroadenosine > D-PIA > NECA. This order of

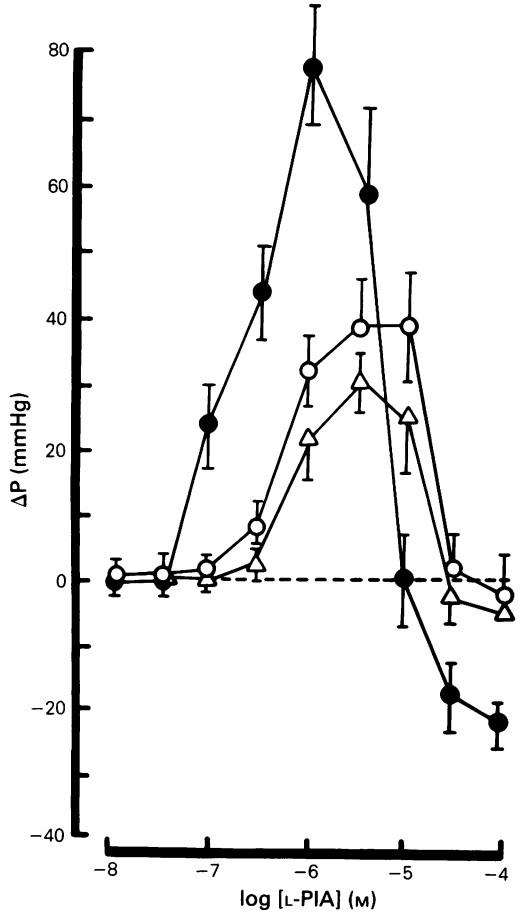

Figure 7 Effects of 8-(2-amino-4-chlorophenyl)-1,3-dipropylxanthine (PACPX) on vasoconstrictor responses to L-N^6-phenyl isopropyl adenosine (L-PIA) in isolated perfused kidneys. Ordinates: as for Figure 3. Abscissae: log molar concentration of L-PIA in the perfusate. All kidneys were pretreated with methoxamine/forskolin (adenyl cyclase activated) and 8-SPT (10 μM) to optimize L-PIA vasoconstriction. Responses in the absence (●, $n = 5$) and presence of PACPX 0.1 μM (○, $n = 4$) and 0.3 μM (△, $n = 4$) are shown. Vertical lines represent s.e.mean.

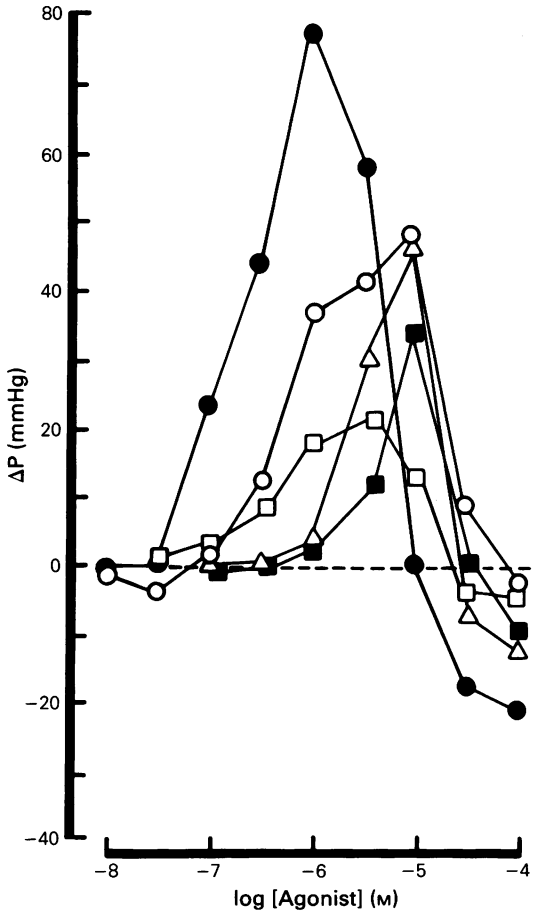

Figure 8 Effects of purine agonists on adenyl cyclase activated perfused kidneys pretreated with 8-*p*-sulphophenyltheophylline (10 μM). Ordinates: as for Figure 3. Abscissae: log molar concentrations of agonist in the perfusate. Responses to L-N^6-phenyl-isopropyl adenosine L-PIA (●, $n = 5$), N^6-cyclohexyladenosine (○, $n = 4$), 2-chloroadenosine (△, $n = 4$), D-PIA (■, $n = 4$) and N^6-ethylcarboxamide adenosine (□, $n = 4$) are shown.

potency was consistent with studies on A_1-receptors (Bruns *et al.*, 1980; Paton, 1981). The second is the relative potency of L-PIA and D-PIA as vasoconstricting agonists. The significantly greater potency and maximal vasoconstriction of L-PIA compared with its isomer suggests activation of A_1-receptors (relative potency of L-vs D-PIA on A_1-receptors 50 to 100) since the relative potency of these stereoisomers at A_2-receptors has been found to be much less (approximately 5 fold).

Discussion

In whole animals, purines have been shown to reduce kidney function (Haddy & Scott, 1968; Osswald, 1975;

Osswald *et al.*, 1975; 1978) but the mechanisms responsible or the receptors mediating these effects have not been fully defined. Specifically, it is unclear to what extent the purine effects *in vivo* result from purine-mediated reduction in neurotransmission (Hedqvist *et al.*, 1978), the release of renin (Tagawa & Vander, 1970; Osswald *et al.*, 1978; Murray & Churchill, 1984) or direct vascular effects. These experiments in the rat isolated kidney were designed to examine possible direct effects of purines on renal vascular tone. In this regard, the kidney was utilized simply as an isolated tissue for the kidney vasculature. Adenosine was not utilized in these studies because of the complicating effects of metabolic uptake and degradation processes for this purine known to be present in tissues (i.e. Kenakin & Leighton, 1985) including the kidney (Trimble & Coulson, 1984).

Initial studies indicated that 2-chloroadenosine and L-PIA produced a modest vasodilatation. Such direct vasodilatation has been observed in other blood vessels such as cat cerebral arteries (Edvinsson & Fredholm, 1983), rabbit aorta (Ghai & Mustafa, 1982), and bovine coronary artery (Mustafa & Askar, 1985). However, while renal vasoconstriction has been observed *in vivo*, no evidence of vasoconstriction was seen in these preparations of rat isolated kidney. In view of the proposed inhibitory effects of purines on adenyl cyclase via the A_1-receptor, it appeared possible that a basal stimulation of adenyl cyclase was required in the kidney as a prerequisite to the observation of A_1-receptor-mediated responses. This could have constituted a difference between the *in vitro* kidney preparation and normal kidneys *in vivo*, the latter being under neural and hormonal control. *In vivo*, it would be likely that the adenyl cyclase in the renal vasculature was functioning at a basal level of activation in the control of renal blood flow.

The pretreatment of isolated kidneys with methoxamine and forskolin appeared to provide a preparation in which the activation of adenyl cyclase by forskolin cancelled the α-adrenoceptor-mediated vasoconstriction, presumably by elevating intracellular levels of cyclic AMP. This procedure was not meant to mimic a normal physiologically functioning kidney (although vascular α- and β-adrenoceptor activation by catecholamines would produce a similar condition) but rather to produce an optimum preparation of renal vasculature for detection of adenyl cyclase inhibition by purines. The observation that 2-chloroadenosine and L-PIA produced vasoconstriction under these experimental conditions was consistent with the activation of A_1-purinoceptors. This was used as an initial working hypothesis and experiments aimed at receptor classification were initiated.

Although renal vasoconstriction to 2-chloroadenosine and L-PIA was observed, the effects were mild and a biphasic dose-response curve resulted. This indicated the possibility of concomitant activation of A_1- and A_2-purinoceptors producing opposing and therefore self-cancelling effects on renal perfusion pressure. This possibility was investigated by studying the effects of the purinoceptor antagonist 8-SPT on the agonist responses to 2-chloroadenosine and L-PIA. The rationale for this approach was to observe the effect of shifting the concentration range over which the A_1- and A_2-receptors were observed. Although the relative receptor occupancy of A_1- and A_2-receptors would not be expected to differ, given the relative lack of receptor specificity of 8-SPT (Fredholm & Persson, 1982), the intrinsic efficacies of the agonists at the different receptors and the relative efficiencies of stimulus-response coupling of the two receptors may have allowed one of the responses to dominate at greater concentrations of purine agonist. The considerable potentiation of vasoconstriction after pretreatment of the kidneys with 8-SPT indicated that this could be the case in rat kidney.

Given that a potentiated vasoconstriction was obtained after 8-SPT, the nature of the receptor mediating this response was studied with the selective A_1-receptor antagonist PACPX (Daly *et al.*, 1985) and a series of purine agonists. The antagonism of vasoconstriction by PACPX and the relative order of potency of the agonists as vasoconstrictor agents were consistent with the hypothesis that this was a A_1-receptor-mediated effect. Unfortunately, the biphasic nature of the dose-response curves made proper receptor classification by Schild analysis or quantitation of potency-ratios (Kenakin & Leighton, 1985) impossible.

In conclusion, the aim of classifying the receptors mediating renal vasoconstriction *in vitro* was partially satisfied. *In vitro* conditions for the study of A_1- and A_2-receptor-mediated responses were defined by partial activation of adenyl cyclase but quantitative estimates of agonist affinity and intrinsic efficacy and antagonist affinity for these receptors were not obtained. More selective drugs for these putative receptors may allow more satisfactory data to be obtained from this preparation.

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