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RESEARCH PAPER

Regional vascular responses to ATP and ATP analogues in the rabbit kidney *in vivo*: roles for adenosine receptors and prostanoids

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Background and purpose: Our knowledge of the effects of P2-receptor activation on renal vascular tone comes mostly from *in vitro* models. We aimed to characterise the pharmacology of ATP in the renal circulation *in vivo*.
Experimental approach: In pentobarbitone anaesthetized rabbits, we examined total renal and medullary vascular responses to ATP (0.2 and 0.8 mg kg^{-1}), β,γ-methylene ATP (β,γ-mATP, 7 and 170 μg kg^{-1}), α,β-mATP (0.2 and 2 μg kg^{-1}) and adenosine (2 and 6 μg kg^{-1}) using transit-time ultrasound and laser Doppler flowmetry, respectively. We also determined whether adenosine receptors, NO or prostanoids contribute to the actions of the purinoceptor agonists.
Key results: Renal arterial boluses of ATP, β,γ-mATP, and adenosine produced biphasic changes; ischaemia followed by hyperaemia, in total renal and medullary blood flow. α,β-mATP induced only ischaemia. The adenosine receptor antagonist 8-(*p*-sulphophenyl)theophylline reduced the responses to adenosine and the hyperaemic responses to ATP and β,γ-mATP only. NO synthase inhibition (N_ω-nitro-L-arginine) did not significantly alter responses to the P2 receptor agonists. Subsequent cyclooxygenase inhibition (ibuprofen) reduced the ATP- and β,γ-mATP-induced increases in renal blood flow. All other responses remained unchanged.
Conclusions and implications: In the rabbit kidney *in vivo*, α,β-mATP sensitive receptors mediate vasoconstriction. β,γ-mATP and ATP induce vasodilation at least partly through adenosine receptors. ATP induced renal vasodilatation is independent of NO and partly dependent on prostanoids in the bulk of the kidney, but not in the vasculature controlling medullary blood flow.
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Keywords: ATP; adenosine; purinoceptors; α,β-methylene ATP; β,γ-methylene ATP; renal circulation; renal medulla; 8-(*p*-sulphophenyl)theophylline; NO; ibuprofen

Abbreviations: CBF, cortical blood flow; HR, heart rate; MAP, mean arterial pressure; α,β-mATP, α,β-methylene ATP; β,γ-mATP, β,γ-methylene ATP; MBF, medullary blood flow; RBF, renal blood flow; 8-SPT, 8-(*p*-sulphophenyl)theophylline; L-NNA, N_ω-nitro-L-arginine

Introduction

ATP is emerging as an important factor in the regulation of renal haemodynamics (Inscho, 2001). ATP acts via P2X receptors located on vascular smooth muscle cells, to mediate vasoconstriction (Nori *et al*., 1998; Lewis and Evans, 2001; Wang *et al*., 2002) (Figure 1). Within the renal circulation, the $P2X_1$ receptor subtype is localized predominantly along the preglomerular vasculature (Chan *et al*., 1998), where it has been shown to mediate vasoconstriction (Inscho, 2001; Inscho *et al*., 2003) and may contribute to renal blood flow (RBF) autoregulation (Majid *et al*., 1999; Inscho *et al*., 2003). While P2X receptors on vascular smooth muscle cells are mediators of vasoconstriction, P2X receptors on endothelial cells of other vascular beds can mediate vasodilation (Yamamoto *et al*., 2006). Whether there is a role for P2X receptors in renal vasodilatation remains to be determined. ATP can activate $P2Y_1$ and $P2Y_2$ receptors on vascular endothelium to mediate vasodilation (Kunapuli and Daniel, 1998; Wangensteen *et al*., 2000) (Figure 1). There are also P2Y receptors on vascular smooth muscle cells in the kidney where they can mediate vasoconstriction (Inscho, 2001).

Most of our knowledge of the effects of P2-receptor activation on renal vascular tone, including that described in the preceding paragraph, comes from *in vitro* models. To our knowledge, there are only two previous studies that have examined renal vascular responses to ATP or ATP analogues *in vivo*. Majid *et al*. (1999) observed renal vasoconstriction

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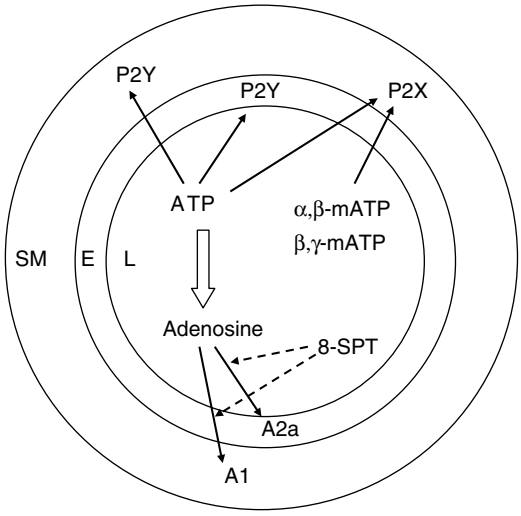

Figure 1 Schematic diagram of our current understanding of the receptors activated within the renal vasculature following intraluminal administration of ATP, α,β-methyleneATP (α,β-mATP), β,γ-methyleneATP (β,γ-mATP) or adenosine. SM, smooth muscle; E, endothelium; L, lumen; 8-SPT, 8-(*p*-sulphophenyl)theophylline. Solid arrows indicate possible receptor activation. Dotted arrows indicate receptor antagonism. The unfilled arrow represents metabolism of ATP to adenosine. β,γ-mATP, but not α,β-mATP, can also directly activate adenosine A_2 receptors (not shown).

during renal arterial infusion of ATP in anaesthetized dogs during concomitant NO synthase blockade. Similarly, Malmström *et al*. (2001) showed that intravenous (i.v.) boluses of α,β-methylene ATP (α,β-mATP) reduced renal vascular conductance in the pig.

In order to further characterize the vascular pharmacology of ATP on the renal circulation *in vivo*, we determined the renal vascular responses to renal arterial bolus administration of ATP and the more stable P2X-receptor agonists β,γ-methylene ATP (β,γ-mATP) and α,β-mATP in the rabbit kidney *in vivo*. ATP can be rapidly metabolized to adenosine, potentially resulting in adenosine receptor-mediated vasoconstriction and/or vasodilation (Figure 1). Owing to the difficulties in quantifying the rate of conversion of ATP to adenosine in the renal circulation, we also tested whether the responses to ATP and the analogues could be attenuated by adenosine receptor antagonism. Finally, we also determined the role of NO and prostanoids in the vasodilatory responses to these agents. We measured total RBF, and recorded perfusion in the medulla using laser Doppler flowmetry, since responses to vasoactive agents often differ in the medulla versus the bulk of the kidney, the cortex (Evans *et al*., 2004).

Methods

Experiments were performed on 14 male rabbits of a crossbred English strain (3.0 ± 0.1 kg) and were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. The protocols were approved in advance by the Monash University Department of Physiology/Central Animal Services Animal Ethics Committee. Animals were meal fed and allowed water *ad libitum* until the experimental procedures began.

Surgical procedures

These procedures were similar to those used previously (Eppel *et al*., 2004, 2006). Induction of anaesthesia was by i.v. administration of pentobarbitone sodium (90–150 mg) and was immediately followed by endotracheal intubation and artificial ventilation. Anaesthesia was maintained by a continuous pentobarbitone infusion (30–50 mg h^{-1}). During surgery Hartmann's solution (compound sodium lactate, Baxter Healthcare, Toongabbie, NSW, Australia) was infused i.v. at a rate of 0.18 ml kg^{-1} min^{-1} to replace fluid losses. This infusion was replaced with a mixture of Hartmann's (80%) and a polygeline/electrolyte solution (20%; Haemaccel, Hoechst, Melbourne, Victoria, Australia) once surgery was completed. Body temperature was maintained at 36–38°C. Arterial pressure was monitored in a central ear artery.

The left kidney was approached via a retroperitoneal incision and stabilized in a cup. The kidney was denervated. A catheter was placed in a side branch of the renal artery (suprarenolumbar artery) (Kalyan *et al*., 2002). A transit-time ultrasound flow probe (type 2SB, Transonic Systems, Ithaca, NY, USA) was placed around the left renal artery for measurement of RBF. For measurements of medullary blood flow (MBF), a 26 gauge needle-type laser Doppler flow probe (DP4s, Moor Instruments, Millwey, Devon, UK) was inserted into the kidney using a micromanipulator, so that its tip lay 9–10 mm below the midregion of the lateral surface of the kidney, within the inner medulla. For measurements of cortical blood flow (CBF), a standard plastic probe (DP2b, Moor Instruments) was placed on the dorsal surface of the kidney and secured with gauze packing. The laser Doppler system provides a signal, in flux units, proportional to the product of erythrocyte velocity and concentration in a small volume of tissue (<1 mm^3). In the kidney, the signal predominantly reflects erythrocyte velocity (Eppel *et al*., 2003a). A 60–90 min equilibration period was allowed before the experimental protocols commenced.

Protocol 1: effects of adenosine receptor antagonism on responses to P2 receptor agonists

Intrarenal arterial boluses of ATP (0.2. and 0.8 mg kg^{-1}), β,γ-mATP (7 and 170 μg kg^{-1}), α,β-mATP (0.2 and 2 μg kg^{-1}) and adenosine (2 and 6 μg kg^{-1}) were administered during an initial control period in four rabbits. The boluses were given in random order with the exception of the highest dose of α,β-mATP, which was always given last. After each bolus, renal perfusion was allowed to recover to baseline levels, before administering the next bolus. Assuming a RBF of 25 ml min^{-1} and a transit time of the bolus through the renal circulation of 1–5 s, we estimate that the maximal concentrations of exogenous ATP and adenosine in the renal circulation after bolus administration were 0.3–6 mg ml^{-1} and 3–60 μg ml^{-1}, respectively. Once all agonist doses had been administered, infusion of the adenosine receptor antagonist 8-(*p*-sulphophenyl)theophylline (8-SPT;

50 mg kg^{-1} plus 50 mg kg^{-1} h^{-1}) then commenced. 8-SPT was dissolved in 154 mM NaCl (saline) and delivered i.v. at a rate of 5 ml kg^{-1} plus 5 ml kg^{-1} h^{-1}. After a 20 min equilibration period, responses to the P2 receptor agonists and adenosine were determined for the second time. A vehicle control group was not included in this Protocol. However, responses to ATP and its analogues were observed to be stable over time in Protocol 2.

Protocol 2: effects of NO synthase and cyclooxygenase inhibition on responses to P2 receptor agonists

Two groups of five rabbits were studied. Responses to renal arterial bolus administration of ATP (0.2 and 0.8 mg kg^{-1}), β,γ-mATP (7 and 170 μg kg^{-1}) and α,β-mATP (0.2 and 2 μg kg^{-1}) were determined during an initial control period as for Protocol 1. In one group, i.v. infusion of the NO synthase inhibitor N_ω-nitro-L-arginine (L-NNA; 20 mg kg^{-1} plus 5 mg kg^{-1} h^{-1}) then commenced. The second group received vehicle treatment instead (saline, 4 ml kg^{-1} plus 1 ml kg^{-1} h^{-1}). After a 20 min equilibration period, responses to the P2 receptor agonists were determined for the second time. Finally, infusion of the cyclooxygenase inhibitor ibuprofen (12.5 mg kg^{-1} plus 12.5 mg kg^{-1} h^{-1}) commenced in the L-NNA pretreated group. The other group received the corresponding vehicle treatment (saline, 1 ml kg^{-1} plus 1 ml kg^{-1} h^{-1}). After a 15 min recovery period, responses to the P2 receptor agonists were determined for a third time.

Statistical analyses

Data acquisition was identical to that described previously (Eppel *et al*., 2004, 2006). Post-mortem levels of CBF (8±1 units) and MBF (19±2 units) were subtracted before subsequent data analysis. Data are presented as mean±s.e. *P*-values $\leqslant 0.05$ were considered statistically significant. Baseline levels of haemodynamic variables were determined by averaging them over each of the 30 s control periods before each agonist bolus. The peak decreases and the peak increases in response to each dose of ATP, β,γ-mATP, α,β-mATP and adenosine were determined. The data are presented as percentage changes from control (averaged over the 10 s before the bolus was administered).

Table 1 Basal levels of haemodynamic variables during each of the two experimental periods in Protocol 1 ($n=4$)

	Control period	*8-SPT treatment*
MAP (mmHg)	71±2	75±2
HR (beats min^{-1})	224±12	212±11
RBF (ml min^{-1})	27±4	23±4
CBF (units)	243±34	210±20
MBF (units)	28±7	25±8

Abbreviations: CBF, cortical blood flow measured by laser Doppler flowmetry; HR, heart rate; MAP, mean arterial pressure; MBF, medullary blood flow measured by laser Doppler flowmetry; RBF, renal blood flow; 8-SPT, 8-(*p*-sulphophenyl)theophylline.
Results are presented as mean±s.e.
Levels of these variables were not significantly different after, relative to before, 8-SPT treatment as determined by paired *t*-test.

All data were then subjected to analysis of variance (ANOVA) (Snedecor and Cochran, 1967), or repeated measures ANOVA (Ludbrook, 1994), the factors comprising rabbit, group (L-NNA and ibuprofen treated or vehicle treated), treatment (control period versus treatment period), and dose of agonist applied. This allowed us to test (i)

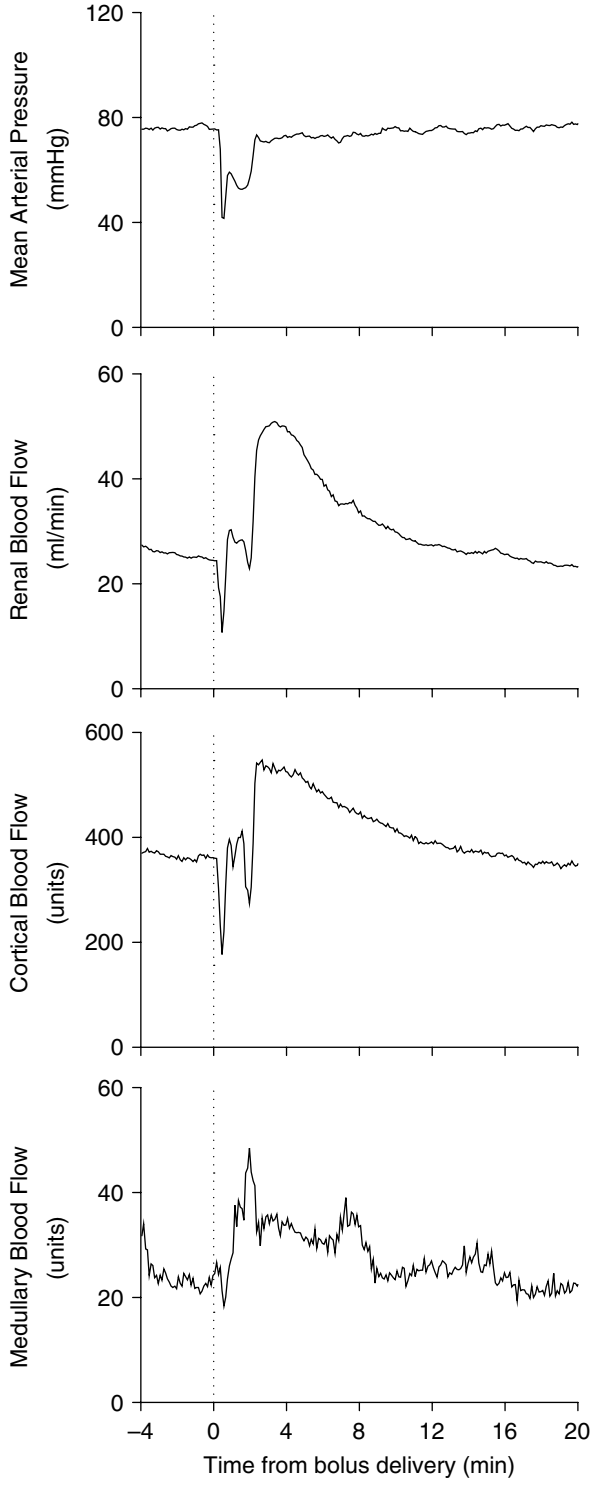

Figure 2 Typical arterial pressure and renal haemodynamic responses to an intrarenal bolus of ATP (0.8 mg kg^{-1}). Dotted line indicates time of injection.

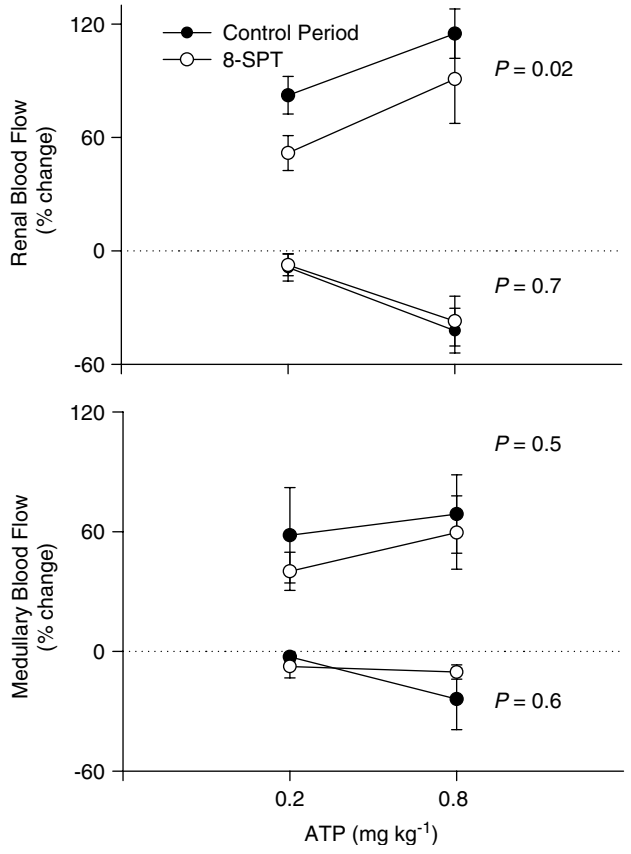

Figure 3 Renal haemodynamic responses to intrarenal bolus administration of ATP before and during administration of 8-SPT ($n=4$). The symbols and error bars represent the mean $\pm$s.e. of peak increases (above the zero line) and peak decreases (below the zero line) of each variable. $P_{treatment}$ values represent the outcomes of analysis of variance, testing for effects of 8-SPT treatment on the responses to ATP.

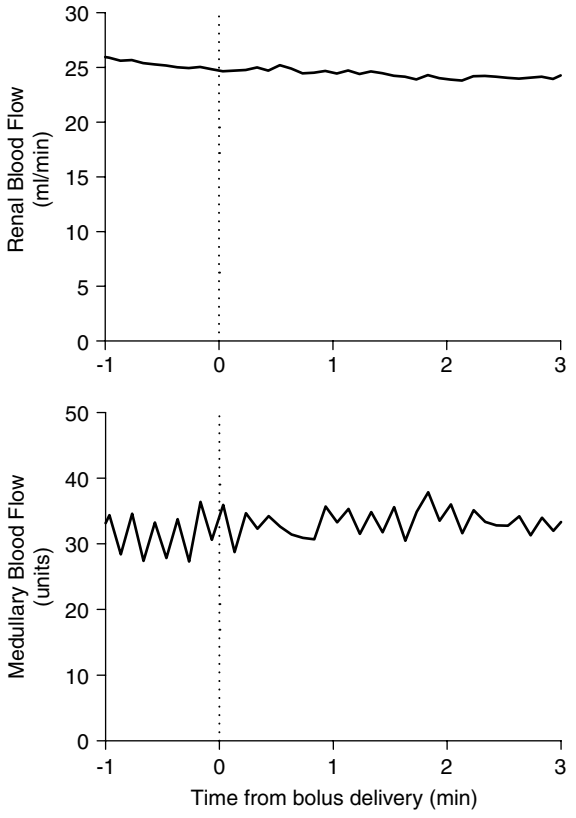

Figure 4 Typical renal haemodynamic responses to an intrarenal arterial bolus of saline (154 mM NaCl, 0.03 ml kg^{-1}). Dotted lines indicate time of bolus administration.

whether baseline variables, or responses to receptor agonists, differed during the control period according to the treatment that was to follow (P_{group}), (ii) whether haemodynamic variables responded in a manner dependent on the dose of receptor agonist (P_{dose}) and (iii) whether baseline variables, or responses to receptor agonists were affected by each of the treatments ($P_{treatment}$ or $P_{group \times treatment}$). *P*-values were conservatively adjusted to account for multiple comparisons using the Bonferroni method (Ludbrook, 1998).

Drugs

ATP, α,β-mATP, β,γ-mATP, adenosine, ibuprofen, L-NNA and 8-SPT were purchased from Sigma Chemical Company (St Louis, MO, USA). Pentobarbitone sodium was from Rhone Merieux (Nembutal, Pinkenba, QLD, Australia) or from Sigma.

Results

Protocol 1: renal responses to P2 receptor agonists and adenosine

During the control period, basal levels of mean arterial pressure (MAP), heart rate (HR), RBF, CBF and MBF (Table 1) were similar to those previously observed in our laboratory (Eppel *et al*., 2004, 2006). Under control conditions, boluses of ATP delivered into the renal artery produced multiphasic responses in RBF, CBF and MBF. These responses were characterized by transient reductions (-42 ± 12% for RBF at the highest dose of 0.8 mg kg^{-1}), followed by increases ($+112\pm13$% for RBF at the highest dose) and finally a recovery phase (Figures 2 and 3). ATP also transiently reduced MAP by -6 ± 3% at the lowest dose of 0.2 mg kg^{-1} and by -35 ± 5% at the highest dose of 0.8 mg kg^{-1}, but had no apparent effect on HR (data not shown). The time course of the renal haemodynamic responses to ATP was dose-dependent. For the greatest dose administered (0.8 mg kg^{-1}), the initial reductions in RBF and MBF were observed to peak within 1 min of injection. The peak increases in RBF and MBF were reached within 5 min after injection and full recovery was observed within 15–20 min of injection. The reductions in MAP typically commenced 2–15 s after the reductions in RBF commenced. MAP returned to basal levels before the onset of renal hyperaemia. RBF and CBF responses were indistinguishable, as we have consistently found previously (Eppel *et al*., 2003a, b, 2004). Therefore, only RBF and MBF responses will be presented. Renal arterial boluses of saline vehicle (0.03 ml kg^{-1}) had a negligible impact on renal haemodynamics (Figure 4) and MAP (data not shown).

Renal arterial boluses of β,γ-mATP (7–170 μg kg^{-1}) also produced multiphasic responses in RBF and MBF, similar to

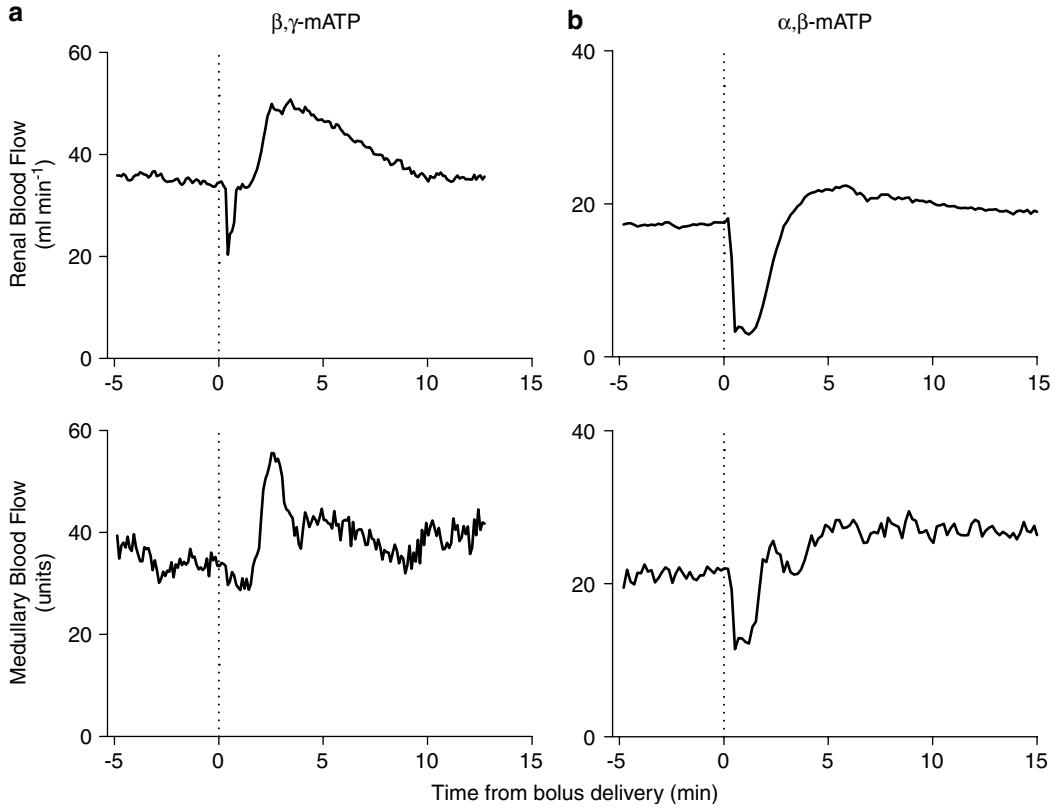

Figure 5 Typical renal haemodynamic responses to intrarenal boluses of (**a**) β,γ-mATP (170 μg kg^{-1}) and (**b**) α,β-mATP (2 μg kg^{-1}). Dotted lines indicate time of injection.

those to ATP. As for ATP, these responses were also characterized by transient reductions, followed by hyperaemia and finally a recovery phase (Figures 5a and 6). β,γ-mATP (170 μg kg^{-1}) also transiently reduced MAP by 15 ± 3% but did not appreciably alter HR (data not shown).

The renal responses to boluses of α,β-mATP were distinct from those of ATP and β,γ-mATP, in that reductions in RBF and MBF were observed (2 μg kg^{-1} of α,β-mATP reduced RBF by -26 ± 11%) but there was little, if any, increase in RBF or MBF in response to α,β-mATP (2 μg kg^{-1} of α,β-mATP increased RBF by $+3\pm2$%) (Figure 5B). α,β-mATP had no appreciable impact on MAP or HR (data not shown).

Boluses of adenosine induced transient reductions in RBF and MBF (RBF was reduced by -48 ± 7% at the highest dose of 6 μg kg^{-1}) followed by increases (RBF increased by $+30\pm6$% at the highest dose) (Figure 7). For the greatest dose administered (6 μg kg^{-1}), the initial ischaemia was observed to peak within 10–20 s of injection. The peak increases in RBF and MBF were reached within 2 min after injection and full recovery was observed within 8 min of injection. Renal arterial bolus injection of adenosine had no appreciable impact on MAP or HR.

Protocol 1: effects of adenosine receptor antagonism on responses to P2 receptor agonists and adenosine

Treatment with 8-SPT did not significantly alter basal haemodynamics (Table 1). 8-SPT did, however, significantly attenuate adenosine-induced reductions and increases in RBF and adenosine-induced reductions in MBF (Figure 8). 8-SPT significantly reduced β,γ-mATP-induced increases in RBF and MBF, but not β,γ-mATP-induced decreases in these variables (Figure 6). 8-SPT slightly, but significantly, attenuated the ATP-induced increases in RBF (Figure 3). For example, during the control period an ATP bolus of 0.8 mg kg^{-1} increased RBF by 115 ± 13%. During the 8-SPT treatment, ATP increased RBF by only 90 ± 23%. 8-SPT did not significantly alter ATP-induced reductions in RBF or MBF. 8-SPT had no significant effect on any of the renal vascular responses to α,β-mATP (data not shown).

Protocol 2: effects of NO synthase and cyclooxygenase inhibition on responses to P2 receptor agonists

During the control period, basal levels of MAP, HR, RBF and MBF (Table 2) were similar to those observed in Protocol 1. They did not vary systematically across the two groups of rabbits ($P_{group}\geqslant0.06$). Vehicle treatment had little effect on basal haemodynamics (Table 2) or on responses to ATP (Figure 9), β,γ-mATP, or α,β-mATP (data not shown). Infusion of L-NNA significantly increased MAP by 51 ± 10% and decreased HR by 19 ± 5% but apparent reductions in RBF ($P_{treatment}=0.11$) and MBF ($P_{treatment}=0.32$) were not statistically significant. When compared to vehicle infusion, L-NNA infusion did not significantly affect responses of RBF and MBF to ATP (Figure 9), β,γ-mATP or α,β-mATP (data not shown; $P_{group\times period}\geqslant0.5$). Ibuprofen infusion in L-NNA pretreated rabbits had no significant impact on basal

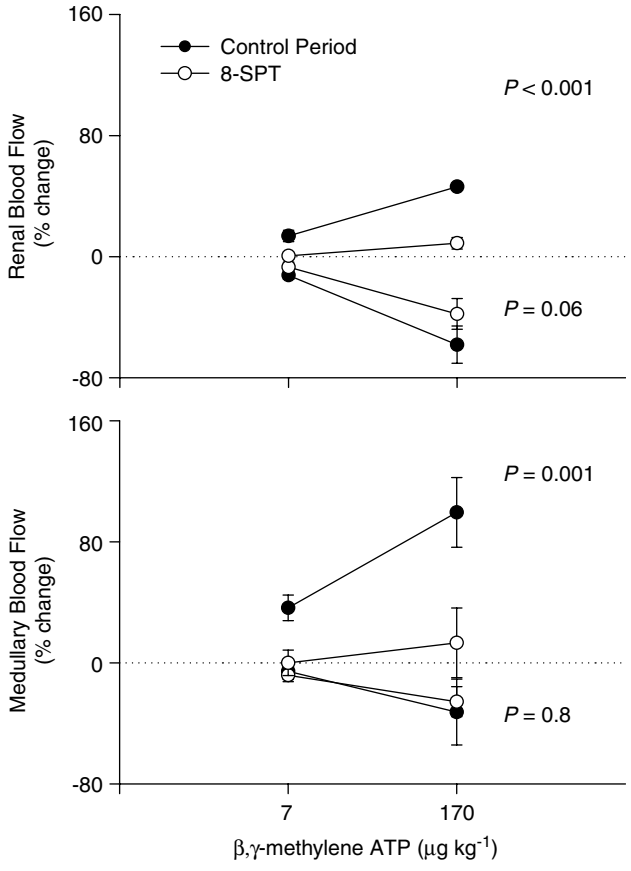

Figure 6 Renal haemodynamic responses to intrarenal bolus administration of β,γ-mATP before and during administration of 8-SPT ($n=4$). The symbols, error bars and *P*-values are as for Figure 3.

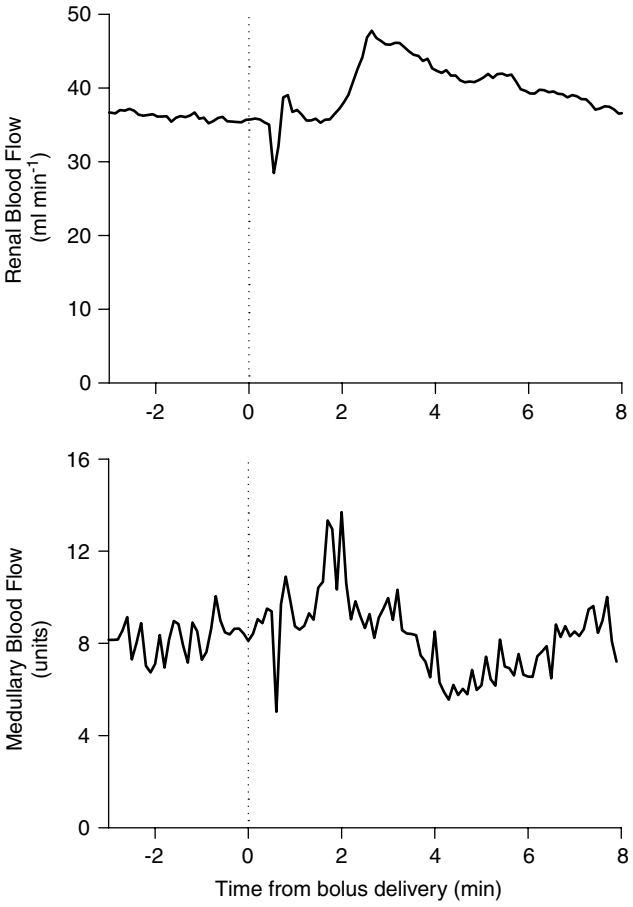

Figure 7 Typical renal haemodynamic responses to an intrarenal bolus of adenosine ($6\,\mu g\,kg^{-1}$). Dotted line indicates time of injection.

haemodynamics, but attenuated ATP- and β,γ-mATP-induced increases in RBF. For example, RBF increased by $150\pm13\%$ in response to ATP ($0.8\,mg\,kg^{-1}$) during the control period but by only $69\pm10\%$ during ibuprofen infusion ($P_{group\times period}<0.001$; Figure 9). Similarly, RBF increased by $79\pm20\%$ in response to β,γ-mATP ($170\,\mu g\,kg^{-1}$) during the control period but only by $31\pm10\%$ during ibuprofen treatment ($P_{group\times period}=0.04$; data not shown). In contrast, increases in MBF in response to ATP or β,γ-mATP were not significantly altered by ibuprofen infusion ($P_{group\times period}\geqslant0.4$). Ibuprofen did not significantly alter the magnitude of reductions in RBF or MBF in response to ATP, β,γ-mATP or α,β-mATP.

Discussion

There were three main findings from the present study. Firstly, responses to ATP, β,γ-mATP and adenosine comprised reductions (ischaemia) followed by increases (hyperaemia) in total RBF and MBF *in vivo*, indicating that they induce vasoconstriction and vasodilation in the vasculature controlling both cortical and medullary perfusion. In contrast, α,β-mATP induced only reductions in RBF and MBF. Hence, as expected, α,β-mATP sensitive receptors mediate only vasoconstriction. The second main finding was that hyperaemic, but not ischaemic, responses to β,γ-mATP and ATP were inhibited by the adenosine receptor antagonist 8-SPT. These data demonstrate that renal vasodilation, but not vasoconstriction induced by β,γ-mATP and ATP are, at least in part, mediated by adenosine receptors in the rabbit kidney *in vivo*. The last main finding relates to the role of endothelial-derived factors in the vasodilatory responses to ATP and its analogues. We observed that NO synthase blockade did not reduce the renal hyperaemic responses to ATP, nor β,γ-mATP. Hence the renal vasodilation induced by ATP and β,γ-mATP is independent of NO. Cyclooxygenase blockade concomitant with NO synthase blockade reduced the ATP- and β,γ-mATP-induced increases in RBF, but not MBF. Therefore, prostanoids appear to play a more significant role in ATP- and β,γ-mATP-induced vasodilation in the cortical than the medullary circulation.

We chose to use the stable analogues α,β-mATP and β,γ-mATP as P2X receptor agonists. These agents act with greatest potency at $P2X_1$- and $P2X_3$-receptor subtypes (Chen *et al*., 1995; Evans *et al*., 1995; Ralevic and Burnstock, 1998; Norenberg and Illes, 2000; North and Surprenant, 2000; Liu *et al*., 2001) and do not have appreciable potency at P2Y-receptors (Webb *et al*., 1993; Kerstan *et al*., 1998; Ralevic and Burnstock, 1998). β,γ-mATP, but not α,β-mATP, can also

activate adenosine receptors directly, without undergoing significant dephosphorylation to adenosine (Bailey and Hourani, 1990; Hourani *et al*., 1991). Our data demonstrating that adenosine receptor antagonism reduced renal vascular responses to β,γ-mATP but not to α,β-mATP are consistent with this. Further, direct activation of adenosine receptors by β,γ-mATP is likely also to explain why α,β-mATP and β,γ-mATP had quite different effects on the renal vasculature; monophasic versus biphasic. Adenosine receptor antagonism significantly attenuated β,γ-mATP-induced hyperaemia but not ischaemia. We conclude from this that β,γ-mATP-induced hyperaemia is likely to be mainly mediated by adenosine receptors, whereas β,γ-mATP-induced ischaemia is likely to be chiefly mediated by P2 receptors, such as the $P2X_1$ subtype.

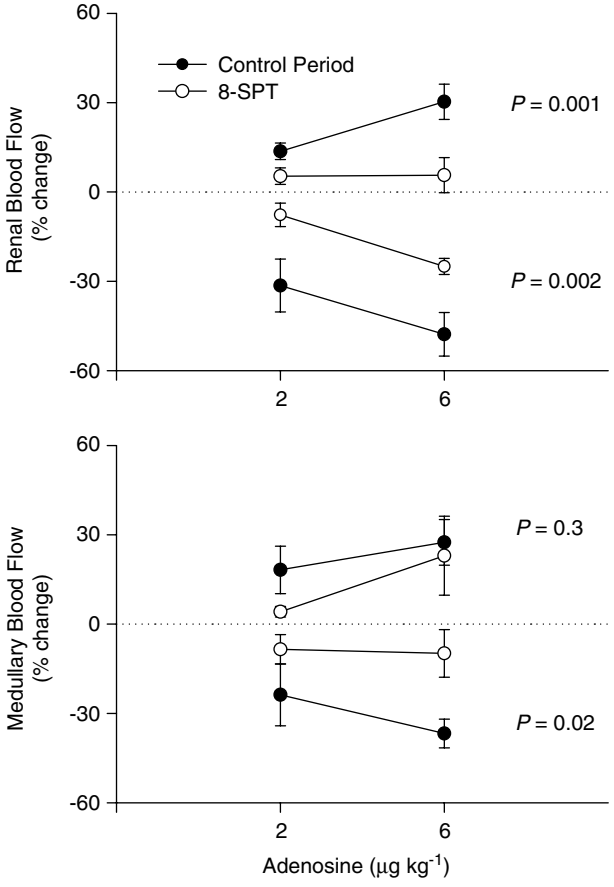

Figure 8 Renal haemodynamic responses to intrarenal boluses administration of adenosine before and during administration of 8-SPT ($n=4$). The symbols, error bars and *P*-values are as for Figure 3.

Renal A_1 and A_{2a} receptors mediate vasoconstriction and vasodilation, respectively (Rump *et al*., 1999; Nishiyama *et al*., 2001; Jackson *et al*., 2002). Given that adenosine boluses delivered under control conditions induced both ischaemia and hyperaemia it is likely that both receptor populations were activated under our experimental conditions. 8-SPT is an A_1 and A_2 receptor antagonist (Daly *et al*., 1985). Therefore, as expected, 8-SPT reduced the magnitude of both ischaemic and hyperaemic responses to adenosine in the current study. 8-SPT also reduced the magnitude of the hyperaemic response to β,γ-mATP, suggesting that β,γ-mATP acts at least partly at A_{2a} receptors. Adenosine receptor antagonism slightly reduced the increase in RBF induced by ATP. This could be explained by rapid metabolism of ATP to adenosine within the renal circulation (Bailey and Hourani, 1990; Hourani *et al*., 1991). Adenosine receptor antagonism had no significant impact on the renal ischaemic effects of ATP, suggesting that adenosine receptors do not make a major contribution to ATP-induced vasoconstriction and that ATP-induced renal vasoconstriction is most likely to be mediated by P2 receptors.

We observed profound hyperaemic responses to ATP and β,γ-mATP. This led us to test the effects of blockade of NO synthase and cyclooxygenase on these responses. NO synthase blockade, with L-NNA at a dose that has previously been shown to maximally inhibit NO production in rabbits (Denton and Anderson, 1994; Evans *et al*., 1994), did not inhibit the increases in RBF and MBF in response to ATP or β,γ-mATP, indicating that NO is not essential for these responses. In contrast, ibuprofen treatment attenuated increases in RBF induced by β,γ-mATP and ATP in rabbits pretreated with a NO synthase blocker. Hence, the renal vasodilator effects of β,γ-mATP and ATP appear to be at least partly mediated by prostanoids. However ATP and β,γ-mATP produced considerable increases in RBF even after administration of both L-NNA and ibuprofen. Furthermore, these treatments did not blunt increases in MBF induced by ATP or β,γ-mATP. Thus, mechanisms independent of NO and cyclooxygenase also appear to contribute to purinoceptor-mediated vasodilation in the kidney, particularly within the medullary circulation. One possible mechanism

Table 2 Basal levels of haemodynamic variables during each of the three experimental periods in Protocol 2 ($n=5$ per group)

	Group 1			*Group 2*		
	Control period	*Vehicle 1 treatment*	*Vehicle 2 treatment*	*Control period*	*L-NNA treatment*	*L-NNA + Ibuprofen*
MAP (mmHg)	66±3	73±2	71±2	65±4	96±3***	88±7
HR (beats min^{-1})	286±2	263±7*	246±8	261±15	210±14*	226±21
RBF (ml min^{-1})	26±3	24±2	23±2	25±1	19±2	22±3
MBF (units)	41±11	30±6	33±7	39±12	16±4	29±10

Abbreviations: ANOVA, analysis of variance; HR, heart rate; MAP, mean arterial pressure; MBF, medullary blood flow measured by laser Doppler flowmetry; RBF, renal blood flow.
Results are presented as mean±s.e.
*$P\leqslant0.05$, ***$P\leqslant0.005$ versus the previous period. These *P*-values were derived from specific contrasts made from partitioned ANOVA, and were conservatively adjusted to account for multiple comparisons using the Bonferroni method (Ludbrook, 1998).

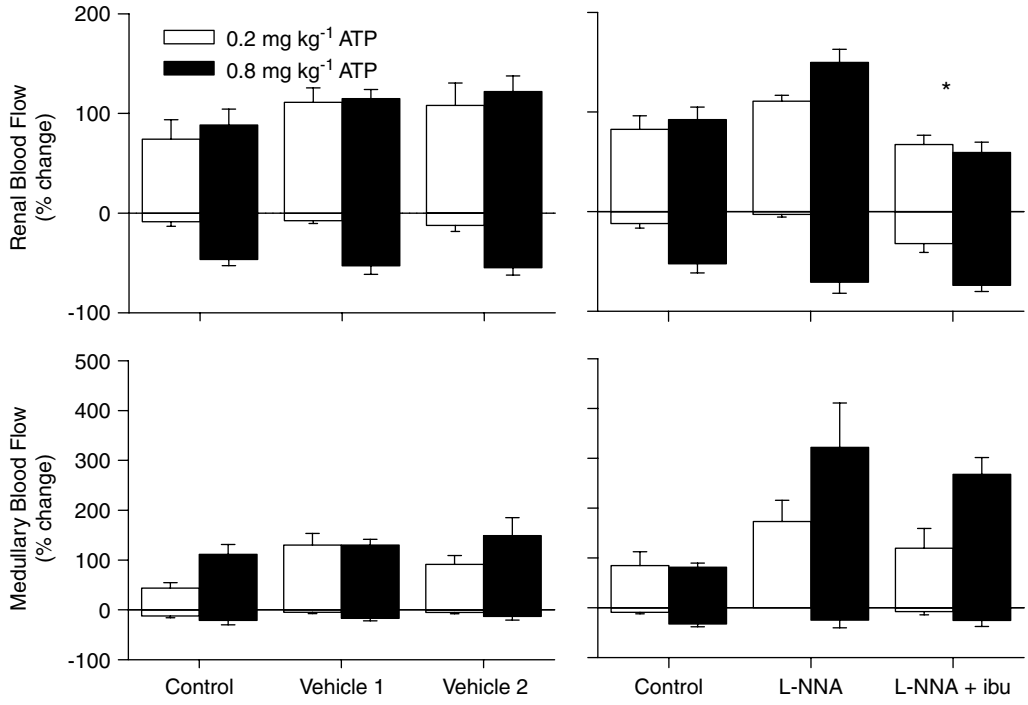

Figure 9 Renal haemodynamic responses to intrarenal bolus administration of ATP before and during administration of L-NNA, L-NNA plus ibuprofen (ibu) or vehicle treatment ($n=5$ per group). The columns and error bars represent the mean $\pm$s.e. of peak increases (above the zero line) and peak decreases (below the zero line) of each variable. The asterisk denotes a significant ($P<0.05$) interaction between group and treatment as determined by analysis of variance. This interaction term tested whether the effects of ibuprofen infusion on responses to ATP differed from those of the corresponding vehicle.

involves endothelium-derived hyperpolarizing factor which contributes to P2Y-mediated vasodilatation in the rat isolated kidney preparation (Wangensteen *et al*., 2000). The receptor subtypes mediating ATP-induced vasodilation remain to be determined but are probably $P2Y_1$ and $P2Y_2$ receptors (Kunapuli and Daniel, 1998; Wangensteen *et al*., 2000).

Our conclusion that ATP-induced vasodilation in the rabbit renal circulation is independent of NO and partly dependent on prostanoids contrasts with the findings of other studies of renal perfusion. For example, in the dog, renal arterial infusion of ATP in the presence of endogenous NO results in renal vasodilation, but ATP infusion in the presence of NO synthase blockade results in vasoconstriction (Majid *et al*., 1999). Similarly, in the isolated perfused rat kidney, ATP-mediated vasodilation is dependent on NO but independent of prostanoids (Wangensteen *et al*., 2000). Therefore, there are clearly species-related differences in the factors mediating ATP-induced vasodilation. Our results indicate that in the rabbit kidney, ATP-induced vasodilation is partly mediated by its breakdown product adenosine. The lack of effect of NO synthase blockade on responses to ATP therefore suggests that adenosine-mediated vasodilation is also largely NO independent. Although this is in contrast to the observations of the effects of adenosine in the kidneys of mice and other species (Hansen *et al*., 2005), it is consistent with the results of a previous study of rabbit renal arteries (Rump *et al*., 1999). Therefore, there seem to be species-related differences in the factors mediating renal vascular responses to adenosine.

Our observations of the effects of ATP and β,γ-mATP on renal haemodynamics were confounded to some extent by associated depressor responses, despite the fact that the agents were administered directly into the renal artery. However, the transient depressor responses to these agents commenced after the renal hyperaemic effects commenced. Furthermore, bolus renal arterial administration of α,β-mATP did not reduce MAP, but did produce renal ischaemia. Thus, the renal haemodynamic effects of these agents are likely to chiefly reflect their direct effects within the renal vasculature.

In conclusion, we have confirmed in the rabbit kidney *in vivo*, that there are receptors sensitive to α,β-mATP in the blood vessels controlling both cortical and medullary perfusion, probably of the $P2X_1$ subtype, which mediate vasoconstriction. There are also adenosine receptors that mediate vasoconstriction and dilatation in both vascular territories. The adenosine receptors that mediate vasodilation, possibly of the A_{2a} subtype, may also be activated by β,γ-mATP directly and by ATP after metabolism to adenosine. ATP-induced renal ischaemia is not mediated by adenosine receptors. ATP-induced vasodilation is independent of NO, and partly dependent on prostanoids, but only in the cortex. Other as yet unidentified mediators also contribute to ATP-induced vasodilatation, particularly in the medullary circulation. Responses of blood flow to ATP, ATP analogues and adenosine were similar, at least qualitatively, in the medulla compared to the cortex. However, the mechanisms mediating their actions appear to differ between the two vascular beds.

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Conflict of interest

The authors state no conflict of interest.

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