Effects of Purines and Pyrimidines on the Rat Mesenteric Arterial Bed

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The effects of the purines, adenosine 5'-triphosphate (ATP) and guanosine 5'-triphosphate (GTP), and the pyrimidines, uridine 5'-triphosphate (UTP), cytidine 5'-triphosphate (CTP), and thymidine 5'-triphosphate (TTP), on vascular resistance were investigated in the rat mesenteric arterial bed. In preparations at basal tone, these agents produced dose-related vasoconstriction with a potency order of ATP > CTP > UTP >> TTP = GTP. When tone was raised with norepinephrine (30 μM), these agents caused dose-related vasodilatation with the potency order of UTP = ATP > TTP = GTP. CTP did not elicit vasodilatation. Removal of the endothelium with sodium deoxycholate resulted in an increased responsiveness of the mesenteric bed preparation to the vasoconstrictor effects of each of the purines and pyrimidines tested. The selective P2x-purinoceptor--desensitizing agent α,β-methylene ATP inhibited vasoconstrictor responses to ATP and to CTP but had no effect on vasoconstrictor responses elicited by UTP, TTP, and GTP. In raised-tone preparations, vasodilator responses to ATP, UTP, TTP, and GTP were abolished after removal of the endothelium with sodium deoxycholate. Responses to acetylcholine were also abolished; those to sodium nitroprusside were unimpaired. An inhibitor of the formation of nitric oxide from L-arginine, Nω-nitro-L-arginine methyl ester (30 μM), which antagonizes responses mediated by endothelium-derived relaxing factor (nitric oxide), attenuated vasodilatation to ATP, UTP, and acetylcholine but not to sodium nitroprusside. In addition to confirming the presence of P2x- and P2y-purinoceptors in the rat mesenteric arterial bed, these results demonstrate the presence of discrete “pyrimidinoceptors,” which mediate vasoconstriction and vasodilatation by receptors located on the smooth muscle and on endothelial cells, respectively. Furthermore, it is shown that the vascular relaxations to ATP and UTP occur largely via production of endothelium-derived relaxing factor. It is probable that, like P2x-purinoceptors, UTP pyrimidinoceptors comprise a heterogeneous population, subtypes of which have been partially characterized according to their different actions and locations within the vasculature. (Circulation Research 1991;69:1583–1590)

Adenosine 5'-triphosphate (ATP) produces powerful systemic effects; it influences many biological processes, being released from nerve endings, platelets, and endothelial cells in physiological and pathophysiological processes. In the cardiovascular system its ability to cause vasoconstriction or vasodilatation is mediated through activation of subtypes of the P2x-purinoceptor, P2x- and P2y-purinoceptors, respectively. In many systems, including the rat mesenteric arterial bed, P2x-purinoceptors are present on the vascular smooth muscle; P2y-purinoceptors are located on endothelial cells, although in some vessels P2y-purinoceptors are also located on the smooth muscle. The naturally occurring nucleotides, cytidine 5'-triphosphate (CTP), thymidine 5'-triphosphate (TTP), and uridine 5'-triphosphate (UTP), which are pyrimidines, and guanosine 5'-triphosphate (GTP), which is a purine, have also been shown to have effects on the vasculature. Of these, probably the most studied has been UTP, which may be released from blood platelets. Several differences between the effects of UTP and ATP have led to a proposal for the existence of specific “pyrimidinoceptors,” distinct from purinoceptors. The basis for this proposal has come primarily from studies on the effects of ATP and UTP in the perfused liver, in HL-60 cells, and in neutrophils and macrophages, with limited studies on blood vessels. These are summarized in a review article by Seifert and Schultz. Among other discriminating differences, it has been shown in the rabbit ear artery that, whereas

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selective desensitization of the \( \mathrm{P}_{2X} \)-purinoceptor with \( \alpha,\beta \)-methylene ATP (\( \alpha,\beta \)-meATP)\(^{11} \) abolishes contractile responses to ATP, there is potentiation or only partial desensitization of responses to UTP.\(^9 \) Differential desensitization has also been observed after pretreatment with UTP and other agents in the rabbit basilar artery\(^{12} \) and in the rat tail and femoral arteries and dog saphenous vein.\(^{13} \)

The isolated rat mesenteric arterial bed was used to study the effects of ATP, UTP, and other purine and pyrimidine nucleotides on vascular tone. Previous characterization of \( \mathrm{P}_{2Y} \)-purinoceptor subtypes has shown this preparation to be amenable to the study of smooth muscle-mediated and endothelium-mediated responses.\(^3 \) In this preparation we aimed to produce a preliminary characterization of pyrimidinoceptors based on the nature of the responses and the location in the vasculature of the receptor subtypes. We further aimed to distinguish between purinoceptors and pyrimidinocinceptors by looking at the effects of \( \mathrm{P}_{2X} \)-purinoceptor desensitization with \( \alpha,\beta \)-meATP and at the effects of inhibition of endothelium-derived relaxing factor (EDRF)\(^{14} \) formation using \( N^w \)-nitro-L-arginine methyl ester (L-NAME).\(^{15} \)

Materials and Methods

Adult male Wistar rats (300–400 g) were used in the study. Rats were given heparin (1,000 units i.p.) and killed by stunning and exsanguination. Mesenteric beds were prepared for perfusion essentially as described by McGregor.\(^{16} \) A midline abdominal incision was made, and the superior mesenteric artery was exposed and cannulated. The superior mesenteric vein was severed, and perfusion was begun with Krebs’ solution containing (mM) \( \mathrm{NaCl} \) 133, KCl 4.7, \( \mathrm{NaH}_{2} \mathrm{PO}_{4} \) 1.35, \( \mathrm{NaHCO}_{3} \) 16.3, \( \mathrm{MgSO}_{4} \) 0.61, glucose 7.8, and \( \mathrm{CaCl}_{2} \) 2.52. Bovine serum albumin was added (5 g/l) to raise the colloid osmotic pressure and prevent tissue edema.\(^{17} \) The perfusate was gassed with 95% \( \mathrm{O}_{2} \)–5% \( \mathrm{CO}_{2} \) and maintained at 37°C. The vascular bed was isolated by cutting as closely as possible to the gut, and then perfusion was interrupted briefly to allow the bed to be transferred onto gauze in a heated chamber. Perfusion was begun again and adjusted to 4.8 ml/min. The preparation was also superfused at 1 ml/min with Krebs’ solution of the same composition. The preparation was allowed to equilibrate for 30 minutes before experimentation. Responses were measured as changes in perfusion pressure (mm Hg) with a pressure transducer (model P23, Gould, Cleveland, Ohio) on a side arm of the perfusion cannula and were recorded on a polygraph (model 79D, Grass Instrument Co., Quincy, Mass.).

Removal of Endothelium

The endothelium was removed by perfusing the preparation with 2 ml of a 2 mg/ml solution of sodium deoxycholate in saline over a period of 30 seconds. This caused a transient increase in perfusion pressure. The preparation was washed through for 10–15 minutes after addition of sodium deoxycholate solution. In experiments with raised tone, norepinephrine (NE) was washed out before removal of the endothelium and reintroduced after sodium deoxycholate treatment, after washout, when perfusion pressure had returned to baseline. The success of this treatment was assessed by using the endothelium-dependent relaxing agent acetylcholine (ACh). The integrity of the smooth muscle was checked with the direct smooth muscle relaxant sodium nitroprusside (SNP).

Drug Administration

Drugs were applied as bolus injections of 50 \( \mu \)l into an injection port proximal to the tissue. Vasoconstrictor responses to agents were examined on the preparation at basal tone. Vasodilator responses were examined after the tone of the preparation had been raised by the addition of NE to the perfusate to a final concentration of 30 \( \mu \)M. Pressor responses to doses of ATP, UTP, CTP, TTP, and GTP were examined before and after removal of the endothelium and after desensitization of \( \mathrm{P}_{2X} \)-purinoceptors with \( \alpha,\beta \)-meATP (1 \( \mu \)M, added to the perfusate 15 minutes before drug application). Responses to the purines and pyrimidines were also investigated in the raised-tone preparation before and after the endothelium had been removed.

The mechanism of the vasodilator response to ATP and UTP was investigated by using L-NAME.\(^{15} \) After control vasodilator responses had been established in the raised-tone preparation, NE was removed from the perfusate, and the preparation was perfused with L-NAME (30 \( \mu \)M) for 40–45 minutes, after which time the tone was again raised with NE. Since L-NAME caused a supersensitivity of the preparation to NE, careful titration of NE into the perfusate was required to avoid excessive and detrimental rises in perfusion pressure but yet to produce a level of tone equivalent to the control level. The new lower final concentration required to raise the tone was found to be 1–8 \( \mu \)M NE.

Drugs

ATP (sodium salt), UTP (sodium salt), GTP (sodium salt), CTP (sodium salt), TTP (sodium salt), \( \alpha,\beta \)-meATP (lithium salt), SNP, ACh (chloride), NE (bitartrate), and L-NAME were obtained from Sigma Chemical Co., Poole, UK. All were made up in distilled water except for NE, which was made up as a stock solution of 10 mM in 0.1 mM ascorbic acid.

Statistical Analysis

Results are given as mean±SEM. Statistical significance was evaluated by Student’s \( t \) test. A value of \( p<0.05 \) was taken as significant. The rank order of potency of agents was determined empirically.
Results

Isolated rat mesenteric beds were perfused at a constant flow rate of 4.8 ml/min. Basal perfusion pressure was 18.2±0.8 mm Hg (n=38).

The Effect of Purines and Pyrimidines on Rat Mesenteric Arterial Beds at Basal Tone

In preparations at basal tone, ATP, UTP, and CTP elicited dose-dependent contractions; TTP and GTP were without effect except at the highest doses used (Figures 1 and 2a). The potency order of these agents, as determined empirically, was ATP>CTP>UTP>TTP=GTP. In the absence of the endothelium, vasoconstrictor responses to each of these agents were significantly greater than control responses, as evidenced by a shift to the left of the dose–response curves (Figure 3). Removal of the endothelium caused greater potentiation of responses to UTP than to ATP. The tone of the preparations was subsequently raised to test the success of endothelium removal; responses to doses of ACh up to and including 5×10^{-9} mol were abolished. The preparation produced dose-related relaxations to SNP at doses from 5×10^{-11} to 5×10^{-8} mol.

The Effect of Purines and Pyrimidines on Preconstricted Rat Mesenteric Arterial Beds

Addition of NE (30 μM) to the perfusate raised the tone of the preparations to a final perfusion pressure of 86.5±5.9 mm Hg (n=28). In preparations in which tone had been raised with NE, dose-dependent relaxations were elicited by ATP, UTP, TTP, and GTP (Figures 1 and 2b). Their rank order of potency, as determined empirically, was UTP=ATP>TTP=GTP. CTP did not cause relaxation. ATP and UTP were equally effective in eliciting relaxations.

Figure 1. Representative tracings showing vasoconstrictor (upper tracings, preparation at basal tone) and vasodilator (lower tracings, preparation with tone raised with 30 μM norepinephrine) responses of the rat mesenterial arterial bed to doses of ATP, CTP, UTP, TTP, and GTP. Doses (expressed as −log mol) were applied as 50 μl bolus injections. In the upper tracings, each dose is labeled; in the lower tracings, alternate doses are labeled starting from the lowest dose at the right. Intermediate doses, which are not labeled, are, from left to right, as follows (−log mol): 9.3, 8.3, and 7.3 for ATP; 7.8 and 6.8 for CTP; 8.8, 7.8, and 6.8 for UTP, TTP, and GTP. Note that CTP did not produce relaxation.

Figure 2. Dose–response curves showing vasoconstrictor (panel a) and vasodilator (panel b) responses of the rat mesenterial arterial bed to purines and pyrimidines. Vasoconstrictor responses are shown as increases in perfusion pressure (mm Hg) and are the means of 14–20 preparations. Vasodilator responses were examined in the raised-tone preparation, in which tone was raised by the addition of norepinephrine (30 μM) to the perfusate. Shown are responses to ATP (●), UTP (▲), GTP (△), CTP (●), and TTP (○). Vasodilator responses are shown as decreases in perfusion pressure (mm Hg) and are the mean of 6–11 preparations. Symbols plus vertical bars indicate mean±SEM.
of the preparation to 97.6±8.3 mm Hg (n=13), which was not significantly different from the control. Removal of the endothelium abolished vasodilator responses to ATP, UTP, TTP, and GTP, and contractions were often produced instead. Relaxations to ACh at doses up to and including 5×10⁻⁵ mol were abolished. Dose-dependent relaxations of the preparation to SNP (doses of 5×10⁻¹¹ to 5×10⁻⁶ mol) were not attenuated by removal of the endothelium.

The Effect of Desensitization With α,β-meATP on Pressor Responses to Purines and Pyrimidines

Perfusion with α,β-meATP (1 μM) abolished responses to lower doses of ATP and greatly reduced the magnitude of the responses to higher doses. Responses to CTP were also abolished by α,β-meATP. Responses to UTP, GTP, and TTP were unaffected by α,β-meATP (Figure 4).

The Effect of L-NAME on Vasodilator Responses of Rat Mesenteric Arterial Beds to ATP, UTP, ACh, and SNP

Perfusion with L-NAME (30 μM) caused an enhanced sensitivity of preparations to NE when this was reapplied to raise the tone of the preparations. Careful titration of NE into the perfusate was required to allow the tone to be raised to 88.4±11.0 mm Hg (n=8), which was not significantly different from the control preparations. The concentration of NE required to produce this increase was 1–8 μM. L-NAME produced a significant attenuation of responses to ACh at all doses up to and including 5 nmol (Figure 5). Responses to SNP were not attenuated, but the lower doses were, in fact, enhanced in the presence of L-NAME (results not shown). L-NAME also attenuated vasodilator responses to ATP and UTP (Figures 6 and 7). Probably because of the difficulty in manipulating the tone to compare with pre-L-NAME tone, there was a large variation between preparations in the degree of effectiveness of L-NAME as an antagonist. There was a tendency
for it to be more effective against ATP than UTP, although this was not significant. There was a more pronounced tendency for ATP to produce vasoconstriction of the raised-tone preparation in the presence of L-NAME (Figure 6).

Discussion

The results of this study demonstrate the presence of the recently described pyrimidinoinceptors,8,9 distinct from purinoceptors, in the rat mesenteric arterial bed. In this preparation, the low perfusion pressures obtained at "basal tone" are not representative of normal conditions in vivo but allow assessment of vasoconstrictor mechanisms with minimum contribution of opposing dilator effects. NE added to the perfusate to raise the tone allowed us to look selectively at vasodilator mechanisms. In vivo, the net response is the resultant of vasodilator and vasoconstrictor effects, the relative contribution of which will vary according to vascular tone. As with ATP, the pyrimidines UTP and TTP and the purine GTP can elicit both vasoconstriction and vasodilatation in the mesenteric arterial bed, after action at receptors located on the vascular smooth muscle and on the endothelium, respectively. CTP produces only contraction. Desensitization to α,β-meATP was used to distinguish between the P2X-purinoceptors and pyrimidinoinceptors mediating vasoconstriction, whereas EDRF was shown to be common to the mechanism of action of the vasodilator activity of UTP and ATP.

The many potent and diverse effects of ATP have long been recognized (see Reference 1 for review). Action-specific P2-purinoceptor subtypes have been described that, in the cardiovascular system, can mediate contraction (the P2X-purinoceptor) and re-
laxation (the P$_{2\nu}$-purinoceptor). In contrast there are comparatively few reports of the vascular effects of the other naturally occurring nucleotides. As with ATP, both vasoconstrictor and vasodilator effects have been described for UTP; UTP causes vasoconstriction in several preparations including the rabbit ear artery, isolated rat mesenteric vessels, and the isolated perfused rat liver, whereas endothelium-dependent vasodilatations have been described in the pig aorta and human pial vessels. Both vasoconstriction and vasodilatation due to UTP have been shown in the perfused rat hind limb. Since UTP is a constituent of platelets and brain tissue, it has been suggested that this agent may have a role as a mediator of cerebral vasospasm. The effects of CTP, GTP, and TTP on the vasculature tend to be less pronounced. A lack of effect of TTP and CTP on human pial vessels has been described, although CTP and GTP have been shown to cause vasoconstriction of the rat hind limb and endothelium-dependent relaxation of the pig aorta. GTP and CTP potentiate vasoconstriction to exogenous NE in the rat mesenteric arterial bed, a process that has also been described for ATP in this preparation.

One of the first studies to investigate specifically the possibility of discrete vascular purinoreceptors and pyrimidinoreceptors was that of von Kügelgen et al. in the rabbit ear artery. These workers used the selective P$_{2\nu}$-desensitizing agent αβ-meATP to demonstrate that, although vasoconstrictor responses to ATP were reduced by 88%, those to UTP were enhanced or only partially reduced. They concluded that, in their preparation, UTP elicited vasoconstriction by receptors distinct from the P$_{2\nu}$-purinoceptor. In the rat mesenteric arterial bed, we also found that, although vasoconstrictor responses to ATP were virtually abolished by prior desensitization with αβ-meATP, those to UTP, GTP, and TTP were unaffected, which implies a vasoconstrictor action at receptors other than the P$_{2\nu}$-purinoceptor. Vasoconstrictor responses to CTP, however, were abolished by αβ-meATP. It is possible that the stereoc- hemical similarity of ATP and CTP at the amino group (N$^4$ for ATP, N$^4$ for CTP) is a crucial link between the antagonistic effect of αβ-meATP and the pressor responses elicited by these agents. CTP differed from the other purines and pyrimidines in that it elicited powerful contractions, but only at relatively high doses, as reflected by its steep dose–response curve. It is possible that its effects were mediated indirectly after release of ATP by high concentrations of this agent, which would account for the fact that its responses were abolished by αβ-meATP.

In a previous study, we have shown that, as in many other isolated vessels and vascular beds, the receptors for ATP-mediated vasoconstriction of the rat mesenteric arterial bed are located on the vascular smooth muscle. The present study confirmed these findings and showed that this was also the case for receptors mediating vasoconstriction to UTP, GTP, TTP, and CTP, because responses were not abolished after removal of the endothelium. In the absence of the endothelium, pressor responses to all of these agents were significantly greater than in the controls, as reflected in a shift to the left of the dose–response curves. An increased responsiveness to various agents after removal of the endothelium has previously been observed in this and in other preparations. The enhanced responsiveness may be due to either 1) an increased local concentration of these agents at their smooth muscle receptors by virtue of the removal of a diffusion barrier (the endothelium) or 2) the removal of modulatory effects of the endothelium. Since responses to CTP, which does not elicit relaxation in this preparation, were also enhanced, it is less likely that this effect was due to the removal of an opposing relaxant effect exerted at endothelial receptors.

The rank order of vasodilator potency of the purines and pyrimidines in the rat mesenteric bed was found to be UTP > ATP > TTP > GTP. CTP had no vasodilator effects in this preparation or in the rat hind limb preparation, in contrast to the pig aorta, in which it elicits endothelium-dependent relaxation. ATP and UTP were equipotent in eliciting vasodilatation; however, at the highest doses, responses to ATP showed a tendency to become smaller. It is likely that at these doses the balance of the response shifts in the favor of the P$_{2\nu}$-purinoceptor–mediated contraction, opposing the P$_{2\nu}$-purinoceptor–mediated vasodilatation. Since UTP is less potent as a vasoconstrictor than ATP, this opposing effect is less pronounced, resulting in more potent relaxations compared with ATP at high doses. In the present study we found that, as in the pig aorta and human pial vessels, relaxation of the rat mesenteric arterial bed by UTP was endothelium dependent. Relaxations to ATP, TTP, and GTP were also dependent on an intact endothelium.

ATP can stimulate prostaglandin and prostacyclin production from perfused vascular beds and from endothelial cells in culture, as well as cause the production of EDRF. UTP has also been shown to stimulate prostacyclin production in endothelial cells and in the rat liver. The vascular relaxations produced by ATP and UTP could, therefore, be mediated by production of either of these endothelium-derived substances, although there is evidence that in the case of ATP relaxation is more likely to proceed via EDRF. We used this information to investigate, and possibly distinguish between, the
vasodilator mechanisms of ATP and UTP by using L-NAME, an inhibitor of the pathway of conversion of l-arginine to nitric oxide and, hence, an inhibitor of relaxations mediated by EDRF (identified as nitric oxide). A more direct approach to distinguish between P2Y-purinoceptor-mediated and pyrimidinoceptor-mediated relaxations could not be adopted because of the absence of specific antagonists to pyrimidinoceptors and because the P2Y-purinoceptor antagonist, reactive blue 2, caused a drop in tone of the raised-tone preparation.

L-NAME has been successfully used in the rat mesenteric bed to antagonize relaxant responses to ACh (Reference 15 and the present study). In the current study, it significantly attenuated relaxations to both ATP and UTP, suggesting that, for both of these, relaxation takes place largely through the formation of EDRF and not through prostacyclin. The tendency for L-NAME to produce a greater antagonism of vasodilatation to ATP than UTP may be due to an unmasking of the more potent effects of ATP as a contractile agent at P2X-purinoceptors, although it may also be because a more significant proportion of the relaxation to UTP is mediated through a non-EDRF pathway. The potentiation of responses to NE in the presence of L-NAME may be due to inhibition of a continuous basal release of endogenous EDRF, allowing constriction to occur with greater potency. Responses to the endothelium-independent vasodilator SNP were enhanced by L-NAME. The reason for the enhancement of responses to SNP is not known but has also been observed by us in the isolated perfused rabbit liver preparation and is the subject of a separate communication.

In conclusion, we have demonstrated the presence of pyrimidinoceptors, distinct from P2-purinoceptors, in the rat mesenteric arterial bed. As with P2-purinoceptors, activation of action-specific pyrimidinoceptors located on the smooth muscle and on endothelial cells elicits vasoconstriction and vasodilatation, respectively. Further characterization of these receptors awaits the development of pyrimidine agonists and antagonists in the same way as has been done for ATP and other purines. However, there is strong evidence that pyrimidinoceptors are a heterogeneous population comprising at least two subtypes. Although the physiological relevance of pyrimidines in the regulation of rat mesenteric resistance vessels is not clear from the present study, it is feasible that they may be released under certain physiological or pathophysiological conditions to contribute to the regulation of vascular tone. A preliminary characterization has been made of the receptors involved, and a model system has been described for testing agonists and antagonists of these agents.

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**KEY WORDS**  
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