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Prominent sympathetic purinergic vasoconstriction in the rabbit splenic artery: potentiation by 2,2′-pyridylisatogen tosylate

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1 Vasoconstrictions induced by transmural electrical field stimulation were frequency-dependent from 2 to 32 Hz in the rabbit isolated splenic artery. All contractions were abolished in the presence of tetrodotoxin 1 μM or guanethidine 100 μM. Stimulation at a frequency of more than 32 Hz induced both neurogenic and myogenic responses.

2 Prazosin (1 μM) did not significantly affect vascular contractions to electrical stimulation. Desensitization of P_{2X}-purinoceptors with α,β-methylene ATP (α,β-meATP, 3 μM) abolished the contractions to stimulation at 2–8 Hz and inhibited more than 80% of the vascular response at 16 Hz, but it did not significantly change the responses at 32 Hz. Contractile responses at 32 Hz were inhibited by a combination of prazosin and α,β-meATP. Effects of pyridoxal-phosphate-6-azophenyl-2′, 4′-disulphonic acid tetrasodium salt (a selective P_{2X}-purinoceptor antagonist) and suramin (a competitive P_2-purinoceptor antagonist) on the neurogenic responses were investigated in this study.

3 2,2′-Pyridylisatogen tosylate (PIT, 0.3–3 μM) significantly potentiated the vasoconstrictions to electrical stimulation at 2–32 Hz in a concentration-dependent manner. Potentiated responses were restored to the control level 30 min after washing. Concentration-dependent response curves for noradrenaline (NA) or α,β-meATP were not significantly changed by 3 μM PIT, and vasoconstriction by adenosine 5′-triphosphate (ATP, 300 μM) was unaffected by PIT. Coomassie brilliant blue-G (1 μM), which shares the potentiating effect on a recombinant P_{2Y}-purinoceptor with PIT (King *et al*., 1996), did not inhibit or potentiate the purinergically-mediated component of the response to sympathetic nerve stimulation. The selective α_2-adrenoceptor antagonist yohimbine (1 μM) also potentiated the vascular responses to electrical stimulation.

4 The present results indicate that ATP evokes postjunctional contractile responses at low and high frequency electrical stimulation of sympathetic nerves supplying the rabbit splenic artery. PIT potentiates the responses to sympathetic (purinergic) nerve stimulation; this appears to be mainly via prejunctional rather than postjunctional actions.

Keywords: Sympathetic nerves; purinergic transmission; prejunctional modulation; 2,2′-pyridylisatogen tosylate; vasoconstriction; rabbit splenic artery

Introduction

It is generally accepted that adenosine 5′-triphosphate (ATP) is a cotransmitter with noradrenaline (NA) in sympathetic nerves supplying various blood vessels (Burnstock, 1990; von Kügelgen & Starke, 1991). Evidence for this concept in the vasculature was obtained largely from *in vitro* experiments with rabbit blood vessels, including aorta (Su, 1975; 1978), pulmonary artery (Katsuragi & Su, 1980; 1982), ear artery (Kennedy *et al*., 1986), jejunal artery (Ramme *et al*., 1987), saphenous artery (Burnstock & Warland, 1987) and hepatic artery (Brizzolara & Burnstock, 1990). The proportion of the purinergic to the adrenergic component varies considerably among different arteries (ear artery, Kennedy *et al*., 1986; saphenous artery, Burnstock & Warland, 1987; hepatic artery, Brizzolara & Burnstock, 1990). It has been shown that the sole transmitter released by sympathetic nerves supplying rabbit mesenteric arteries and guinea-pig submucosal arterioles is ATP, while NA acts as a prejunctional modulator of ATP release (Ramme *et al*., 1987; Evans & Surprenant, 1992). Similarly, we demonstrated recently that ATP is the sole transmitter of contractile responses to sympathetic nerve stimulation at 1 Hz, while NA co-released with ATP acted as a prejunctional modulator in the canine isolated and perfused splenic artery (Ren *et al*., 1996).

The present study was designed to investigate contractile responses to sympathetic nerve stimulation in rabbit splenic artery. We analysed neurogenic vasoconstriction, by use of a selective P_{2X}-purinoceptor antagonist, pyridoxal-phosphate-6-azophenyl-2′, 4′-disulphonic acid tetrasodium salt (PPADS) (Ziganshin *et al*., 1994), a competitive P_2-purinoceptor antagonist, suramin (Dunn & Blakeley, 1988; Leff *et al*., 1990), and desensitisation of P_{2X}-purinoceptors with α,β -methylene ATP (α,β-meATP) (Warland & Burnstock, 1987). Another known P_2-purinoceptor antagonist, 2,2′-pyridylisatogen tosylate (PIT) (Spedding *et al*., 1975), was recently shown to be a forerunner of a new class of therapeutic agents because of its potentiating action in low concentrations of ATP-responses at P_{2Y}-purinoceptors (King *et al*., 1996). Potentiation of the response to ATP by PIT at low concentration was also obtained in the guinea-pig terminal ileum (Kazic & Milosavljevic, 1977). Thus, we also investigated whether PIT potentiated the purinergic component of the responses to sympathetic nerve stimulation in the rabbit splenic artery.

Methods

Arterial preparations

Male New Zealand white rabbits (3.0–3.5 kg) were killed by an overdose of pentobarbitone sodium (Sagatal) injected via the ear vein, then exsanguinated. The splenic artery was excised and cleaned of excess connective tissue and fat. Ring segments with endothelium (4 mm in length) were mounted horizontally in a 10 ml organ bath by carefully inserting a

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tungsten wire through the lumen of the vessel ring and anchoring it to a stationary support. Another wire similarly inserted, was connected to a Grass FT03C force-displacement transducer and responses were recorded on a pen recorder (Grass). The preparation was placed under a resting tension of 1.0 g and allowed to equilibrate for 1 h in a physiological solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$, 16.3, $MgSO_4$ 0.61, glucose 7.8 and $CaCl_2$ 2.52, pH 7.2 (Bülbring, 1953). The solution was maintained at 37°C and aerated with 95% O_2 and 5% CO_2.

Transmural electrical stimulation was delivered to the tissue by means of two platinum wire electrodes placed parallel to, and on each side of the vessel, by a Grass S11 stimulator. A voltage of 60–70 V and a pulse width of 0.09–0.1 ms were used in the experiments and the neurogenic origin of the responses was confirmed by the abolition of all contractions with 1 μM tetrodotoxin (TTX). The preparation was stimulated over a frequency range of 2–32 Hz for 1 s at 5 min intervals. This frequency-dependent response curve was repeated 4 or 5 times at 30 min intervals in each preparation. The first set of data was not used in the present experiments.

NA was added cumulatively to the organ bath to produce agonist concentration-response curves. The concentration-response curve for NA was repeated 3 times in each preparation at 1 h intervals. The first set of data was not used in analysis. The P_{2X}-purinoceptor agonist α,β-meATP, which rapidly desensitizes its own receptors, was added non-cumulatively at 30 min intervals. Only one concentration-response curve for α,β-meATP was generated per preparation. α,β-MeATP (10 μM) was added to the organ bath and the preparation repeatedly washed for 1 h before the concentration-response curve for α,β-meATP was constructed; this initial response was used to standardize the experimental data.

Antagonists and inhibitors were added to the organ bath 20 min before carrying out the next experimental procedure (electrical stimulation, concentration-dependent response curve for NA or administration of α,β-meATP), except for TTX (10 min before). Desensitization of the P_{2X}-purinoceptor was achieved by several exposures (generally 3 times) of the vessel to α,β-meATP (1 μM) at 5 min intervals until no further contraction was elicited and tone had returned to baseline. The selective α-$_1$-adrenoceptor antagonist prazosin (1 μM) was used to block an adrenergic component of the neurogenic response (Brizzolara & Burnstock, 1990). The selective P_{2X}-purinoceptor antagonist, PPADS (30 μM, Ziganshin, 1994), the P_{2X}-purinoceptor desensitising agent, α,β-meATP (Sneddon & Burnstock, 1985), and the competitive P_2-purinoceptor antagonist, suramin (300 μM, Dunn & Blakeley, 1988; Leff *et al.*, 1990), were used to block the purinergic component of the neurogenic response. The effects of each antagonist, inhibitor and desensitization of P_{2X}-purinoceptor with α,β-meATP on the contractile responses to NA and to α,β-meATP (as an agonist) were tested.

The effects of PIT (0.3–3 μM) and Coomassie brilliant blue-G (1 μM), which both potentiate P_2-purinoceptor-regulated functional responses (King *et al.*, 1996), on the vascular responses to electrical stimulation and to exogenous NA, ATP or α,β-meATP were observed.

In order to determine whether there was prejunctional modulation in the present experimental system (Story *et al.*, 1981; Bulloch & Starke, 1990), the selective α_2-adrenoceptor antagonist, yohimbine (1 μM, Ramme *et al.*, 1987), was tested. TTX (1 μM) and guanethidine (100 μM) were applied to analyse whether the contractile response was generated by neurotransmitters from sympathetic nerves.

Drugs

α,β-Methylene adenosine 5′-triphosphate (α,β-meATP, lithium salt), (−)-noradrenaline bitartrate (NA), adenosine 5′-triphosphate (ATP, sodium salt), Coomassie brilliant blue-G, prazosin hydrochloride, tetrodotoxin (TTX), and yohimbine hydrochloride were all obtained from Sigma Chemical Company. Guanethidine sulphate (Ismelin) was obtained from CIBA Laboratories, and pentobarbitone sodium was supplied by May and Baker Ltd. Pyridoxal-phosphate-6-azophenyl-2′, 4′-disulphonic acid tetrasodium salt (PPADS) was purchased from Tocris Cookson Ltd. Suramin was a gift from Bayer Plc. 2,2′-Pyridylisatogen tosylate (PIT) was synthesized by Dr. D. Billington, Institut de Recherches Servier, France. All drugs were dissolved in distilled water, except for PIT which was dissolved in 0.1 N HCl and then brought back to pH 7.4 by adding 0.5 N NaOH. Ascorbic acid (100 μM) was added to the NA solution.

Statistical analysis

Vascular responses to drugs and electrical stimulation are expressed as the maximal changes in tension (mg or g) from their control levels, unless mentioned otherwise (see Results). Values presented are the mean ± s.e.mean. An analysis of variance (general linear model) was used to evaluate any differences between frequency-dependent response curves for electrical stimulation or concentration-response curves for drug. If the F statistic was significant, we compared the individual datum with its respective control value by simultaneous multiple comparisons, by use of Dunnett's test (Wallenstein *et al.*, 1980). The EC_{50} value for a drug was calculated from the mean (concentration) ± s.e.mean which produced 50% of its maximal response. Comparison of NA maximal responses and its EC_{50} values before and after treatment were analysed by unpaired t tests. Comparison of a pair of responses to ATP before and after treatment was analysed by paired t tests. P values less than 0.05 were considered statistically significant.

Results

Effects of TTX and guanethidine on the contractile responses to electrical stimulation

Electrical stimulation (2–32 Hz) produced a frequency-dependent response curve and reproducible response curves were obtained on 3 or 4 successive occasions without significant difference. The contractile responses to stimulation were abolished by treatment with TTX (1 μM) or guanethidine (100 μM) (Figure 1). NA (0.01–100 μM) gave reproducible concentration-dependent responses curves (Figure 2a). TTX (1 μM) changed neither the maximal response nor the EC_{50} value of NA significantly (Table 1). Guanethidine (100 μM)

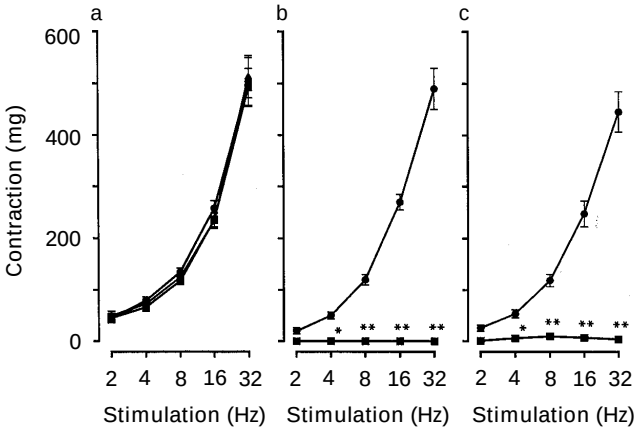

Figure 1 Effects of tetrodotoxin and guanethidine on the vascular responses to electrical sitmulation in the rabbit splenic artery. (a) Frequency-dependent response curves of time control; (●) the first time; (■) the second time; (▲) the third time. (b) (●) Control; (■) 1 μM tetrodotoxin. (c) (●) Control (■) 100 μM guanethidine. Points represent the mean values and vertical lines show s.e.mean. Asterisks represent statistical significance vs the respective control value: $^*P<0.05$; $^{**}P<0.01$, $n=7-8$.

significantly shifted the concentration-dependent response curve for NA to the left in a parallel manner, but it did not affect the maximal contractile response to NA (Figure 2c).

Effects of prazosin, P_{2X}-purinoceptor desensitization and P_2-purinoceptor antagonists on the contractile responses induced by electrical stimulation, NA and α,β-meATP

Prazosin (1 μM) did not affect the vascular responses to stimulation at 2–16 Hz. In eight preparations stimulated at 32 Hz, the contractile responses in three preparations were enhanced or not changed, and in the other 5 responses were partially inhibited by prazosin. Desensitization of P_{2X}-purinoceptors with α,β-meATP (3 μM) abolished the contractile responses to stimulations at 2–8 Hz. The response to stimulation at 16 Hz was significantly inhibited by 86.3±5%. In eight preparations stimulated at 32 Hz, the contractile responses in three preparations were enhanced, and in the other 5 responses were partially inhibited by desensitization of P_{2X}-purinoceptors with αβ-meATP. A combination of both prazosin and α,β-meATP blocked all responses to electical stimulation at 2–16 Hz, and significantly inhibited responses at 32 Hz by 86.3±5% (Figure 3a) and 93±2.4% (Figure 3b). Vasoconstrictor responses to NA (0.1–100 μM) were completely inhibited by prazosin (1 μM) except for the response to 100 μM NA which was inhibited by 98±0.8% (Figure 2b). In preparations desensitized with α,β-meATP (3 μM), α,β-meATP (0.01–30 μM, as an agonist) failed to elicit further contractile responses (data not shown), but the concentration-dependent response curve for NA was not changed significantly (Table 1).

Treatment with the selective P_{2X}-purinoceptor antagonist PPADS (30 μM) or a combination of both PPADS and prazosin changed the contractile responses to electrical stimulation at 2–32 Hz in a similar manner to desensitisation with α,β-meATP or the combination of both α,β-meATP and prazosin (Figure 4a). Suramin (100 μM) did not change the neurogenic contraction induced by electrical stimulation (data not shown). Suramin (300 μM) significantly inhibited the contractile responses to electrical stimulation at 2, 4, 8, 16 and 32 Hz by 84%, 69%, 46%, 49% and 52%, respectively. A combination of both prazosin and suramin significantly inhibited responses at 4 and 8 Hz (Figure 4b).

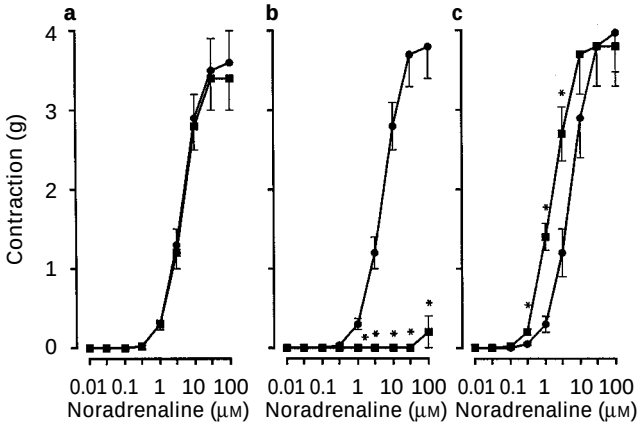

Figure 2 Effects of prazosin and guanethidine on the vascular responses to noradrenaline in the rabbit splenic artery. (a) Concentration-dependent response curves for noradrenaline; (●) the first time; (■) the second time. (b) (●), Control; (■), 1 μM prazosin. (c) (●) Control; (■) 100 μM guanethidine. Points represent the mean values and vertical lines show s.e.mean. Asterisks represent statistical significance vs the respective control value: $*P<0.05$, $n=7-10$.

Table 1 Effects of P_2-purinoceptor antagonists, αβ-methylene ATP, PIT, guanethidine and tetrodotoxin on the vasoconstrictive responses to NA in the rabbit splenic artery

Antagonist (μM)	*Maximal responses* (g) *Control*	*Treatment*	*EC_{50} values* (μM) *Control*	*Treatment*
Suramin (300)	3.87±0.46	3.68±0.38	3.5±0.4	4.1±0.3
PPADS (30)	4.0±0.3	3.90±0.4	3.2±0.6	3.1±0.6
α,β-meATP (3)	3.19±0.38	3.13±0.37	3.2±0.5	3.3±0.6
PIT (3)	3.34±0.56	3.37±0.58	3.2±0.6	3.1±0.6
Guaneth (100)	3.99±0.5	3.86±0.51	3.6±0.4	1.4±0.1**
TTX (1)	3.22±0.22	3.04±0.23	3.4±0.6	3.6±0.6

$**P<0.01$, $n=6-8$. PIT = 2,2′-pyridylisatogen tosylate.

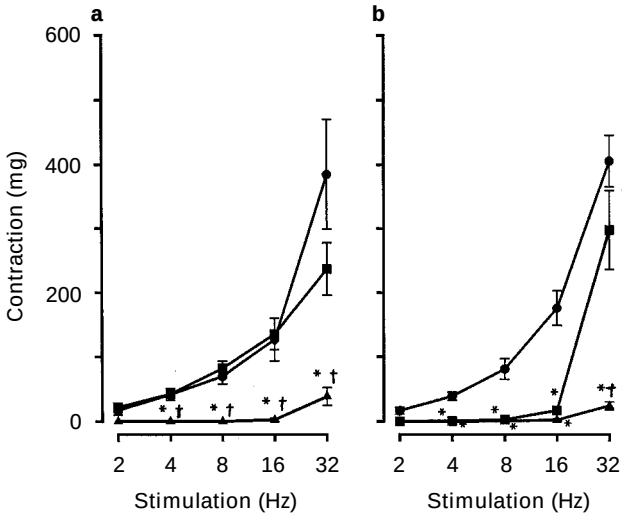

Figure 3 Effects of 1 μM prazosin (■, a), desensitization of P_{2X}-purinoceptor with 3 μM α,β-meATP (■, b), and a combination of both drugs (▲, a and b) on the contractile responses (●, control) to electrical stimulation. Points represent the mean values and vertical lines show s.e.mean. *Represents statistical significance vs the respective control value. †Represents statistical significance vs prazosin treatment (a) or α,β-meATP treatment (b): $*P<0.05$; $\dagger P<0.05$, $n=8$.

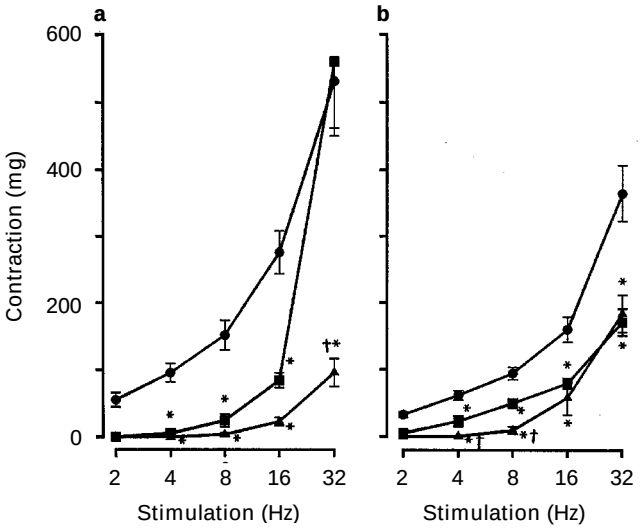

Figure 4 Effects of 30 μM PPADS (■, a), 300 μM suramin (■, b), and a combination of both PPADS and 1 μM prazosin (▲, a) or both suramin and prazosin (▲, b) on the contractile responses (●, control) to electrical stimulation. Points represent the mean values and vertical lines show s.e.mean. *Represents statistical significance vs the respective control value. †Represents statistical significance vs PPADS treatment (a) or suramin treatment (b): $*P<0.05$; $\dagger P<0.05$, $n=7$.

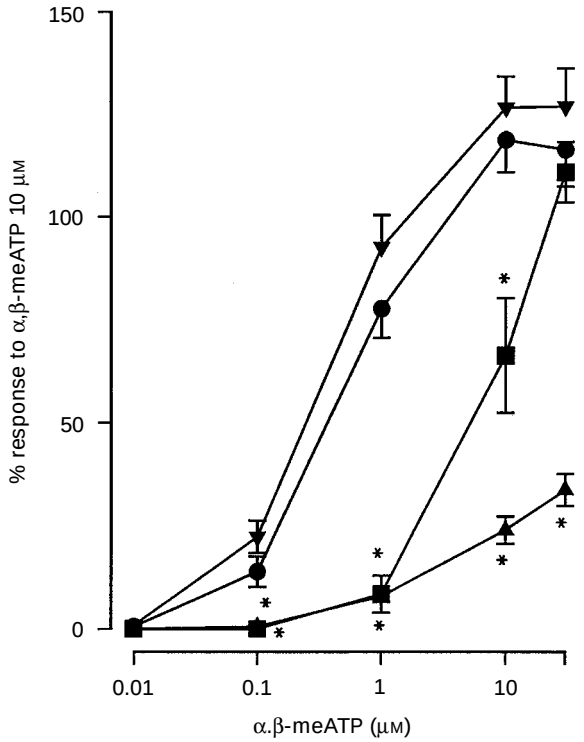

Figure 5 Effects of 300 μM suramin (■), 30 μM PPADS (▲) and 3 μM 2,2′-pyridylisatogen tosylate (▼) on the vascular contractile response curve for α,β-methylene ATP (●). The responses are expressed as percentage of those to an initial administration of 10 μM α,β-meATP 1 h before the response curve in each preparation was constructed. Points represent the mean values and vertical lines show s.e.mean. Asterisks represent statistical significance vs the respective control value: $^*P<0.05$, $n=5$.

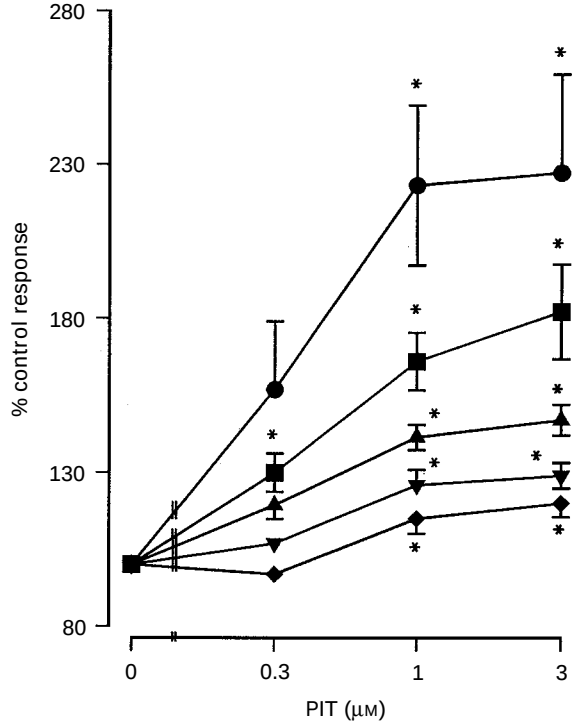

Figure 6 Effects of 2,2′-pyridylisatogen tosylate (PIT, 0.3–3 μM) on vasoconstrictions induced by electrical stimulation at 2 (●), 4 (■), 8 (▲), 16 (▼) and 32 Hz (◆) Hz in the rabbit splenic artery. Responses are expressed as percentage of the response to the same frequency of stimulation in the absence of PIT. Points represent the mean values and vertical lines show s.e.mean. Asterisks represent statistical significance vs the respective control value: $^*P<0.05$, $n=8$.

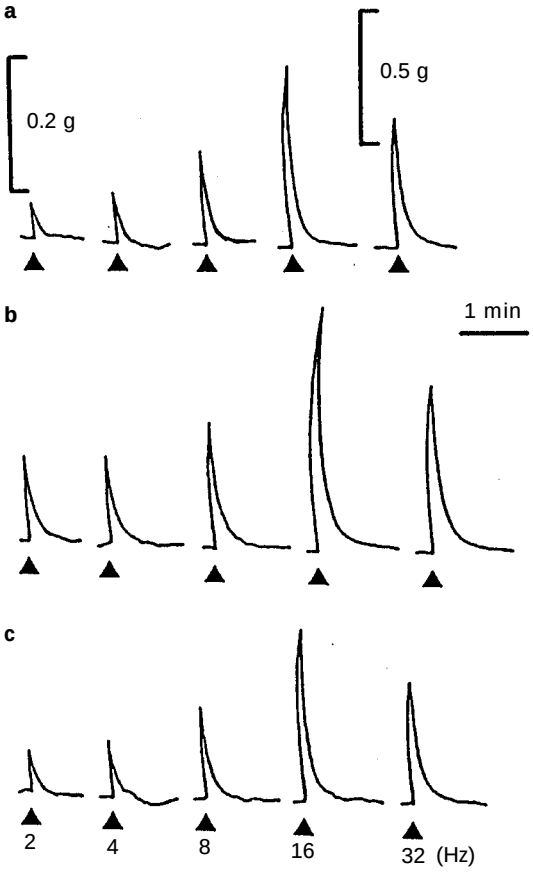

Figure 7 Single traces showing frequency-dependent contractile responses to electrical stimulation, 2–32 Hz, in the rabbit splenic artery in the absence (a) and presence (b) of 3 μM 2,2′-pyridylisatogen tosylate and (c) 30 min after washing out the drug.

A non-cumulative concentration-response curve for α,β-meATP (as an agonist) was constructed from 0.01 to 30 μM. Maximal contractions to α,β-meATP were observed at 10 μM, the EC_{50} was 0.6 ± 0.07 μM ($n=5$). Concentration-response curves for α,β-meATP were significantly shifted to the right by either PPADS or suramin. Only PPADS reduced the maximal contraction of α,β-meATP (Figure 5). Neither PPADS nor suramin affected the maximal response and the EC_{50} value for NA (Table 1).

Effects of PIT, Coomassie brilliant blue-G and yohimbine on electrical stimulation-induced contraction and vascular responses to NA, ATP and α, β-meATP

Figure 6 shows the effects of PIT on neurogenic responses. Each response, after treatment with PIT, was expressed as percentage of its respective control. PIT (0.3–3 μM) did not change the resting tension of the preparation, but it potentiated the contractile responses to electrical stimulation at 2–32 Hz, significantly and concentration-dependently. The potentiation by PIT of the neurogenic response was more prominent at low rather than high frequencies of stimulation (Figure 6). Potentiated responses were restored to control level after washing for 30 min (Figure 7). The maximal contractile response to NA and its EC_{50} value were not changed by 3 μM PIT (Table 1). The concentration-response curve for α,βmeATP in the preparations treated by 3 μM PIT was not significantly different from the control curve (Figure 5). PIT (3 μM) did not affect the contractile responses to 300 μM ATP (Figure 8). Coomassie brilliant blue-G (1 μM) did not cause any significant effects on the vasoconstriction induced by electrical stimulation ($n=4$, data not shown). Vascular responses induced by electrical stimulation at 2–32 Hz were

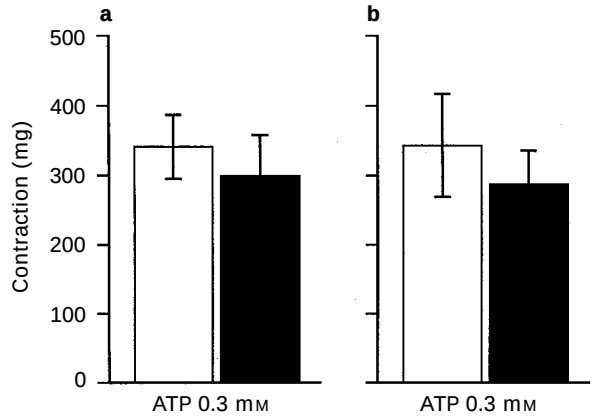

Figure 8 Effects of (a) vehicle and (b) 3 μM 2,2′-pyridylisatogen tosylate (solid columns) on the vascular contractile response induced by ATP. Control responses to ATP (open columns). Columns represent the mean values with s.e.mean; $n=8$.

potentiated by the selective α_2-adrenoceptor antagonist yohimbine (1 μM) from control values of 11 ± 10 (mg, $n=4$), 29 ± 15, 68 ± 11, 137 ± 15 and 339 ± 37, to values of 20 ± 20, 63 ± 12, 133 ± 12 ($P<0.05$), 315 ± 21 ($P<0.05$) and 510 ± 17 ($P<0.05$).

Discussion

The results of this study lead to two main conclusions. First, electrical stimulation of sympathetic nerves supplying the rabbit splenic artery induces a vasoconstriction, which is largely the result of the action of the purinergic cotransmitter, ATP; while NA is responsible for negative autofeedback modulation of transmitter release via prejunctional α_2-adrenoceptors. Second, PIT potentiates sympathetic purinergic cotransmission, apparently at a prejunctional site.

Although the arteries were stimulated by electrical stimulation with a short train (1 s), which favoured the purinergic component of the vasoconstriction, prazosin significantly inhibits most of the contractile response at 2–100 Hz in the rabbit ear artery (Kennedy *et al*., 1986), a portion of the response at 8–32 Hz in the rabbit hepatic artery (Brizzolara & Burnsock, 1990), a portion of the response in the rabbit jejunal artery (10 Hz, Evans & Cunnane, 1992) and saphenous artery (32–64 Hz, Warland & Burnstock, 1987). However, the contractile responses in the rabbit splenic artery to electrical stimulation with 2–16 Hz were not changed by prazosin, but were abolished by desensitization of P_{2X}-purinoceptors with α,β-meATP. The response induced by 32 Hz stimulation was not significantly reduced by prazosin alone, but blocked by a combination of both α,β-meATP and prazosin. This concentration of prazosin blocked the endogenous NA-induced vasoconstriction, while desensitisation of the P_{2X}-purinoceptor with α,β-meATP did not affect the responses to NA. Similar results were observed in the experiments in which a selective P_{2X}-purinoceptor antagonist, PPADS, was used. Furthermore, the neurogenic contraction was blocked by guanethidine, which is a sympathetic neurone blocking agent (Burnstock & Warland, 1987; Ren *et al*., 1994). These results suggest that, in common with mesenteric and submucosal vessels (Ramme *et al*., 1987 Evans & Surprenant, 1992), ATP released by sympathetic nerves is involved predominantly in postjunctional contraction of the rabbit splenic artery, and there is no an apparent adrenergic component of the neurogenic contraction induced by electrical stimulation over a wide range of frequencies, 2–32 Hz, in the present experimental conditions. On the other hand, the selective α_1-adrenoceptor antagonist prazosin blocked the responses to NA, while yohimbine significantly potentiated the neurogenic contractions, indicating that only postjunctional α-$_1$-adrenoceptors are responsible for the contraction to NA and endogenous NA co-released by electrical stimulation inhibits the release of neurotransmitters at prejunctional α_2-adrenoceptors, similar to the splenic artery of the dog (Ren *et al*., 1994; 1996).

The spleen and liver have a large potential for blood mobilisation and serve as the major blood volume reservoir in some animals (Brooksby & Donald, 1972). It has been demonstrated that after the induction of cardiogenic shock in the dog, hepatic volume is increased whereas splenic volume decreased, which probably involves active dilatation of the hepatic vessels and active splenic contraction, respectively (Risöe *et al*., 1991). Previous work in our laboratory showed that there were no clear contractile responses in the rabbit hepatic artery to electrical stimulation at a frequency less than 8 Hz (Brizzolara & Burnstock, 1990), but electrical stimulation at 2–8 Hz caused an obvious contraction in the rabbit splenic artery. Maximal contraction induced by NA (3–4 g) and α,β-meATP (2.9 g) in the rabbit splenic artery was 2 and 4 times, respectively, the strength of the responses of the hepatic artery (Brizzolara & Burnstock, 1990). Therefore, the results of this study and a previous study of the hepatic artery show further evidence of a difference in sensitivity of the sympathetic nerves and a difference in reactivity of the smooth muscles to drugs, consistent with the pathological changes in cardiogenic shock in the dog (Risöe *et al*., 1991). It has been speculated that the purinergic component of sympathetic cotransmission is involved in the rapid and powerful vasoconstriction in the defence reaction (Burnstock, 1988).

A relatively small inhibitory effect of suramin on the purinergic contraction elicited by electrical stimulation might be due to its property of competitive antagonism and its inhibitory action on ecto-ATPase (Crack *et al*., 1994). Indeed, the maximal response to α,β-meATP (agonist) was obviously reduced by PPADS, but not changed by suramin (Figure 5). The potentiation by guanethidine of NA-induced contractile responses could be explained by its inhibitory action on neuronal uptake of catecholamines (Lundborg & Stitzel, 1968; Khan & Wakade, 1979), which would result in an increase in the concentration of NA at the vascular neuromuscular junctions.

It has been shown that PIT ($\geqslant12.5$ μM) selectively suppresses the relaxant responses of the guinea-pig taenia caeci to ATP in a time- and concentration-dependent manner (Spedding *et al*., 1975; Spedding & Weetman, 1978). However, lower concentrations (0.5–2.5 μM) of PIT, enhanced the contractions to exogenous ATP, KCl and electrical stimulation in the guinea-pig terminal ileum (Kazic & Milosavljevic, 1977). Recently, King *et al*. (1996) found that PIT at a concentration of 0.1–3 μM potentiated ATP-evoked inward membrane currents in cRNA-injected *Xenopus* oocytes expressing chick P_{2Y_1}-purinoceptors. A direct relaxant response to low concentrations (less than 2.5 μM) of PIT was also observed in the taenia caeci (Spedding *et al*., 1975; Spedding & Weetman, 1978).

The present study showed that PIT (0.3–3 μM) significantly potentiated the contractions induced by electrical stimulation of sympathetic (purinergic) nerves, but did not affect the vascular contractile responses to α,β-meATP (as an agonist), exogenous ATP and NA in the rabbit splenic artery (Figures 5, 6, 7 and 8). All the known actions of PIT at low concentrations do not account for the apparent discrepancy between the effect of PIT on the response to electrical stimulation and that to exogenous drugs. By analogy, a potentiation of P_{2Y}-purinoceptor-mediated responses by PIT (King *et al*., 1996) and an action of directly relaxing smooth muscles by PIT (Spedding *et al*., 1975) should have reduced the contractile response to electrical stimulation in the present study. Instead, we did not observe any significant changes in resting tension when the preparation was incubated with PIT and the responses to ATP were not affected by PIT. It was also reconfirmed that PIT at lower concentrations failed to potentiate ATP-induced relax-

ant responses in the guinea-pig taenia caeci (Spedding *et al*., 1994). An action of PIT on ecto-ATPase is excluded because it inhibits the ecto-ATPase only weakly and not in a concentration-dependent manner (King *et al*., 1996).

Antagonism by PIT against ATP-induced relaxation at postjunctional P_{2Y}-purinoceptors (Spedding *et al*., 1975; King *et al*., 1996) could provide an explanation for the potentiation of neurogenic responses to electrical stimulation in the present study. However, a concentration of more than 12.5 or 20 μM was needed for development of its antagonism to P_{2Y}-purinoceptors, and this action was irreversible (Spedding *et al*., 1994; King *et al*., 1996). In the present study 0.3–3 μM PIT significantly potentiated the responses to electrical stimulation at 2–32 Hz in a concentration-dependent manner, and this potentiation recovered to the control level quickly after washing. In addition, a more potent antagonist of P_2-purinoceptors, Coomassie brilliant blue-G (Soltoff *et al*., 1989), which shares the potentiating effect on P_2-purinoceptor with PIT (King *et al*., 1996), was ineffective in the splenic artery. Thus, a prejunctional modulation by PIT of the release of purinergic transmitters from sympathetic nerves rather than antagonism against the postjunctional P_{2Y}-purinoceptors might also account for the potentiation by PIT of the purinergic responses to electrical stimulation. It is interesting to note that Su (1978) found that PIT potentiated the contractile response to electrical stimulation in the rabbit saphenous vien, although the author himself did not present the data and did not comment on this finding in his publication. Further study is needed to clarify the mechanism of prejunctional potentiation by PIT of sympathetic purinergic cotransmission. Since PIT has weak affinity for an adenosine (A_1) receptor (King *et al*., 1996), further investigation should be designed to clarify whether the potentiation by PIT is related to its action at prejunctional P_1-purinoceptors.

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References

BRIZZOLARA, A.L. & BURNSTOCK, G. (1990). Evidence for nodradrenergic-purinergic cotransmission in the hepatic artery of the rabbit. *Br. J. Pharmacol*., **99,** 835–839.

BROOKSBY, G.A. & DONALD, D.E. (1972). Release of blood from the splanchnic circulation in dogs. *Circ. Res*., **31,** 105–118.

BÜLBRING, E. (1953). Measurements of oxygen consumption in smooth muscle. *J. Physiol*., **122,** 111–134.

BULLOCH, J.M. & STARKE, K. (1990). Presynaptic α_2-autoinhibition in a vascular neuroeffector junction where ATP and noradrenaline act as co-transmitters. *Br. J. Pharmacol*., **99,** 279–284.

BURNSTOCK, G. (1988). Local purinergic regulation of blood pressure. (The First John T. Shepherd Lecture). In *Vasodilatation: Vascular Smooth Muscle, Peptides, Autonomic Nerves, and Endothelium*. ed. Vanhoutte, P.M. pp. 1–14. New York: Raven Press.

BURNSTOCK, G. (1990). Noradrenaline and ATP as cotransmitters in sympathetic nerves. *Neurochem. Int*., **17,** 357–368.

BURNSTOCK, G. & WARLAND, J.J.I. (1987). A pharmacological study of the rabbit saphenous artery *in vitro*: a vessel with a large purinergic contractile respsonse to sympathetic nerve stimulation. *Br. J. Pharmacol*., **90,** 111–120.

CRACK, B.E., BEUKERS, M.W., MCKECHNIE, K.C.W., IJZERMAN, A.P. & LEFF, P. (1994). Pharmacological analysis of ecto-ATPase inhibition: evidence for combined enzyme inhibition and receptor antagonism in P2X-purinoceptor ligands. *Br. J. Pharmacol*., **113,** 1432–1438.

DUNN, P.M. & BLAKELEY, A.G.H. (1988). Suramin: a reversible P_2-purinoceptor antagonist in the mouse vas deferens. *Br. J. Pharmacol*., **93,** 243–245.

EVANS, R.J. & CUNNANE, T.C. (1992). Relative contributions of ATP and noradrenaline to the nerve evoked contraction of the rabbit jejunal artery. Dependence on stimulation parameters. *Naunyn-Schmiedeberg's Arch. Pharmacol*., **345,** 424–430.

EVANS, R.J. & SURPRENANT, A. (1992). Vasoconstriction of guinea-pig submucosal arteriols following sympathetic nerve stimulation is mediated by the release of ATP. *Br. J. Pharmacol*., **106,** 242–249.

KATSURAGI, T. & SU, C. (1980). Purine release from vascular adrenergic nerves by high potassium and a calcium ionophore A-23187. *J. Pharmacol. Exp. Ther*., **215,** 685–690.

KATSURAGI, T. & SU, C. (1982). Augmentation by theophylline of [^{3}H]purine release from vascular adrenergic nerves: Evidence for presynaptic autoinhibition. *J. Pharmacol. Exp. Ther*., **220,** 152–156.

KAZIC, T. & MILOSAVLJEVIC, D. (1977). Influence of pyridylisatogen tosylate on contractions produced by ATP and by purinergic stimulation in the terminal ileum of the guinea-pig. *J. Pharm. Pharmacol*., **29,** 542–545.

KENNEDY, C., SAVILLE, V.L. & BURNSTOCK, G. (1986). The contributions of noradrenaline and ATP to the responses of the rabbit central ear artery to sympathetic nerve stimulation depend on the parameters of stimulation. *Eur. J. Pharmacol*., **122,** 291–300.

KHAN, M.T. & WAKADE, A.R. (1979). Relationship between accumulation, storage and overflow of noradrenaline in the rat salivary gland after chronic treatment with guanethidine. *Br. J. Pharmacol*. **66,** 223–228.

KING, B.F., DACQUET, C., ZIGANSHIN, A.U., WEETMAN, D.F., BURNSTOCK, G., VANHOUTTE, P.M. & SPEDDING, M. (1996). Potentiation by 2,2′-pyridylisatogen tosylate of ATP-responses at a recombinant P_{2Y1} purinoceptor. *Br. J. Pharmacol*., **117,** 1111–1118.

LEFF, P., WOOD, B.E. & O'CONNOR, S.E. (1990). Suramin is a slowly-equilibrating but competitive antagonist at P2X-receptors in the rabbit isolated ear artery. *Br. J. Pharmacol*. **101,** 645–649.

LUNDBORG, P. & STITZEL, R.E. (1968). Studies on the dual action of guanethidine in sympathetic nerves. *Acta Physiol. Scand*., **72,** 100–107.

RAMME, D., REGENOLD, J.T., STARKE, K., BUSSE, R. & ILLES, P. (1987). Identification of the neuroeffector transmitter in jejunal branches of the rabbit mesenteric artery. *Naunyn-Schmiedeberg's Arch. Pharmacol*., **336,** 267–273.

REN, L.-M., NAKANE, T. & CHIBA, C. (1994). Characteristics of the responses of isolated and perfused canine splenic arteries to vasoactive substances and to periarterially electrical stimulation. *Jpn. J. Pharmacol*., **64,** 19–25.

REN, L.-M., NAKANE, T. & CHIBA, C. (1996). Purinergic and adrenergic transmission and their presynaptic modulation in canine isolated perfused splenic arteries. *Eur. J. Pharmacol*., **295,** 61–68.

RISÖE, C., HALL, C. & SMISETH, O.A. (1991). Blood volume changes in liver and spleen during cardiogenic shock in dogs. *Am. J. Physiol*., **261,** *(Heart Circ. Physiol*., **30),** H1763–H1768.

SNEDDON, P. & BURNSTOCK, G. (1985). ATP as a co-transmitter in rat tail artery. *Eur.J. Pharmacol*., **106,** 149–152.

SOLTOFF, S.P., MCMILLIAN, M.K. & TALAMO, B.R. (1989). Coomassie brilliant blue G is a more potent antagonist of P_2 purinergic responses than reactive blue 2 (cibacron blue 3GA) in rat parotid acinar cells. *Biochem. Biophys. Res. Commun*., **165,** 1279–1285.

SPEDDING, M., DACQUET, C., BILLINGTON, D., WEETMAN, D.F. KING, B.F. & BURNSTOCK, G. (1994). 2-2′-Pyridylisatogen, an ATP receptor antagonist, revisited. *Br. J. Pharmacol*., **113**, 59P.

SPEDDING, M., SWEETMAN, A.J. & WEETMAN, D.F. (1975). Antagonism of adenosine 5′-triphosphate-induced relaxation by 2-2′-pyridylisatogen in the taenia of guinea-pig caecum. *Br.J. Pharmacol.*, **53,** 575–583.

SPEDDING, M. & WEETMAN, D.F. (1978). The mechanism of the relaxant effect of 2-2′-pyridylisatogen on the isolated taenia of the guinea-pig caecum. *Br. J. Pharmacol.*, **63,** 659–664.

STORY, D.F., MCCULLOCH, M.W., RAND, M.J. & STANDFORD-STARR, C.A. (1981). Conditions required for the inhibitory feedback loop in noradrenergic transmission. *Nature*, **293,** 62–65.

SU, C. (1975). Neurogenic release of purine compounds in blood vessels. *J. Pharmacol. Exp. Ther.*, **195,** 159–166.

SU, C. (1978). Purinergic inhibition of adrenergic transmission in rabbit blood vessels. *J. Pharmacol. Exp. Ther.*, **204,** 351–361.

VON KÜGELGEN, I. & STARKE, K. (1991). Noradrenaline-ATP co-transmission in the sympathetic nervous system. *Trends Pharmaco. Sci.*, **12,** 319–324.

WALLENSTEIN, S., ZUCKER, C.L. & FLEISS, J.L. (1980). Some statistical methods useful in circulation research. *Circ. Res.,* **47,** 1–9.

WARLAND, J.J.I. & BURNSTOCK, G. (1987). Effects of reserpine and 6-hydroxydopamine on the adrenergic and purinergic components of sympathetic nerve responses of the rabbit saphenous artery. *Br.J. Pharmacol.*, **92,** 871–880.

ZIGANSHIN, A.U., HOYLE, C.H.V., LAMBRECHT, G., MUTSCHLER, E., BÄUMERT, H.G. & BURNSTOCK, G. (1994). Selective antagonism by PPADS at P_{2X}-purinoceptors in rabbit isolated blood vessels. *Br. J. Pharmacol.,* **111,** 923–929.

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