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The P_2-purinoceptor antagonist suramin is a competitive antagonist at vasoactive intestinal peptide receptors in the rat gastric fundus

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1 The P_2-purinoceptor antagonist, suramin, was used to investigate the possible involvement of adenosine 5′-triphosphate (ATP) in the inhibitory non-adrenergic non-cholinergic (NANC) innervation of the rat gastric fundus.

2 ATP (1–30 μM) produced biphasic responses consisting of concentration-dependent relaxations followed by concentration-dependent contractions. Suramin (200 μM) significantly reduced relaxations and abolished contractions to ATP.

3 Under NANC conditions, electrical field stimulation (EFS) induced frequency-dependent relaxations. Suramin (200 μM) and the peptidase α-chymotrypsin (1 u ml^{-1}) had the same effects on EFS-induced relaxations: their duration was reduced, but their magnitude was unaffected.

4 Cumulative relaxations to vasoactive intestinal peptide (VIP; 0.1–100 nM), and to the VIP analogue pituitary adenylate cyclase activating peptide 1–27 (PACAP; 0.2–100 nM), were almost completely abolished by α-chymotrypsin (1 u ml^{-1}), and were inhibited by suramin (3–200 μM) in an apparently competitive manner. Schild plot analysis indicated that suramin had pA_2 values of 5.1 ± 0.2 (Hill slope $= 0.9 \pm 0.2$) and 5.6 ± 0.1 (Hill slope $= 1.0 \pm 0.1$), against VIP and PACAP, respectively.

5 Concentration-dependent relaxations to nitric oxide (1–30 μM) and cumulative relaxations to isoprenaline (0.1–300 nM) were not affected by suramin (200 μM).

6 No conclusions can be made regarding the possible involvement of ATP in EFS-induced NANC relaxations. The results suggest that suramin acts as a competitive antagonist at VIP receptors in the rat gastric fundus.

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Keywords: ATP; pituitary adenylate cyclase activating peptide; rat gastric fundus; suramin; vasoactive intestinal peptide; VPAC receptors

Abbreviations: ANOVA, analysis of variance; ATP, adenosine 5′-triphosphate; EFS, electrical field stimulation; MANOVA, multiple analysis of variance; α,β-methylene ATP, α,β-methylene adenosine 5′-triphosphate; NANC, non-adrenergic non-cholinergic; NO, nitric oxide; PACAP, pituitary adenylate cyclase activating peptide; PSS, physiological salt solution; SNP, sodium nitroprusside; VIP, vasoactive intestinal peptide

Introduction

Dunn & Blakely (1998) first reported that the trypanocidal drug suramin was a P_{2X}-purinoceptor antagonist. They demonstrated that suramin inhibits contractions to the stable adenosine 5′-triphosphate (ATP) analogue α,β-methylene ATP, without affecting contractions to either carbachol or noradrenaline in the mouse vas deferens. It has since been shown that suramin is a non-selective antagonist of P_{2X}- and P_{2Y}-purinoceptor subtypes in numerous smooth muscle preparations (Hoyle *et al*., 1990; Voogd *et al*., 1993). Furthermore, suramin inhibits contractile responses to the P_2-purinoceptor agonists ATP and α,β-methylene ATP without affecting those to noradrenaline (Dunn & Blakely, 1988; Mallard *et al*., 1992), carbachol (Dunn & Blakely, 1988; Hoyle *et al*., 1990) or histamine (Hoyle *et al*., 1990), and inhibits relaxant responses to ATP without affecting those to sodium nitroprusside (SNP; Brizzolara *et al*., 1993) or phenylephrine (Den Hertog *et al*., 1989), suggesting that its antagonism is specific for P_2-purinoceptors. As such, suramin has been used extensively to investigate ATP as a putative inhibitory non-adrenergic, non-cholinergic (NANC) neurotransmitter in various smooth muscle preparations, including the rabbit portal vein (Brizzolara *et al*., 1993), human jejunum (Xue *et al*., 1999), duodenum (Glasgow *et al*., 1998) and colon (Pluja *et al*., 1999), and the guinea-pig stomach (Ohno *et al*., 1993) and taenia coli (Den Hertog *et al*., 1989; Hoyle *et al*., 1990).

The rat gastric fundus has an inhibitory NANC innervation that is mediated by nitric oxide (NO) and vasoactive intestinal peptide (VIP) (Li & Rand, 1990; D'Amato *et al*., 1992). However, in the presence of blockade of both NO and VIP a residual relaxation remains, suggesting the involvement of a third inhibitory NANC neurotransmitter in this tissue (Li & Rand, 1990; D'Amato *et al*., 1992; Jenkinson & Reid, 1995). It has long been hypothesized that the purine ATP may be a NANC neurotransmitter in the rat gastric fundus (Burnstock *et al*., 1970), however, previous investigators have produced evidence both for and against this proposal (Lefebvre, 1986; Belai *et al*., 1991; Lefebvre *et al*., 1991). Clarification of the possible involvement of ATP in NANC relaxations in the rat gastric fundus requires the use of a specific purinoceptor antagonist.

The aim of the present study was to examine the effect of suramin on relaxant responses to ATP and NANC nerve stimulation in the rat gastric fundus, and to test its specificity against a range of agonists in this tissue.

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Methods

Tissue preparation

Male Sprague-Dawley rats (380–600 g) were stunned and killed by decapitation, the stomach was immediately removed, and two longitudinal strips were prepared from the ventral part of the fundus as previously described (Jenkinson & Reid, 1995). Each fundus strip was mounted in a 6-ml water-jacketed organ bath under a resting tension of 1 g in physiological salt solution (PSS) of the following composition (mM): NaCl 118; KCl 4.7; $CaCl_2$ 2.5; KH_2PO_4 1.03; $MgSO_4$ 0.45; $NaHCO_3$ 25.0; D-(+)-glucose 11.1; disodium edetate 0.067; and ascorbic acid 0.14. The PSS was maintained at 37°C, and gassed with 5% CO_2 in O_2. Intramural nerves were electrically stimulated using a Grass S88 or S11 stimulator *via* two platinum wire electrodes, one placed on either side of the strip, with square wave pulses of 1 ms duration and supramaximal voltage (17 V cm^{-1}). The PSS contained atropine (3 μM) and guanethidine (5 μM) throughout experiments to block cholinergic and noradrenergic responses to EFS, respectively. Changes in tissue length were measured using a Ugo Basile isotonic transducer and recorded using a MacLab data acquisition system.

Experimental protocol

Each fundus strip was allowed to equilibrate for at least 30 min before serotonin (10 μM) was added to produce a sustained increase in tone of 10.4 ± 0.3 mm ($n=68$). After a further 30-min equilibration period, a control response curve was obtained to either ATP (1–30 μM), EFS (0.5–4 Hz, 30 s train), VIP (0.1–100 nM), pituitary adenylate cyclase activating peptide 1–27 (PACAP; 0.1–100 nM), NO (1–30 μM), SNP (3 nM–30 μM) or isoprenaline (0.1–300 nM). Relaxant responses to ATP, EFS and NO were obtained at 5-min intervals in random order, whereas relaxations to VIP, PACAP, SNP and isoprenaline were obtained in a cumulative manner.

A second response curve was then obtained in the absence (time-control experiments) or presence of suramin (3–200 μM) or α-chymotrypsin (1 u ml^{-1}). Tissues were exposed to suramin for at least 50 min, and to α-chymotrypsin for at least 20 min before further responses were elicited. Relaxations induced by VIP varied markedly between experimental groups, for example the response to VIP at 10 nM varied between 6.2 ± 0.5 mm ($n=5$) and 13.2 ± 1.7 mm ($n=5$). Therefore, responses to VIP obtained from the second curve have been expressed as a percentage of the initial maximum response obtained to 100 nM VIP in the same tissue to normalize for differences in responsiveness between experimental groups. The reason for the variability in responsiveness to VIP is not known.

Analysis of results

Data are expressed as means ± s.e.mean and n indicates the number of animals tested. Differences between means were assessed by unpaired Student's t-test, or by one-way multiple analysis of variance (MANOVA) followed by Student-Newman-Keuls test. Analyses were performed using the statistical software package Sigma Stat 1.0 (Jandel Scientific, U.S.A.). Probability values less than 0.05 ($P<0.05$) were taken to indicate statistical significance.

Analysis of antagonism

The nature of antagonism and pA_2 values for suramin were determined using the method of Arunlakshana & Schild (1959). Accordance with competitive antagonism was assessed by comparing the slope of the Schild plot (Hill slope) with unity by Student's t-test.

Drugs and drug solutions

The following drugs were used in the study: adenosine 5′-triphosphate disodium salt (ATP, Sigma, U.S.A.), atropine sulphate (Sigma, U.S.A.), α-chymotrypsin (bovine pancreas, Sigma, U.S.A.), guanethidine sulphate (Ciba-Geigy, Australia), 5-hydroxytryptamine creatinine sulphate (serotonin; Sigma, U.S.A.), isoproterenol hydrochloride (isoprenaline, Sigma, U.S.A.), nitric oxide gas (NO; CIG, Australia), pituitary adenylate cyclase activating peptide 1–27 (PACAP, ovine, Auspep, Australia), sodium nitroprusside (SNP; Sigma, U.S.A.), tetrodotoxin (Sigma, U.S.A.), vasoactive intestinal peptide (VIP, human; Auspep, Australia). Suramin (Germanin) was kindly donated by Bayer (Leverkusen, Germany). Saturated solutions of NO (2 mM) were prepared on the day of the experiment as described by Feelisch (1991). Briefly, vials of deionized water, deoxygenated by bubbling with argon gas for 1 h, were bubbled with NO gas for 20 min to give saturated solutions of NO. α-Chymotrypsin was dissolved in distilled water on the day of the experiment to give a stock solution of 100 u ml^{-1}. All other drugs were dissolved in distilled water to give stock solutions of 10 mM, or 0.1 mM for VIP and PACAP, and dilutions were made in PSS.

Results

Neither suramin (3–200 μM) nor α-chymotrypsin (1 u ml^{-1}) affected the basal tone of precontracted strips of rat gastric fundus.

Responses to ATP

Addition of ATP (1–30 μM) to precontracted strips of rat gastric fundus produced biphasic responses: rapid concentration-dependent relaxations were followed by more prolonged concentration-dependent contractions (Figure 1a). Relaxations to ATP (1–30 μM) remained consistent over the duration of time-control experiments. Contractions to ATP at 10–30 μM did not differ between the first and second response curves in time-control experiments ($P>0.05$, MANOVA followed by Student-Newman-Keuls test), however, ATP at concentrations below 10 μM did not produce contractions in the second response curve. Therefore, responses to ATP in the presence of suramin (200 μM) have been compared to those obtained from the second response curve in time-control experiments (Figure 1b,c).

Suramin (200 μM) significantly reduced ($P<0.05$, MANOVA) the magnitude of relaxations, and abolished ($P<0.05$, MANOVA) contractions to ATP (1–30 μM; Figure 1). The duration of relaxations to ATP (10–30 μM) appeared to be prolonged in the presence of suramin (Figure 1a).

Relaxations to NANC nerve stimulation

EFS (0.5–4 Hz, 30 s train) produced rapid, frequency-dependent relaxations (Figure 2) that remained consistent in time-control experiments. Both suramin (200 μM) and α-

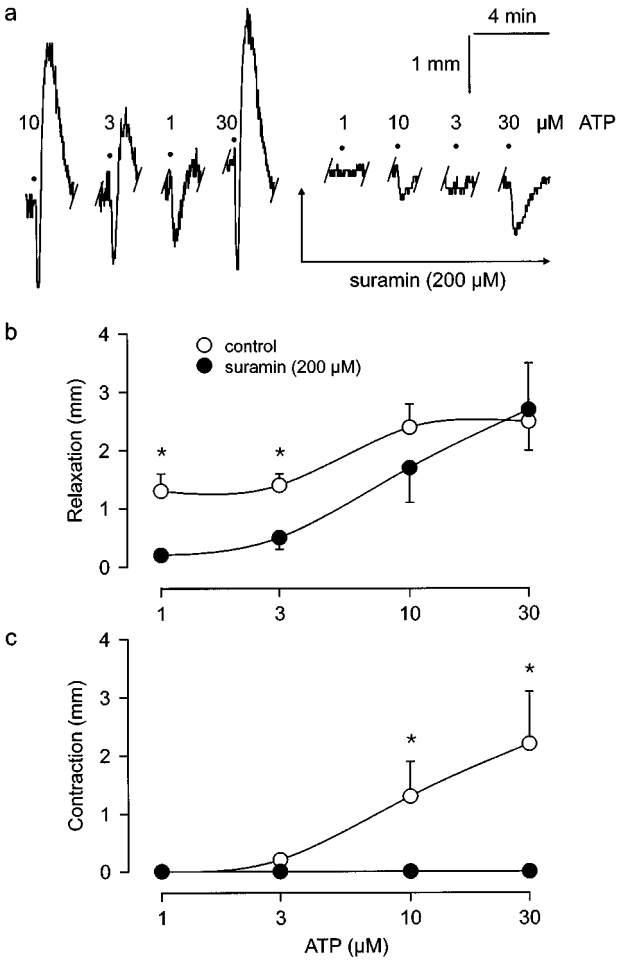

Figure 1 (a) Original trace showing the effect of suramin (200 μM) on responses to adenosine 5′-triphosphate (ATP; ●; 1–30 μM) in a longitudinal strip of rat gastric fundus. (b) Magnitude of relaxant responses and (c) magnitude of contractile responses to ATP (1–30 μM) in longitudinal strips of rat gastric fundus in the absence and presence of suramin (200 μM). Values are mean±s.e.mean for six experiments. *Significant difference between the absence and presence of suramin ($P<0.05$, MANOVA followed by Student-Newman-Keuls test).

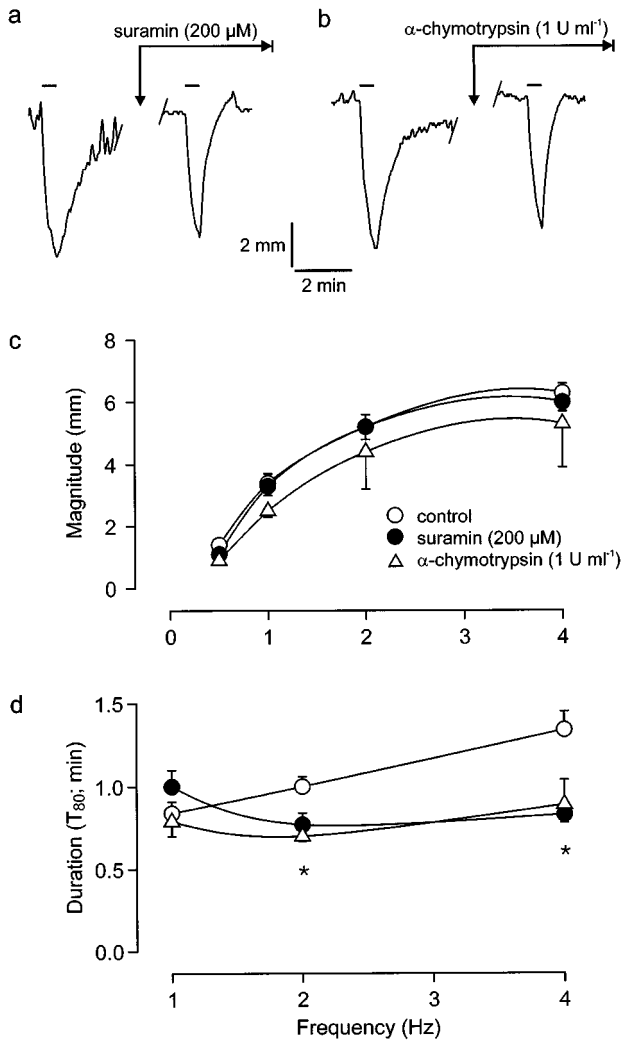

Figure 2 (a,b) Original traces showing the effects of suramin (200 μM) and α-chymotrypsin (1 u ml^{-1}) on relaxant responses to non-adrenergic, non-cholinergic nerve stimulation ((bars)–; 4 Hz, 30 -s train) in longitudinal strips of rat gastric fundus. (c) Magnitude and (d) duration of relaxations to non-adrenergic, non-cholinergic nerve stimulation (0.5–4 Hz, 30 -s train) in longitudinal strips of rat gastric fundus in the absence and presence of α-chymotrypsin (1 u ml^{-1}) or suramin (200 μM). The duration of relaxations was measured as the mean time taken for an 80% reduction in the magnitude of the peak response (T_{80}; see Jenkinson & Reid, 1995). Values are mean±s.e.mean for 6–7 experiments. *Significant difference between the absence and presence of suramin and the absence and presence of α-chymotrypsin ($P<0.05$, MANOVA followed by Student-Newman-Keuls test).

chymotrypsin (1 u ml^{-1}) had no effect ($P>0.05$, MANOVA) on the magnitude of EFS-induced relaxations (Figure 2c). The duration (measured as the mean time taken for an 80% reduction in the magnitude of the peak response, T_{80}; see Jenkinson & Reid, 1995) of relaxant responses to EFS (1–4 Hz) was significantly reduced ($P<0.05$, MANOVA) by either suramin (200 μM) or α-chymotrypsin (1 u ml^{-1}; Figure 2d).

Relaxations to VIP and PACAP

Both VIP (0.1–100 nM) and PACAP (0.1–100 nM) produced slowly-developing, concentration-dependent relaxations of fundus strips (Figures 3 and 4) that did not alter over the time course of time-control experiments. α-Chymotrypsin (1 u ml^{-1}) virtually abolished ($P<0.05$, MANOVA) relaxations to VIP and PACAP (Figures 3b and 4a). Suramin (3–200 μM) inhibited ($P<0.05$, MANOVA) relaxations to VIP and PACAP in an apparently competitive manner (Figures 3 and 4). Maximum responses to VIP and PACAP were not significantly affected ($P>0.05$, MANOVA) by suramin at concentrations below 100 μM; insufficient VIP or PACAP was available to determine whether suramin at concentrations of 100 and 200 μM altered maximum responses to VIP or PACAP (Figures 3b and 4a). Schild plot analysis indicated that suramin had pA_2 values of 5.1±0.2 (Hill slope=0.9±0.2) and 5.6±0.1 (Hill slope=1.0±0.1) against VIP and PACAP, respectively (Figures 3c and 4b); the pA_2 values were not significantly different ($P>0.05$, unpaired Student's *t*-test).

Relaxations to NO and SNP

Addition to exogenous NO (1–30 μM) and SNP (3 nM–30 μM) to precontracted fundus strips produced concentration-dependent relaxations (Figure 5) that remained consistent in time-control experiments. Relaxations to NO

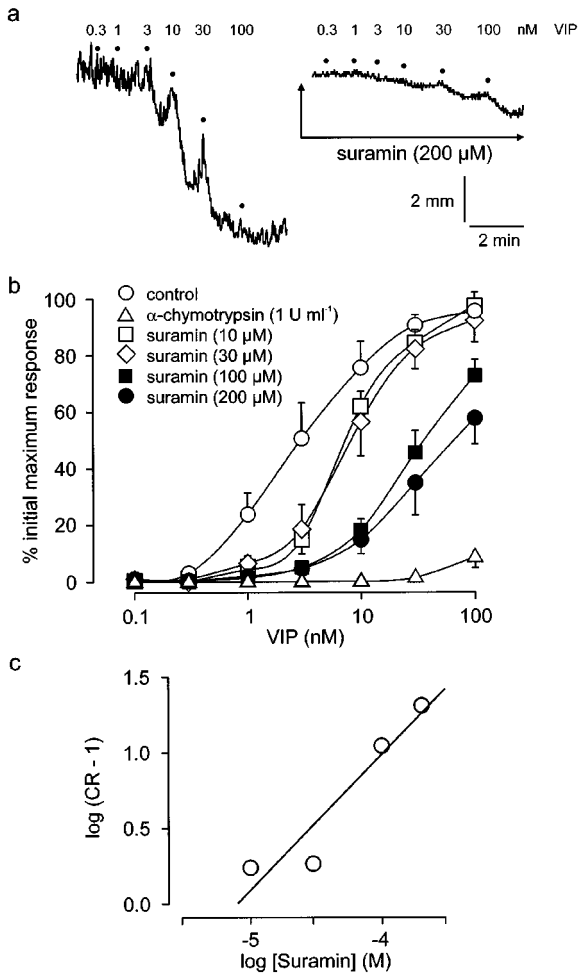

Figure 3 (a) Original trace showing the effect of suramin (200 μM) on relaxant responses to vasoactive intestinal peptide (VIP; ●; 0.1–100 nM) in a longitudinal strip of rat gastric fundus. (b) Magnitude of relaxations to VIP (0.1–100 nM) in longitudinal strips of rat gastric fundus. Relaxations from the second curve in the absence and presence of α-chymotrypsin (1 u ml^{-1}) or suramin (10–200 μM) are expressed as a percentage of the initial maximum relaxation obtained to 100 nM VIP. (c) Schild plot analysis of the antagonism by suramin of relaxant responses to VIP, where CR is the concentration ratio for VIP required to produce the same response in the presence and absence of suramin. The analysis indiated that suramin had a pA_2 value of 5.1±0.2 (Hill slope = 0.9±0.2). Values are mean±s.e.mean for 4–7 experiments.

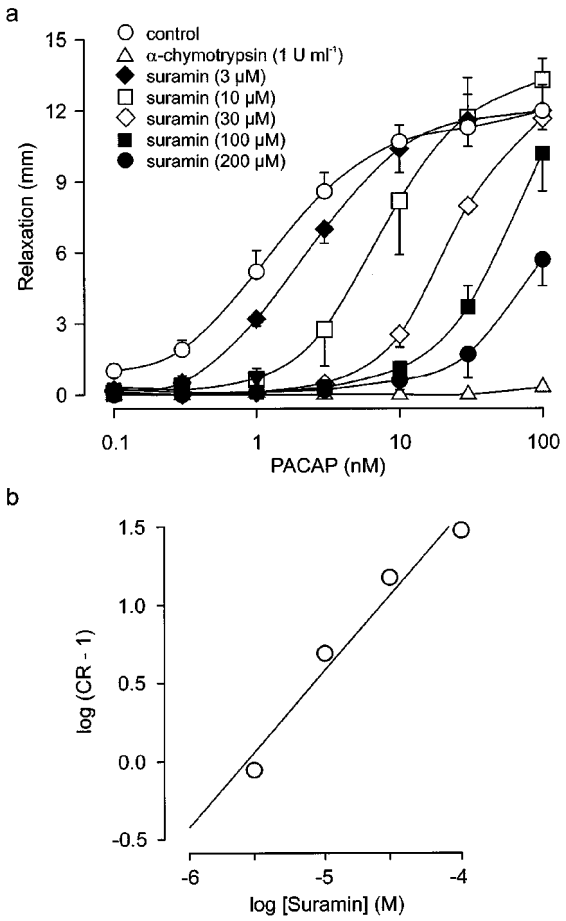

Figure 4 (a) Magnitude of relaxant responses to pituitary adenylate cyclase activating peptide 1–27 (PACAP; 0.1–100 nM) in longitudinal strips of rat gastric fundus in the absence and presence of α-chymotrypsin (1 u ml^{-1}) or suramin (3–200 μM). (b) Schild plot analysis of the antagonism by suramin of relaxant responses to PACAP, where CR is the concentration ratio for PACAP required to produce the same response in the presence and absence of suramin. The analysis indicated that suramin had a pA_2 value of 5.6±0.1 (slope = 1.0±0.1). Values are mean±s.e.mean for 4–6 experiments.

were rapid in onset and short-lived when compared to those to SNP, which were slower in onset and were well maintained. The magnitude of relaxations to NO was not affected ($P>0.05$, MANOVA) by suramin (200 μM; Figure 5a), however, the duration of relaxations to 30 μM NO was markedly reduced by suramin in two of four preparations (data not shown). Relaxations to SNP were slightly, but significantly ($P<0.05$, MANOVA) reduced by suramin (200 μM) or α-chymotrypsin (1 u ml^{-1}) (Figure 5b).

Relaxations to isoprenaline

Isoprenaline (0.1–300 nM) produced slowly-developing, concentration-dependent relaxations that did not alter over the duration of time-control experiments. Relaxations to isoprenaline were not significantly altered ($P>0.05$, MANOVA) by suramin (200 μM; data not shown).

Discussion

No conclusion can be made from the results of this study regarding the possible involvement of ATP in EFS-induced NANC relaxations in the rat gastric fundus, as the P_2-purinoceptor antagonist suramin caused only a limited inhibition of relaxations to ATP and had no effect on the magnitude of NANC relaxations. The limited effect of suramin on relaxations to ATP in the rat gastric fundus suggests that the purinoceptor mediating the relaxations may be a suramin-resistant subtype. Suramin-insensitive P_2-purinoceptors have been reported in rat hepatocytes (Tomura *et al.*, 1992) and in the guinea-pig bladder (Hourani & Chown, 1989), small intestines (Heinemann *et al.*, 1999) and stomach (Xue *et al.*, 1998). The findings of the present study suggest that suramin is not suitable for investigating the involvement of ATP in EFS-induced NANC relaxations in this tissue.

In the present study suramin appeared to increase the duration of relaxations to ATP (see Figure 1a). Two factors may contribute to this anomalous effect of suramin: firstly, suramin inhibits breakdown of ATP by smooth muscle endonucleotidases (Hourani & Chown, 1989), thus increasing

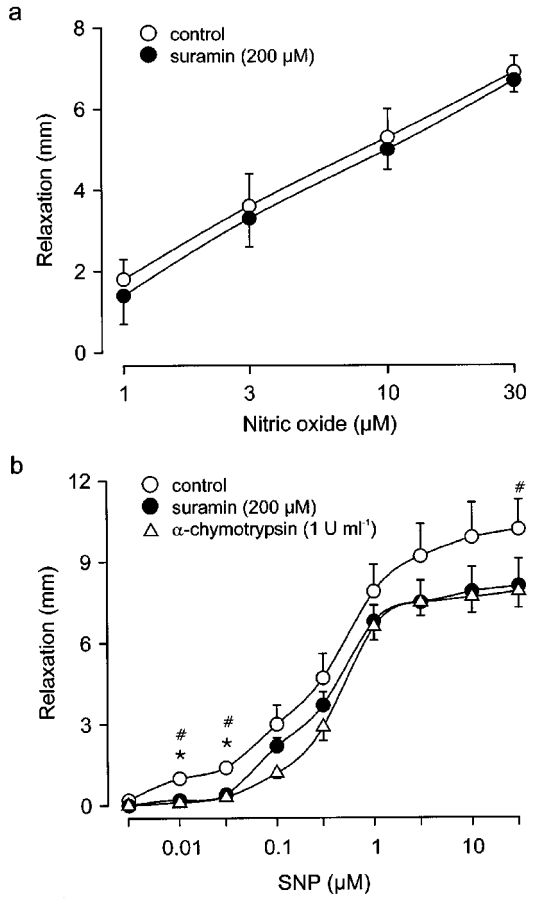

Figure 5 Magnitude of relaxant responses to (a) nitric oxide (1–30 μM) and (b) sodium nitroprusside (SNP; 3 nM–30 μM) in longitudinal strips of rat gastric fundus in the absence and presence of α-chymotrypsin (1 u ml^{-1}) or suramin (200 μM). Values are mean ± s.e.mean for four experiments. *Significant difference between the absence and presence of suramin ($P<0.05$, MANOVA followed by Student-Newman-Keuls test). #Significant difference between the absence and presence of α-chymotrypsin ($P<0.05$, MANOVA followed by Student-Newman-Keuls test).

the half-life of ATP; and secondly, suramin abolished the delayed contractile phase of responses to ATP that would normally oppose the relaxant response.

The effects of suramin on responses to ATP in rat gastric fundus have previously been studied by Matharu & Hollingsworth (1992) who reported very similar findings to those of the present study. Suramin (100 μM) caused a partial inhibition of relaxant responses to ATP (100 nM–1 mM), similar to that observed in the present study, and almost abolished the contractile response to ATP at concentrations lower than 10 μM. Unlike the present study, the contractile response to 10 μM ATP was not abolished by suramin, possibly due to the lower concentration of suramin used in this study.

Suramin significantly reduced the duration of NANC relaxations to EFS in the rat gastric fundus, despite its lack of effect on their magnitude. This is in contrast to its apparent enhancement of the duration of relaxations to ATP, suggesting that this effect is unlikely to be due to interference with a purinergic component of the NANC response. The effects of suramin on the magnitude and duration of EFS-induced NANC relaxations did not differ from those of the peptidase α-chymotrypsin. α-Chymotrypsin almost completely abolished relaxations to VIP, and reduced the duration of relaxations to NANC nerve stimulation with only a slight reduction in magnitude, presumably by inhibiting the VIP-mediated component of the NANC response. These findings suggest that suramin may also interfere with the VIP-mediated component of the NANC response. Suramin markedly inhibited relaxations to VIP and to PACAP, a neuropeptide with approximately 70% homology with VIP and which is an equipotent activator of VIP_1 and VIP_2 receptors (Miyata *et al*., 1989). In contrast, relaxations to isoprenaline and NO were unaffected, suggesting that suramin selectively inhibits VIP-induced relaxations.

The findings of the present study correlate with previous reports that suramin inhibits radiolabelled VIP binding and VIP-induced cyclic AMP accumulation in two human cancer cell lines (Bellan *et al*., 1991), and inhibits relaxations induced by VIP in guinea-pig proximal colon (Briejer *et al*., 1995) and gastric fundus (Ohno *et al*., 1996) and hamster proximal urethra (Pinna *et al*., 1998), and by PACAP in the guinea-pig taenia coli (McConalogue *et al*., 1995). In three of these studies suramin had no effect on the equivalent response induced by isoprenaline (Bellan *et al*., 1991; Briejer *et al*., 1995; McConalogue *et al*., 1995). Suramin has been reported to inhibit receptor coupling to guanine nucleotide-binding proteins (G proteins) in cell membrane preparations (Butler *et al*., 1988; Huang *et al*., 1990). However, suramin is impermeant to intact red blood cells (Fortes *et al*., 1973), and so an interaction with G proteins in intact gastric smooth muscle cells in the present study is unlikely. Therefore, the inhibition by suramin of relaxations to VIP and PACAP probably occurs by an extracellular interaction with VIP/PACAP receptors.

Suramin caused a parallel rightward shift in the concentration-response curve to VIP and PACAP with no change in the maximum response to the agonists at concentrations of suramin below 100 μM. Furthermore, Schild plot analyses of the inhibition by suramin of relaxations to VIP and PACAP produced Hill slopes that did not differ from unity. The pA_2 value of suramin was the same against relaxations induced by VIP and PACAP, suggesting that the two agonists are activating the same VIP receptor in the rat gastric fundus. This is in accord with the work of Usdin *et al*. (1994) who demonstrated that messenger RNA for the VIP_2 receptor, but not for the VIP_1 receptor, is expressed in rat stomach. Moreover, Robberecht *et al*. (1998) showed that the selective VIP_2 receptor agonist, RO25-1553, induced concentration-dependent relaxations in the rat gastric fundus, whereas the selective VIP_1 receptor agonist, [K^{15},R^{16},L^{27}]VIP-(1–7)/GRF-(8–27), was without effect. Therefore, the present results suggest that suramin acts as a competitive antagonist at the VIP_2 receptor subtype present on smooth muscle in the rat gastric fundus. If so, suramin would be the first non-peptide VIP receptor antagonist identified, and may provide an invaluable pointer in the development of more selective non-peptide antagonists. It remains to be determined whether suramin also acts as an antagonist at VIP_1 receptors or PACAP receptors.

Both suramin and α-chymotrypsin slightly inhibited relaxations to the NO-donor sodium nitroprusside, but not those to NO itself. It has previously been demonstrated that exogenous NO and SNP induce VIP release in the guinea-pig small intestine (Grider & Jin, 1993 and canine ileum (Daniel *et al*., 1994), respectively. Furthermore, NO synthase inhibitors greatly reduce VIP release in the canine ileum (Daniel *et al*., 1994), rabbit gastric fundus (Jin & Grider, 1993), rat colon (Grider, 1993), and guinea-pig gastric fundus (Grider *et al*., 1992) and small intestine (Grider & Jin, 1993). A NO-

stimulated VIP release in the rat gastric fundus would explain the effects of suramin and α-chymotrypsin on relaxations to SNP in the present study. Suramin did not affect the magnitude of relaxations to NO, but the duration of relaxations to NO at the highest concentration used (30 μM) was markedly reduced in two of four preparations (data not shown). This suggests that the magnitude of NO-induced relaxations, which is achieved rapidly following application, is due to direct effects of NO on smooth muscle, whereas NO-induced VIP release may play a role in maintenance of the response.

Both VIP and ATP have been proposed as inhibitory NANC neurotransmitters at sites throughout the gastrointestinal tract, as well as in a number of respiratory and genital tissues (Fahrenkrug, 1993; Murthy *et al*., 1995; Hoyle, 1992). Numerous investigators have used suramin to investigate ATP as a putative NANC neurotransmitter (Mallard *et al*., 1992; Dunn & Blakely, 1988; Den Hertog *et al*., 1989; Brizzolara *et al*., 1993; Ohno *et al*., 1993; 1996; Hoyle *et al*., 1990; Xue *et al*., 1999; Glasgow *et al*., 1998; Pluja *et al*., 1999). However, our results strongly suggest that suramin is a competitive antagonist at VIP/PACAP receptors. Therefore we suggest that suramin alone is not suitable for investigating the involvement of ATP in NANC neurotransmission in peripheral smooth muscle preparations.

In summary, the P_2-purinoceptor antagonist suramin did not alter the magnitude of EFS-induced NANC relaxations in the rat gastric fundus, and only reduced relaxations to ATP at sub-maximal concentrations. In contrast, suramin markedly inhibited relaxations to VIP and PACAP in an apparently competitive manner, whereas relaxations to isoprenaline and NO were unaffected. Therefore, no conclusion can be made regarding the possible involvement of ATP in NANC relaxations to EFS in this tissue, and investigators should exercise caution in the use of suramin to identify or characterize purinergic neurotransmission in peripheral smooth muscle preparations.

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