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ORIGINAL ARTICLE

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Vasoconstrictor and vasodilator effects of guanine nucleotides in the rat aorta

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Abstract Although GTP, like ATP and UTP, is stored in platelet dense granules, little is known about its vascular effects. The present study was carried out in order to characterize the effects of GTP and related compounds in the rat aorta.
Contractions were examined in aortic rings at resting tension. In rings with intact endothelium, GTP, GDP, guanosine 5'-O-(3-thiotriphosphate) (GTPγS) and guanosine 5'-O-(2-thiodiphosphate) (GDPβS) caused small contractions. In endothelium-denuded rings, the contractions were unchanged or increased and persisted after desensitization of P2X-receptors by α,β-methylene ATP. *Relaxations* were examined in aortic rings precontracted with noradrenaline. In rings with intact endothelium, GTP (EC_{50} 131 μM), GDP (no maximal effect obtained), GTPγS (EC_{50} 6.8 μM) and guanosine (EC_{50} 822 μM) caused prominent relaxation, whereas GDPβS caused further contraction. In endothelium-denuded rings, the relaxant effect of GTP was greatly reduced, that of GDP and guanosine was unchanged, and that of GTPγS was abolished. Relaxations by GTP and GTPγS in endothelium-intact rings were studied in more detail. The relaxation by GTP was slightly and the relaxation by GTPγS greatly reduced after treatment with N^G-nitro-L-arginine methyl ester. Pre-exposure to a high concentration of the P2Y-receptor agonist 2-methylthio ATP (MeSATP) did not attenuate the effects of GTP and GTPγS. Four compounds previously identified as antagonists at the P2Y- and P2U-receptors of rat aortic endothelium – suramin, reactive blue 2, pyridoxalphosphate-6-azophenyl-2',5'-disulphonate (*iso*-PPADS) and 5,5'-(1,1'-biphenyl-4,4'-diylbisazo)-*bis*-7-amino-6-hydroxy-naphthalene-1,4-disulphonate (NH05) – were tested against GTP and GTPγS. Suramin, reactive blue 2 and *iso*-PPADS were much less potent against GTP and GTPγS than previously found against (the P2Y effect of) MeSATP. Suramin, *iso*-PPADS and NH05 were about as potent against GTP and GTPγS as previously found against (the P2U effect of) UTP and in particular ATP.
It is concluded that guanine nucleotides can cause both contraction and relaxation of the rat aorta. The high concentrations of GTP and GDP required, and in the case of contraction the small size of the response, make a physiological role of the vascular effects of these nucleotides unlikely. GTP and GTPγS elicit endothelium-dependent relaxation through P2U-receptors. GTP in addition relaxes the aorta through smooth muscle receptors, possibly by way of its degradation product guanosine. The stable analog GTPγS is a relatively potent and selective agonist for the endothelial P2U-receptor.

Key words Rat aorta · Endothelium · P2-receptors · P2U-receptor · P2-receptor antagonists · Guanine nucleotides · GTP · Guanosine

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Introduction

Extracellular adenine and possibly uracil nucleotides contribute to the local regulation of vascular tone (for review see Olsson and Pearson 1990; Ralevic and Burnstock 1991a). One possible source of the nucleotides is aggregating platelets. Like ATP and UTP, the guanine nucleotide GTP is stored in platelet dense granules. Although the amount of GTP stored exceeds the amount of UTP (Goetz et al. 1971), little is known about its vascular effects. GTP caused contraction of the rabbit ear artery (von Kügelgen et al. 1987) and the rat mesenteric vasculature (Ralevic and Burnstock 1991b; but see Juul et al. 1993) and induced a small Ca^{2+} transient in rat aortic myocytes (Tawada et al. 1987). In each case GTP was considerably less effective than ATP and UTP. On the other hand, endothelium-dependent relaxation in response to GTP has been demonstrated in the pig aorta (Martin et al. 1985), the guinea-pig coronary (Vials and Burnstock 1993) and the rat mesenteric vasculature

(Ralevic and Burnstock 1991b; Vuorinen et al. 1992, 1994). In the latter, GTP was 10- to 100-fold less potent than ATP and UTP, and the response to GTP was mediated by nitric oxide (Vuorinen et al. 1992). Based on the observation that the P2-receptor antagonist reactive blue 2 (10 μM) attenuated endothelium-dependent relaxations of the rat mesenteric artery elicited by ATP but not relaxations elicited by GTP, Vuorinen et al. (1994) suggested the existence of a guanine nucleotide-specific ('P_G') receptor.

In the present experiments, vasoconstriction and vasodilation by GTP and related compounds was investigated in the rat thoracic aorta. Two aims were pursued: to describe in greater detail the vascular effects of GTP, and to identify new selective agonists for the P2-receptor subtypes previously found in this tissue.

Methods

Male Wistar rats (250 to 300 g) were decapitated. The thoracic aorta was cleaned of adherent tissue and cut into rings of about 4 mm length. In some rings the endothelium was removed by gently rubbing the intimal surface (see Hansmann et al. 1997). The rings were mounted in a 5.9-ml organ bath. Two stainless steel hooks were inserted through the lumen; the lower hook was fixed and the upper one attached to an isometric force transducer (K30, Hugo Sachs Elektronik, Hugstetten, Germany). The incubation medium contained (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 0.9, $NaHCO_3$ 25, glucose 11, ascorbic acid 0.3 and disodium EDTA 0.03. It was saturated with 95% O_2/5% CO_2 and kept at 37°C. Unless stated otherwise, the medium was replaced every 15 min.

During a 60-min equilibration period, the resting tension was twice adjusted to 9.8 mN (Graphtec thermal pen recorder, Ettlingen, Germany). Noradrenaline (1 μM) was then added to the medium twice, 60 and 80 min after the beginning of the experiment. During the plateau of the second noradrenaline contraction, acetylcholine (1 μM) was added in order to examine the condition of the endothelium. The endothelium was considered intact when acetylcholine caused at least 50% relaxation; it was considered removed when acetylcholine failed to elicit relaxation. Rings that did not satisfy these criteria were discarded.

In order to determine concentration-contraction curves, guanosine or the guanine nucleotides were added in a cumulative fashion three times, 110, 170 and 230 min after the beginning of the experiment, to rings at resting tension (after the two initial – 60 and 80 min – responses to noradrenaline). They were washed out when the contraction elicited by the highest concentration was maximal. It took 5 to 10 min to determine a concentration-contraction curve. Three different agonists were studied in each preparation in varying order of application. Contractions were measured at their maximum and expressed as a percentage of the second initial noradrenaline contraction.

In order to determine concentration-relaxation curves, noradrenaline (1 μM usually) was again added to the medium either twice, 105 and 165 min, or three times, 105, 165 and 225 min, after the beginning of the experiment ('precontraction'). Guanosine or the nucleotides were added in a cumulative fashion during the plateau of the noradrenaline response, i.e. from 5 to 7 min after the addition of noradrenaline onwards. They were washed out together with noradrenaline when the relaxation elicited by the highest concentration was maximal. It took 5 to 15 min to determine a concentration-relaxation curve. Unless stated otherwise, only one agonist was studied per preparation. Relaxations were measured at their maximum and expressed as a percentage of the respective noradrenaline precontraction. The cumulative protocol did not underestimate agonist potencies: addition of single concentrations of GTPγS (10 or 32 μM) after noradrenaline precontraction elicited similar or smaller relaxation compared to the same concentration in cumulative concentration-relaxation curves.

For the computation of maximal effects and EC_{50} values of agonists (concentrations producing 50% of the respective maximum), logistic curves were fitted to weighted mean contraction or relaxation values by means of Eq. 25 of Waud (1976) and non-linear regression. When a concentration-response relationship was bell-shaped (Figs. 3–5), the responses to concentrations higher than the maximally relaxant one were taken to be identical with the maximal response. Apparent antagonist K_d values were derived by one of two procedures. If, in a pair of antagonist and agonist, the antagonist did not change the maximum of the agonist concentration-response curve, the apparent K_d was derived from the shift of the curve to the right at the level of the EC_{50}, using Eq. 4 of Furchgott (1972). If, in an antagonist-agonist pair, the antagonist depressed the maximum of the agonist concentration-response curve, the apparent K_d value was derived from a double reciprocal plot according to pp. 335 and 342 of Kenakin (1993; cf. Hansmann et al. 1997).

The following drugs were used: suramin hexasodium (Bayer, Wuppertal, Germany); 2-methylthio ATP tetrasodium (MeSATP), 8-(*para*-sulphophenyl)theophylline (8-SPT), reactive blue 2 (Biotrend, Köln, Germany); pyridoxalphosphate-6-azophenyl-2',5'-disulphonate tetrasodium (*iso*-PPADS; Cookson Chemicals, Southampton, UK); acetylcholine chloride, guanosine, guanosine diphosphate trilithium (GDP), guanosine triphosphate tetralithium (GTP), guanosine 5'-O-(2-thiodiphosphate) trilithium (GDPβS), guanosine 5'-O-(3-thiotriphosphate) tetralithium (GTPγS), guanylylimidodiphosphate trisodium (β,γ-NHGTP), α,β-methylene ATP dilithium (α,β-MeATP), α,β-methylene GDP disodium (α,β-MeGDP), β,γ-methylene GTP trisodium (β,γ-MeGTP), N^G-nitro-L-arginine methyl ester (L-NAME), (–)-noradrenaline bi-(+)-tartrate (Sigma, Deisenhofen, Germany); and 5,5'-(1,1'-biphenyl-4,4'-diylbisazo)-*bis*-7-amino-6-hydroxy-naphthalene-1,4-disulphonate tetrasodium (NH05; Syntec, Wolfen, Germany). Antagonists and noradrenaline were dissolved in distilled water. Acetylcholine, guanosine, 8-SPT and the nucleotides were dissolved in medium.

Data are expressed as either the arithmetic mean ± SEM or, in the case of fitted curves, the EC_{50} and maximal effect with the SE as defined by Waud (1976). Differences between fitted curves were tested according to p. 371 of Motulsky and Ransnas (1987). $P<0.05$ was taken as the limit of statistical significance.

Results

Contraction

The ability of guanosine and the guanine nucleotides to elicit contraction was studied in aortic rings at resting tension (i.e. non-precontracted). In preparations with intact endothelium, GTP, GDP, guanosine 5´-O-(3-thiotriphosphate) (GTPγS) and guanosine 5´-O-(2-thiodiphosphate) (GDPβS) caused small, sustained contractions, always below 10% of the response to noradrenaline (1 μM; Fig. 1a; open symbols in Fig. 2). β,γ-Methylene GTP (β,γ-MeGTP), α,β-methylene GDP (α,β-MeGDP), guanylylimidodiphosphate (β,γ-NHGTP) and guanosine were without effect at up to 100 μM (n = 3 each; not shown).

In endothelium-denuded rings, GTP, GDP, GTPγS and the lower concentrations of GDPβS elicited similar or slightly higher contractions. The response to 100 μM GDPβS was much larger than in rings with intact endothelium (Fig. 1a; filled symbols in Fig. 2). β,γ-MeGTP, α,β-MeGDP, β,γ-NHGTP and guanosine again were inactive at up to 100 μM (n = 3 each; not shown).

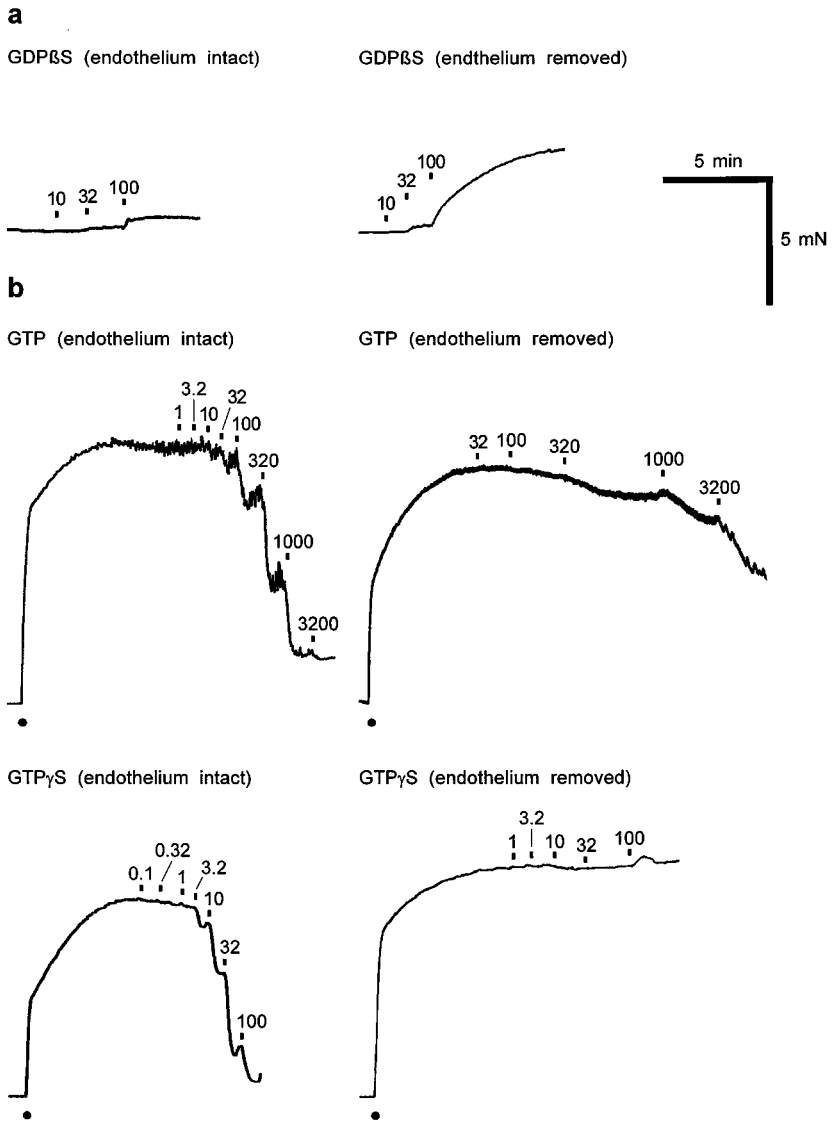

Fig. 1a,b Contraction and relaxation evoked by guanine nucleotides in rings of rat aorta. Typical responses in preparations with intact endothelium (*left-hand tracings*) and endothelium-denuded preparations (*right-hand tracings*). **a** Preparations at resting tone. Increasing concentrations (μM) of GDPβS were administered in a cumulative fashion. **b** Preparations precontracted with noradrenaline (1 μM; ●). Increasing concentrations (μM) of GTP or GTPγS were administered in a cumulative fashion during the plateau of each response to noradrenaline. Representative tracings from 3 to 5 experiments

α,β-Methylene ATP (α,β-MeATP; 100 μM), given in order to desensitize P2X-receptors (see García-Velasco et al. 1995), caused transient contraction in endothelium-denuded aortic rings (26±3% of the response to noradrenaline 1 μM; n = 13). When added 30 min after, and in the continued presence of, 100 μM of α,β-MeATP, GTP (100–3200 μM), GDP (320–3200 μM), GTPγS (10–100 μM) and GDPβS (10–100 μM) elicited contractions similar to those in the absence of α,β-MeATP (n = 3 each; not shown).

Relaxation

The ability of guanosine and the guanine nucleotides to cause relaxation was studied in aortic rings precontracted with noradrenaline. In preparations with intact endothelium, the first precontraction caused by noradrenaline (1 μM) averaged 7.2±0.2 mN (n = 83; for the slight increase of noradrenaline precontractions in the course of such experiments see Hansmann et al. 1997). When added during the plateau of this contraction, increasing concentrations of GTP, GDP, GTPγS and guanosine caused prominent relaxation (Fig. 1b; open symbols in Fig. 3a,b,c,h). Responses to GTP and GTPγS were rapid and transient (Fig. 1b), those to GDP and guanosine were slower and more sustained (not shown). The EC_{50} values (and maximal percentage relaxations) were 131±12 μM (99±2%) for GTP, 6.8±0.7 μM (86±3%) for GTPγS and 822±146 μM (104±5%) for guanosine (from experiments of Fig. 3). The concentration-response curve of GDP did not reach a maximum within the concentration range studied (Fig. 3b). Relaxations caused by β,γ-MeGTP, α,β-MeGDP and β,γ-NHGTP hardly exceeded 10%, and GDPβS did not cause relaxation but further contraction (open symbols in Fig. 3d–g).

In endothelium-denuded rings, the first precontraction to noradrenaline (1 μM) averaged 7.8±0.5 mN (n = 24; not significantly different from endothelium-intact rings). GTP, GDP and guanosine again caused relaxation

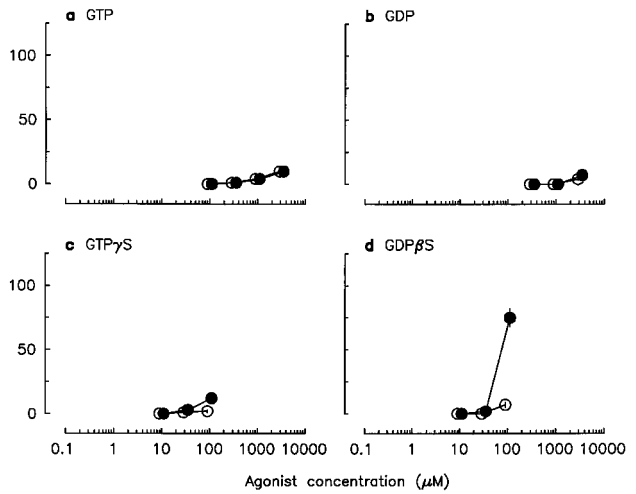

Fig. 2a–d Contraction evoked by guanine nucleotides in endothelium-intact (❍) and endothelium-denuded (●) rat aortic rings at resting tension. Three concentration-contraction curves were determined in each ring, interval 60 min, each for a different agonist, using varying orders of application. GTP (**a**), GDP (**b**), GTPγS (**c**) or GDPβS (**d**) was administered in a cumulative fashion. *Abscissae*, agonist concentration. *Ordinates* show contraction as a percentage of an initial contraction caused by noradrenaline (1 μM). Means ± SEM from 3 experiments each

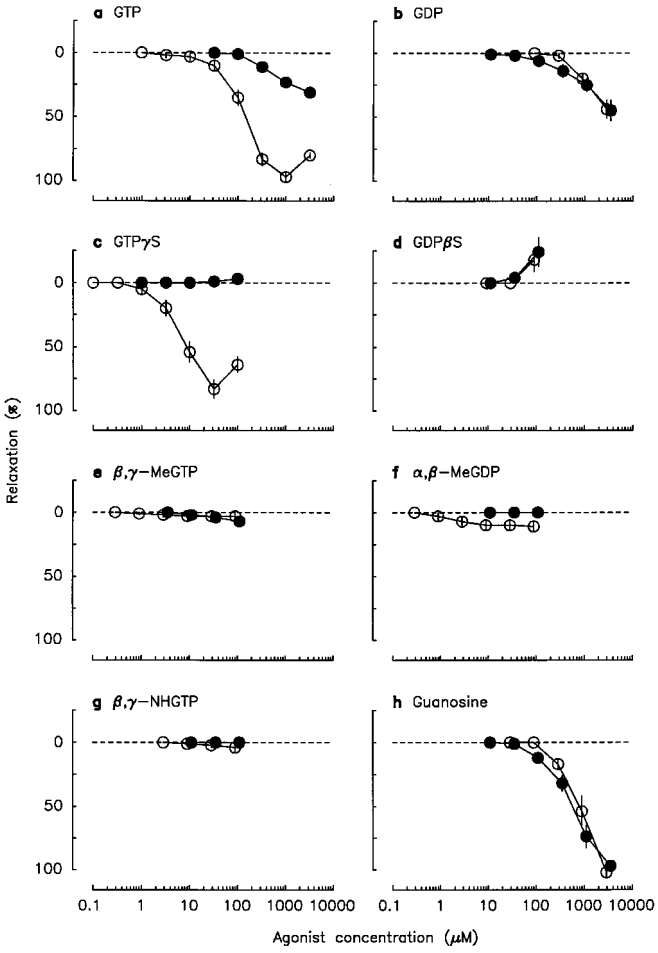

Fig. 3a–h Relaxation evoked by guanine nucleotides in endothelium-intact (❍) and endothelium-denuded (●) rat aortic rings precontracted with noradrenaline. Noradrenaline (1 μM) was added to the medium three times, interval 60 min. GTP (**a**), GDP (**b**), GTPγS (**c**), GDPβS (**d**), β,γ-MeGTP (**e**), α,β-MeGDP (**f**), β,γ-NHGTP (**g**) or guanosine (**h**) was administered in a cumulative fashion during the plateau of each response to noradrenaline. Three different agonists were tested in each preparation, using varying orders of application. *Abscissae*, agonist concentration. *Ordinates* show relaxation as a percentage of the respective response to noradrenaline. Means ± SEM from 3 to 5 experiments

(Fig. 1b; filled symbols in Fig. 3a,b,h). Responses to GDP and guanosine were unchanged in time course (not shown) and magnitude (Fig. 3b,h). The EC_{50} value (and maximal percentage relaxation) for guanosine was 504±55 μM (102±2%; not significantly different from endothelium-intact rings). Responses to GTP were much smaller than in endothelium-intact preparations and also slower (Fig. 1b), resembling in time course the responses to GDP and guanosine, and did not reach an asymptotic maximum within the concentration range studied (Fig. 3a). GTPγS failed to relax endothelium-denuded rings, in marked contrast to rings with intact endothelium (Fig. 1b; Fig. 3c). β,γ-MeGTP, α,β-MeGDP and β,γ-NHGTP caused little, if any, relaxation (Fig. 3e–g). GDPβS caused further contraction also in endothelium-denuded preparations (Fig. 3d).

Relaxations caused by GTP and GTPγS in rings with intact endothelium were examined in greater detail (Figs. 4 and 5). In these experiments, a second and third concentration-response curve for both nucleotides, after addition of solvent, was similar to the first one (Figs. 4a and 5a), except that in the case of GTPγS the maximal relaxation tended to be lower in the third curve (Fig. 5a; $P>0.05$).

The effect of N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase, was studied first. As L-NAME (30 μM) increases the noradrenaline-evoked contraction of the rat aorta (Hansmann et al. 1997), the concentration of noradrenaline, when added in the presence of L-NAME, was reduced to 0.01 μM. Even noradrenaline (0.01 μM) elicited a (second) precontraction in the presence of L-NAME which was higher (9.7±1.0 mN; $n = 8$) than the (second) precontraction to noradrenaline (1 μM) in the absence of L-NAME ($P<0.01$; cf. Hansmann et al. 1997). L-NAME (30 μM) shifted the concentration-relaxation curve of GTP slightly to the right (Fig. 4b; $P<0.01$) and greatly reduced the effect of GTPγS (Fig. 5b).

In order to investigate a possible contribution of P2Y-receptors, nucleotide interactions were studied as follows (Dainty et al. 1991; Hansmann et al. 1997). Immediately after the application of noradrenaline (1 μM; second precontraction), a high concentration of 2-methylthio ATP (MeSATP; 100 μM) was added and this was followed, when the contraction plateaued and without washout, by the usual determination of GTP or GTPγS concentration-relaxation curves. The initial high concentration of Me-

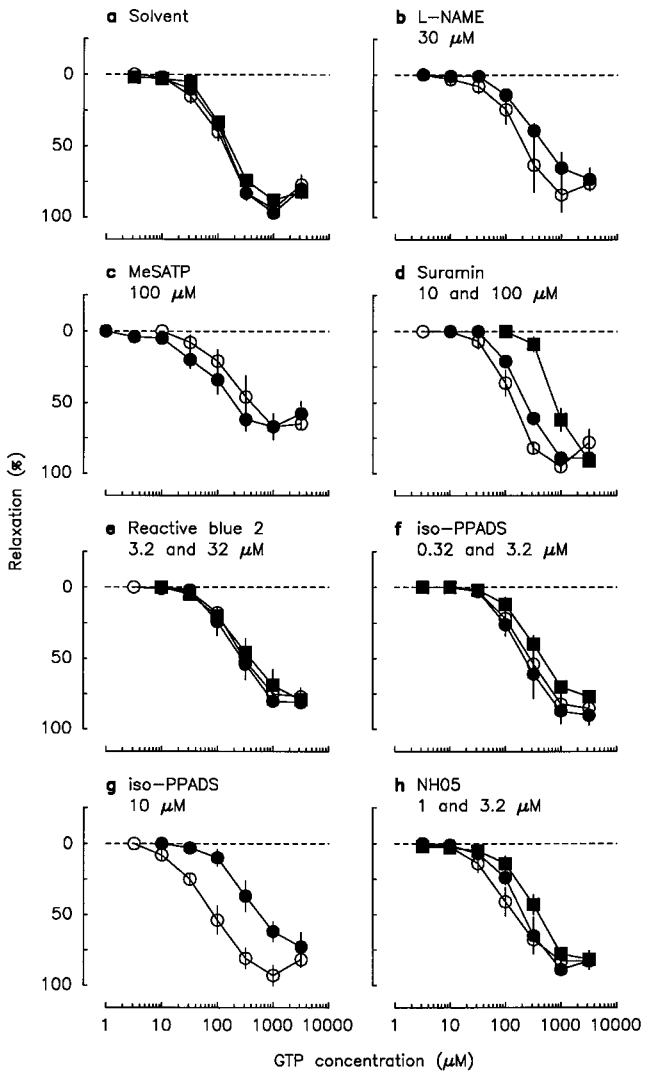

Fig. 4a–h Effect of N^G-nitro-L-arginine methyl ester (L-NAME), MeSATP and P2-receptor antagonists on the GTP-evoked relaxation of rat aortic rings precontracted with noradrenaline. Noradrenaline (1 μM; in the presence of L-NAME 0.01 μM) was added to the medium two (**b**,**c**,**g**) or three (**a**,**d**–**f**,**h**) times, interval 60 min. GTP was administered in a cumulative fashion during the plateau of each response to noradrenaline. L-NAME (30 μM) was added 30 min before the second addition of noradrenaline, i.e. about 35 min before the second GTP concentration-relaxation curve (**b**). MeSATP (100 μM) was added immediately after the second addition of noradrenaline, i.e. about 5 min before the second GTP concentration-relaxation curve (**c**). The P2-receptor antagonists were added at a two increasing concentrations immediately after the first and second GTP concentration-relaxation curve, i.e. about 50 min before the second and third concentration-relaxation curve (**d**–**f** and **h**; *iso*-PPADS in **g** was given only at a single concentration). *Abscissae*, GTP concentration. *Ordinates* show relaxation in first curves (○), second (●) and third (■) curves in the presence of solvent (**a**) or the drugs indicated (**b**–**h**), as a percentage of the respective response to noradrenaline. Means ± SEM from 4 to 7 experiments

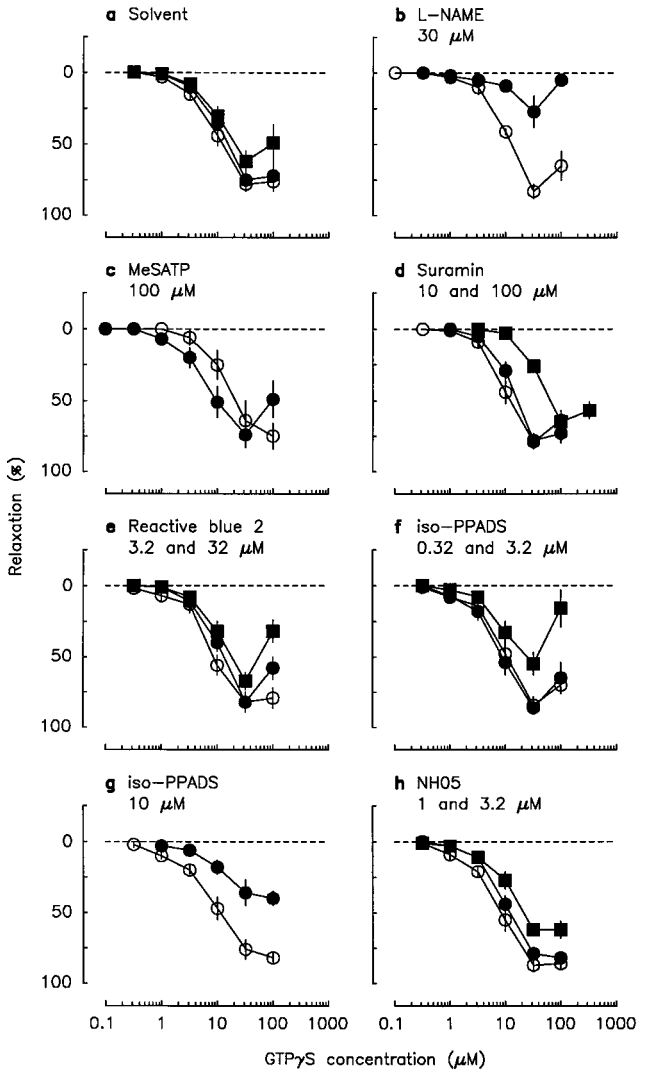

Fig. 5 Effect of N^G-nitro-L-arginine methyl ester (L-NAME), MeSATP and P2-receptor antagonists on the GTPγS-evoked relaxation of rat aortic rings precontracted with noradrenaline. Details are as in the legend to Fig. 4, GTPγS replacing the GTP of Fig. 4. Means ± SEM from 4 to 7 experiments

SATP reduced the noradrenaline response to 6.3±0.9 mN (n = 9; P<0.05 vs. second precontraction without MeSATP; cf. Hansmann et al. 1997). Pre-exposure to MeSATP (100 μM) did not reduce but enhanced the relaxation caused by GTP (Fig. 4c) and GTPγS (Fig. 5c; both P<0.01).

Four P2-receptor antagonists were tested against GTP and GTPγS: suramin, reactive blue 2, pyridoxalphosphate-6-azophenyl-2',5'-disulphonate (*iso*-PPADS), and the trypan blue analogue 5,5'-(1,1'-biphenyl-4,4'-diylbisazo)-*bis*-7-amino-6-hydroxy-naphthalene-1,4-disulphonate (NH05; Wittenburg et al. 1996). All were used at concentrations that did not attenuate the endothelium-dependent relaxation of rat aortic rings produced by acetylcholine (Hansmann et al. 1997). Suramin and *iso*-PPADS shifted the concentration-relaxation curves of GTP and GTPγS to the right at the higher concentrations examined (100 and 10 μM, respectively; Figs. 4d,f,g and 5d,f,g); *iso*-PPADS (10 μM) also reduced the maximum

Table 1 Apparent K_d values of P2-receptor antagonists. K_d values against GTP and GTPγS were calculated from the experiments of Figs. 4 and 5. Values against MeSATP, UTP and ATP are from Hansmann et al. (1997)

Antagonist, μM		Apparent K_d value (μM) against				
		GTP	GTPγS	MeSATP	UTP	ATP
Suramin	10	*n.e.*	*n.e.*	2.4	*n.e.*	*n.e.*
	100	26.3	29.1	4.7	36.5	26.1
Reactive blue 2	3.2	*n.e.*	*n.e.*	0.54		
	32	*n.e.*	*n.e.*		6.5	21.0
iso-PPADS	0.32	*n.e.*	*n.e.*	0.08		
	3.2	*n.e.*	*n.e.*	0.08	1.7	*n.e.*
	10	3.6	4.8 [a]		3.6	3.3
NH05	1	*n.e.*	*n.e.*	0.44	0.21	1.0
	3.2	2.4	*n.e.*	0.35	0.33	1.8

n.e., no effect at concentration indicated
[a] Maximum of concentration-relaxation curve reduced by up to 50%; apparent K_d value determined according to Kenakin (1993)

of the GTPγS concentration-relaxation curve (Fig. 5 g). Reactive blue 2 caused no change (Figs. 4e and 5e; the tendency for a lower maximum in the third control GTPγS concentration-relaxation curve should be remembered: compare Fig. 5e and Fig. 5a). NH05 (3.2 μM) antagonized significantly only GTP but not GTPγS (Figs. 4h and 5h). Apparent antagonist K_d values are summarized in Table 1.

Finally, the interaction of suramin and the P1-purinoceptor antagonist 8-(*para*-sulphophenyl)theophylline (8-SPT) with GTP and guanosine was studied in endothelium-denuded preparations. Solvent or antagonists were added immediately after the first of two agonist concentration-relaxation curves. A second concentration-response curve for GTP and guanosine, after addition of solvent, was similar to the first one (n = 3 each). Suramin (100 μM) and 8-SPT (100 μM) did not alter the relaxation caused by GTP (3.2–3200 μM; n = 3 each). 8-SPT (100 μM) did not alter the relaxation caused by guanosine (32–3200 μM; n = 3; not shown).

Discussion

The stable guanine nucleotides GTPγS and GDPβS are commonly employed to study the function of G-proteins. They do not readily cross the plasma membrane, and cells have to be permeabilized to allow access of GTPγS or GDPβS (e.g., Brock et al. 1988; Shibano et al. 1992). It is unlikely, therefore, that the effects observed in the present study were due to a direct interaction of the guanine nucleotides with G-proteins. This possibility is additionally refuted by the observation that β,γ-MeGTP, α,β-MeGDP, β,γ-NHGTP, which also interact with G-proteins (e.g., Cockcroft and Gomperts 1985), did not mimick the effects of the phosphorothioate analogs, causing no contraction and minimal relaxation. So the main aim of the Discussion will be to interpret the effects of GTP, GDP, GTPγS and GDPβS in terms of actions at the plasma membrane receptors for nucleotides previously identified, or suggested to exist, in the rat aorta.

Contraction

Like several other nucleotides (White et al. 1985; García-Velasco et al. 1995; Hansmann et al. 1997), GTP, GDP, GTPγS and GDPβS caused contraction of rat aortic rings at resting tension. Removal of the endothelium either did not modify or enhanced the contraction (Figs. 1a and 2), indicating that it was mediated by smooth muscle receptors. Which are these receptors?

The smooth muscle of the rat aorta seems to contain at least three receptors for adenine and uracil nucleotides. One is a P2X-receptor (García-Velasco et al. 1995; Pacaud et al. 1995; see Fredholm et al. 1994 and Burnstock and King 1996 for the subclassification of P2-receptors). A second receptor has been suggested to mediate the contraction elicited by UTP and to be a 'pyrimidinoceptor' insensitive to ATP (García-Velasco et al. 1995). The third is a P2U-receptor, sensitive to both ATP and UTP and mediating a rise in intracellular Ca^{2+} in cultured rat aortic myocytes (Kitajima et al. 1994; Pacaud et al. 1995). In support of the existence of two nucleotide receptors in addition to P2X, mRNA transcripts encoding for a 'pyrimidinoceptor' ($P2Y_6$) and for a P2U-receptor ($P2Y_2$; Burnstock and King 1996) have been demonstrated in rat aortic myocytes (Chang et al. 1995).

Desensitization of P2X-receptors with α,β-MeATP did not change the contraction of rat aortic rings elicited by GTP, GDP, GTPγS and GDPβS (as has been shown for GTP in the rat mesenteric vascular bed; Ralevic and Burnstock 1991b), indicating that the contraction-mediating receptor for the guanine compounds is not P2X. In fact GTP is inactive at recombinant P2X-receptors (e.g. $P2X_1$: Valera et al. 1994). This leaves the 'pyrimidinoceptor' ($P2Y_6$) and the P2U-receptor ($P2Y_2$). No unequivocal distinction is possible. GTP is an, albeit weak, agonist at recombinant rat $P2Y_2$-receptors (Chen et al. 1996), and GTP, GDP and GTPγS are agonists at recombinant human $P2Y_2$-receptors (Lazarowski et al. 1995). On the other hand, GTP, GDP and GTPγS are inactive at native $P2Y_6$-receptors in C6-2B rat glioma cells (Lazarowski and Harden 1994; Nicholas et al. 1996). It would seem more likely, therefore, that GTP, GDP and GTPγS contract the rat aorta through the P2U-receptor. For the most effective agonist, GDPβS, lack of data concerning its activity at identified P2-receptors prevents even this preliminary assessment of probabilities.

The naturally occurring nucleotides GTP and GDP caused only minor contraction of the aorta and were less effective than ATP and in particular UTP (cf. García-Velasco et al. 1995; Hansmann et al. 1997), a finding that

argues against a physiological guanine nucleotide-evoked vasoconstriction.

Relaxation

Like several other nucleotides (see White et al. 1985; Dainty et al. 1991; Hansmann et al. 1997), GTP, GDP, GTPγS and guanosine relaxed aortic rings precontracted with noradrenaline. Removal of the endothelium abolished, and L-NAME markedly attenuated, the response to GTPγS (Figs. 1b, 3c and 5b). We have previously shown that at least the major mechanism of the antagonism of L-NAME against the relaxant effect of acetylcholine, adenosine 5'-O-(2-thiodiphosphate) (ADPβS) and UTP in rat aorta is blockade of nitric oxide synthase (Hansmann et al. 1997). The relaxation caused by GTPγS, therefore, was entirely due to activation of endothelial receptors, and nitric oxide was the main mediator released. GTP produced relaxation also in endothelium-denuded preparations, much smaller though than when the endothelium was intact, and its effect was attenuated much less by L-NAME (Fig. 1b, 3a and 4b). The mode of action of GTP therefore comprises an endothelial as well as a non-endothelial component (compare the effect of ATP in Hansmann et al. 1997). The relaxation caused by GDP or guanosine, finally, was not altered by removal of the endothelium at all (Fig. 3b,h), indicating that these two acted entirely through receptors located on the smooth muscle cells. Which are the smooth muscle and the endothelial receptors?

As GTP and GDP are susceptible to enzymatic hydrolysis and guanosine is an effective relaxant, it seems possible that the endothelium-independent relaxation caused by GTP and GDP was mediated by their degradation product guanosine. In support of this view, the response to GTP was slow in endothelium-denuded aortic rings, similar to the responses to GDP and guanosine, and was not altered by the P2 antagonist suramin in these preparations. The relaxation-mediating receptor for guanosine is not known. The lack of effect of 8-SPT argues against an adenosine P1-purinoceptor (cf. Vuorinen et al. 1994). However, the endothelium-independent relaxation of the rat aorta caused by adenosine itself is equally resistant to several adenosine receptor antagonists including 8-SPT (up to 100 μM; Prentice and Hourani 1996). Possibly the – atypical – smooth muscle receptor activated by adenosine is also the site of action of guanosine.

The endothelium-dependent relaxation of the rat aorta in response to extracellular adenine and uracil nucleotides is mediated by P2Y-receptors, which are selectively activated by MeSATP, and P2U-receptors, which are selectively activated by UTP and also ATP (which does not act noticeably through the endothelial P2Y-receptors in this preparation; Dainty et al. 1991; Hansmann et al. 1997). The endothelium-dependent relaxation caused by GTP and GTPγS was not attenuated after pre-exposure to a high concentration of MeSATP (Figs. 4c and 5c), indicating that the site of action of GTP and GTPγS was not the P2Y-receptor (cf. Hansmann et al. 1997). The results obtained with the P2-antagonists confirm this view: as shown in Table 1, suramin, reactive blue 2 and *iso*-PPADS were much less potent against GTP and GTPγS than against MeSATP (data from Hansmann et al. 1997). On the positive side, the K_d values of suramin and *iso*-PPADS against GTP and GTPγS are very similar to those against ATP and UTP (from Hansmann et al. 1997), suggesting an action of GTP and GTPγS through the P2U-receptor. The effects of reactive blue 2 and NH05, both more potent against UTP than against GTP and GTPγS (Table 1), might seem to contradict this view. However, two considerations indicate that the contradiction is only apparent. First, the potency of antagonists against the relaxation effect of UTP may be overestimated due to a simultaneous contraction effect of UTP (Hansmann et al. 1997); in fact the K_d value of NH05 against ATP, which lacks the prominent contraction effect of UTP (Hansmann et al. 1997), was similar to that against GTP (1.0–1.8 *vs*. 2.4; Table 1). Second, the highest concentrations of reactive blue 2 and NH05 that were tested against GTP and GTPγS were close to the K_d values of these antagonists against ATP so that the lack of significant antagonism may well have been chance; higher concentrations of reactive blue 2 and NH05 could not be used because they are non-selective for P2-receptors (as compared to muscarinic receptors; Hansmann et al. 1997). Overall, the agonist interaction and the antagonist experiments are compatible with the view that GTP and GTPγS cause relaxation through the endothelial P2U-receptor.

In agreement with the present data, the endothelium-dependent relaxation of rat mesenteric arteries in response to GTP was not altered by reactive blue 2 (10 μM; Vuorinen et al. 1994). The authors proposed that GTP acted at a specific 'P_G'-receptor. However, the endothelium of the rat mesenteric vasculature is now known to possess P2U-receptors (Windscheif et al. 1994; Ralevic and Burnstock 1996), and our results suggest that, as in the aorta, this subtype mediated the effect of GTP.

A secondary aim of the present study was to detect selective agonists for the P2-receptors of the rat aorta. GTPγS may be one. It elicited prominent endothelium-dependent relaxation, with an EC_{50} (6.8 μM) close to ATP (3.5 μM) and UTP (1.1 μM; Hansmann et al. 1997), but little contraction, and it did not relax endothelium-denuded preparations. GTPγS, hence, is a relatively potent and selective agonist for the P2U-receptor of rat aortic endothelial cells. It has not been tested in other blood vessels in which effects of GTP were examined (see Introduction), but presumably P2-receptor-mediated effects of GTPγS have been observed in rat hepatocytes (e.g. IP_3 formation; Okajima et al. 1987) and human neutrophils (e.g. stimulation of superoxide formation; Seifert et al. 1989). The P2 subtype for these responses has not been characterized but may be P2U, a receptor which both rat

hepatocytes and human neutrophils possess (see O'Connor et al. 1991; Keppens 1993).

With an EC_{50} value of 131 μM, GTP was considerably less potent than ATP and UTP (see above) in eliciting endothelium-dependent relaxation of the rat aorta. GDP also produced relaxation only at very high concentrations. It seems unlikely, therefore, that platelet-derived guanine nucleotides are major physiological vasodilators.

Conclusion

Guanine nucleotides can cause both contraction and relaxation of the rat aorta. The naturally occurring compounds GTP and GDP are weak with respect to either response. Hence, vasoconstriction or vasodilation caused by platelet-derived guanine nucleotides is unlikely to be physiologically relevant. The stable analog GTPγS selectively activates the endothelial P2U-receptor and may therefore be useful in delineating P2-receptor subtypes.

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