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Effects of U46619 on contractions to 5-HT, sumatriptan and methysergide in canine coronary artery and saphenous vein *in vitro*

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1 The aim of this study was to investigate the mechanism of enhanced reactivity to 5-hydroxytryptamine (5-HT) and sumatriptan previously observed in human isolated coronary arteries when active force was raised with the thromboxane A_2-mimetic, U46619.

2 Ring segments of dog isolated coronary artery and saphenous vein were suspended in organ baths and cumulative concentration-contraction curves to 5-HT, sumatriptan and methysergide were constructed in the absence and presence of low concentrations of U46619.

3 In both endothelium-intact and endothelium-denuded rings of coronary artery, precontraction with U46619 to low (< 10% F_{max}; the contraction to a maximum depolarizing 125 mM KCl Krebs solution; KPSS) levels of active force had no effect on either the maximum contraction or sensitivity (pEC_{50}) to 5-HT, sumatriptan and methysergide.

4 Ketanserin (1 μM) had no effect on contractions to sumatriptan and methysergide in endothelium-denuded coronary artery rings, but reduced the maximum contraction to 5-HT by $\approx$ 90% to a value (5% F_{max}) similar to that for sumatriptan and methysergide. Under these conditions, U46619 precontraction had no effect on either pEC_{50} or maximum for 5-HT, sumatriptan or methysergide.

5 In rings of saphenous vein with endothelium and treated with ketanserin (1 μM), 5-HT and sumatriptan caused equal maximum responses of 65% F_{max} which were approximately double that of methysergide (32% F_{max}). The maximum responses and sensitivity to 5-HT, sumatriptan, methysergide and noradrenaline were unaffected by precontraction with U46619.

6 Pretreatment of the saphenous vein with sodium nitroprusside (SNP; 10 μM) caused a small sustained relaxation and significantly depressed the maximal contraction to 5-HT without affecting sensitivity and abolished the contraction curve to sumatriptan and methysergide. When the relaxation response to SNP was reversed with U46619 (1-4 nM), the contraction curves to 5-HT, sumatriptan and methysergide were similar to those obtained prior to relaxation with SNP. In contrast, the same treatment with SNP had little affect on the contraction curve to noradrenaline.

7 In conclusion, the pattern of U46619-enhanced reactivity of 5-HT, sumatriptan and methysergide in SNP-treated dog saphenous vein, highlights the importance of functional antagonism when assessing reactivity to contractile agonists in isolated blood vessels.

Keywords: 5-HT_1-like receptors; 5-HT; sumatriptan; methysergide; dog coronary artery; dog saphenous vein; functional antagonism

Introduction

In isolated rings of human coronary artery, contractions to 5-hydroxytryptamine (5-HT) and the antimigraine drug, sumatriptan, a 5-HT_1-like receptor agonist (Perrin *et al.*, 1989; Humphrey *et al.*, 1990; Ferrari *et al.*, 1991), are enhanced with threshold concentrations of the thromboxane-A_2 mimetic, U46619 (Cocks *et al.*, 1993). Similar findings have also been reported in the rabbit isolated femoral artery (MacLennan & Martin, 1992) and dog coronary artery (Mullane *et al.*, 1982). Whilst this potentiation effect of U46619 is not specific for 5-HT agonists (Cocks *et al.*, 1993), such findings may have important clinical implications as both thromboxane A_2 and 5-HT are released locally from aggregating platelets and amplification of the constrictor response to 5-HT receptor agonists, could lead to coronary vasospasm in arteries hyperreactive to 5-HT (Golino *et al.*, 1989; 1991; Zeiher *et al.*, 1991; McFadden *et al.*, 1991; 1992; Willett *et al.*, 1992).

The mechanism underlying the U46619-mediated potentiation of contractions to 5-HT and sumatriptan in human coronary arteries is unknown. Thus, in the present study, we have attempted to mimic this synergistic interaction in dog isolated vessels, specifically to address the question of whether the potentiation between 5-HT receptor agonists and U46619 observed in the human coronary artery was related to either relative apparent densities of 5-HT receptors or functional antagonism due to altered levels of resting, active force. First, isolated segments of dog coronary artery, where contractions to 5-HT are mediated predominantly by 5-HT_2 receptors with only a small contribution by 5-HT_1-like receptors (Frenken & Kaumann, 1985; Cohen, 1986) and the dog saphenous vein, where the contraction to 5-HT is mediated predominantly by 5-HT_1-like receptors (Apperley *et al.*, 1980; Feniuk *et al.*, 1985; Paiva *et al.*, 1988), were chosen to examine the role of apparent receptor densities. Second, the dog saphenous vein was also used to examine the effect of functional antagonism (induced with sodium nitroprusside) on contractility to 5-HT and sumatriptan in the absence and presence of U46619. Our results not only indicate that functional antagonism alone can account for our previous observations of synergy between U46619 and 5-HT receptor agonists in the human coronary artery (Cocks *et al.*, 1993) but also that the contraction to 5-HT in the human coronary artery is likely to be mediated equally by 5-HT_1-like and 5-HT_2 receptors if functional antagonism is not present.

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Methods

Tissue source

Greyhound dogs of either sex (20–30 kg) were deeply anaesthetized with sodium pentobarbitone (40 mg kg^{-1}, i.v.). The heart and 4–5 cm lengths of the lateral saphenous vein were removed and placed in cold oxygenated Krebs solution. The circumflex coronary artery was dissected free for 5–8 cm from its origin, and together with the saphenous vein, was cleared of connective tissue.

Organ bath studies

Ring segments (3 mm long) of each vessel were suspended on stainless steel wire hooks, 350 μm in diameter, in 25 ml jacketed glass organ baths. The upper wire hook was suspended from a Grass FT03C force transducer via which changes in isometric tension were amplified and then monitored on a single-channel, flat-bed recorder. The lower hook was fixed to a support leg attached to a micrometer. The tissues were maintained in physiological Krebs solution at 37°C and oxygenated with carbogen (95% O_2, 5% CO_2). The Krebs solution was composed of (in mM): Na^+ 144, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128.7, HCO_3^- 25, SO_4^{2-} 1.2 and glucose 11, pH 7.4.

Normalization

In order to compare reactivity between segments of both artery and vein, each ring was set to the same passive stretch conditions prior to construction of concentration-contraction curves. The normalization procedure used was adapted from that developed by Mulvany & Halpern (1977) for small arteries of 150–200 μm internal diameter, suspended as short segments on a myograph. The method involved setting the arteries and veins at a passive tension equivalent to 90% of their internal circumference (0.9 L_{100} or 0.9 L_{20}) if they had been relaxed maximally and exposed to transmural distending pressures of 100 mmHg and 20 mmHg respectively. These pressures were chosen as they approximate those measured *in vivo*. The procedure followed was as described by Angus *et al.* (1986).

Experimental protocol

Following normalization, ring segments were left for 1 h before being contracted with a depolarizing physiological salt solution (KPSS) containing isotonic 124 mM KCl. Once the KPSS-induced contraction had reached a plateau (F_{max}), the tissues were washed and the force allowed to return to baseline. Cumulative (0.5 log unit) concentration-contraction curves to the 5-HT receptor agonists 5-HT, sumatriptan and methysergide were then constructed in coronary arteries in the absence and presence of the thromboxane A_2-mimetic, U46619.

Only one concentration-contraction curve to an agonist was obtained for any one ring of artery. The same protocol was repeated in separate rings of coronary artery denuded of endothelium in order to observe the actions of the 5-HT agonists in the absence of local endothelium-derived vasodilators (e.g. prostacyclin (PGI_2) and endothelium-derived relaxing factor, EDRF; Cocks & Angus, 1983). The endothelium was removed by gentle rubbing with a tapered wooden stick and endothelium removal was tested by challenging each ring with bradykinin (0.1 μM), an endothelium-dependent relaxing agent. Sodium nitroprusside (SNP: 10 μM), an endothelium-independent relaxant, was used to verify the ability of the tissue to relax.

To exclude the actions of the 5-HT agonists at 5-HT_2 receptors, the contraction curves were repeated in endothelium-denuded rings, in the presence of the 5-HT_2 receptor antagonist, ketanserin (1 μM). Ketanserin was added 30 min prior to addition of the agonists.

Cumulative concentration-contraction curves to 5-HT, sumatriptan, methysergide and noradrenaline were constructed for the saphenous vein in the absence and presence of low concentrations of U46619. Contraction curves to the 5-HT agonists were performed in the presence of ketanserin and contraction curves to noradrenaline were generated in the presence of propranolol (1 μM) to prevent β-adrenoceptor-mediated smooth muscle relaxation. In a second series of experiments in the saphenous vein, SNP (10 μM), was added prior to the contractile agonists in order to remove basal levels of resting force. Cumulative (0.5 log unit) concentration-contraction curves to 5-HT, sumatriptan, methysergide and noradrenaline were then constructed in the presence of SNP alone, or together with U46619. Here the concentration of U46619 was raised slowly until the level of resting force in the vein had been restored near to that before the addition of SNP.

Drugs

Drugs used and their sources were: sumatriptan (Glaxo Group Research, Greenford, U.K.); U46619 ([1,5,5-hydroxy-11α, 9α-(epoxymethano) prosta-5Z, 13E-dienoic acid], Upjohn, Kalamazoo, MI, U.S.A.); 5-hydroxytryptamine creatinine sulphate, [5-HT] (Sigma, St. Louis, MO, U.S.A.); methysergide-HML (Sandoz SA, Basle, Switzerland) (−)-noradrenaline bitartrate (Sigma, St. Louis, MO, U.S.A.); sodium nitroprusside dihydrate (Roche, Dee Why, NSW, Australia); ketanserin tartrate (Janssen Pharmaceutica Pty Ltd., Forestville, NSW, Australia); (±)-propranolol HCl (ICI, Villawood, NSW, Australia); bradykinin triacetate (Fluka, Glossop, U.K.). All drugs were dissolved and diluted in distilled water except for U46619 which was made up as a 1 mM stock in absolute ethanol and diluted in water.

Statistical analysis

Agonist-induced contractile responses were normalized to the maximal increase in force to KPSS (F_{max}). When the level of active force was raised with U46619, the responses to the agonists were measured above the U46619 contraction and expressed as a percentage of F_{max}.

The individual contraction curves were fitted to a logistic equation ($E = MA^P/A^P + K^P$) where E is response, M is maximum response, A is agonist concentration, K is EC_{50} and P is the slope parameter at the EC_{50} (Nakashima *et al.*, 1982). From this relationship, computer estimates of the pEC_{50} ($-\log_{10}EC_{50}$) values for each ring were averaged for each treatment group and the standard error of the mean calculated.

A one-way analysis of variance (ANOVA) was used to compare pEC_{50} values, F_{max}, and passive vessel parameters between groups. When significance was found, comparisons between all groups were made with Tukey-Kramer's modified *t* statistic (see Table 1) and comparisons between control and treatment groups with Dunnett's test (see Table 2). Two-tailed paired *t* tests were used to test statistical significance within rings (Table 1). Statistical significance was accepted at $P < 0.05$ for all tests and values are given as mean ± s.e.mean.

Results

Resting vessel parameters

The dog coronary artery ring segments were significantly smaller in diameter compared with the saphenous vein segments at their respective distending pressures (Table 1). The resting passive force recorded immediately after normalization for endothelium-denuded arteries was significantly less than that for the endothelium-intact vessels and in both types of coronary artery, there was a significant spontaneous increase in force 1 h after normalization (Table 1). There was also a similar significant increase in active force in the saphenous vein (Table 1). Depolarization of the vessels with KPSS generated

Table 1 Summary of initial vessel parameters at normalization and contraction in response to KCl depolarization for dog coronary artery and dog saphenous vein

Vessel	*Number of rings*	*D* (mm)	*P* (mmHg)	*Initial force* F_1 (g)	*Force at 60 min* F_2 (g)	*KPSS* (F_{max}) (g)	F_{max}/*Diam* (g mm^{-1})
Dog coronary artery + endothelium	123	3.64±0.06*	59.9±0.7*	5.9±0.02*	7.5±0.23*†	21.1±0.7*	6.04±0.22*
Dog coronary artery - endothelium	160	3.80±0.05*	57.8±0.5*	4.9±0.15*	5.6±0.14*†	14.0±0.5*	3.77±0.15*
Dog saphenous vein + endothelium	312	4.31±0.05*	7.5±0.2*	0.25±0.01*	0.41±0.03*†	18.4±0.3*	4.5±0.09*

Abbreviations used: D, internal diameter estimated for transmural pressure of 100 mmHg for arteries and 20 mmHg for veins; P, equivalent transmural pressure needed to distend vessel at 90% of internal circumference at 100 mmHg (i.e. 0.9 L_{100}) and 20 mmHg (i.e. 0.9 L_{20}).
F_1: force immediately after normalization; F_2: force 60 min after normalization.
*Mean significantly different from means of all other groups ($P<0.001$,Tukey-Kramer test)
†F_2 significantly different from initial (F_1) force ($P<0.001$, Student's paired t-test)

contractile responses (F_{max}) which when normalized for vessel diameter, showed that values obtained in endothelium-intact coronary arteries were significantly greater than in endothelium-denuded vessels or saphenous vein (Table 1).

Reactivity

U46619 (0.0001–0.3 μM) caused concentration-dependent contractions in the coronary artery and saphenous vein (Figure 1). The normalized contraction curve for U46619 in the saphenous vein was calculated from mean, absolute contractions in g to KCl and U46619 as described by He *et al.* (1988). In subsequent studies, U46619 at concentrations of 0.1, 0.3 and 1.0 nM were used to precontract the coronary artery and saphenous vein and these concentrations contracted each tissue to approximately the same position on its contraction curve to U46619 (Figure 1). At a concentration of 1.0 nM, the contraction to U46619 was 15.5% and 17% of its maximum in the dog coronary artery and saphenous vein respectively.

Dog coronary artery

Effect of U46619 5-HT (0.001–10 μM), sumatriptan (0.003–30 μM) and methysergide (0.003–30 μM) caused concentration-dependent contractions in the endothelium-intact coronary artery (Figure 2). U46619 at concentrations of 0.1, 0.3 and 1.0 nM caused a sustained contraction of 2.7±0.5% (28 rings), 3.5±0.7% (26 rings) and 6.8±0.9% (28 rings) F_{max} respectively. Under these conditions, the maximum responses to 5-HT (32.6±8.2% F_{max}), sumatriptan (18.6±2.8% F_{max}) and methysergide (15.8±8.2% F_{max}) were not altered and the contraction curves were simply raised above the contraction to U46619 (Figure 2). In addition, the pEC_{50} values for 5-HT (7.56±0.25) and methysergide (6.90±0.26) were not significantly changed in the presence of U46619. The contraction curve to sumatriptan was biphasic (Figure 2) which may have been due to activation of histamine H_1 receptors at micromolar concentrations of sumatriptan (Maclennan *et al.*, 1991). Due to the biphasic nature of the sumatriptan contraction curves, pEC_{50} values could not be determined. However, when the maximum contraction for the first component of the sumatriptan curve was taken to occur at 1 μM sumatriptan, then there was no significant difference (ANOVA) between either pEC_{50} or maximum relaxation values for any treatment group.

In endothelium-denuded coronary artery rings (which failed to relax to the endothelium-dependent agonist, bradykinin; data not shown), the maximum contractions to 5-HT, sumatriptan and methysergide in the absence of U46619, were 20.9±5.8, 5.3±1.3 and 7.3±1.5% F_{max} respectively (Figure 3a). The pEC_{50} for 5-HT was 7.48±0.25. pEC_{50} values for sumatriptan and methysergide could not be determined as the maximum responses to these agonists were less than 10% F_{max}.

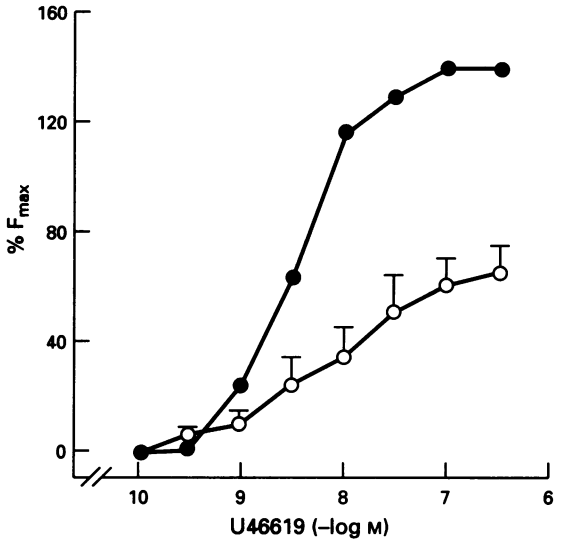

Figure 1 Cumulative concentration-contraction curves to U46619 in dog, isolated coronary artery (○, $n=6$) and saphenous vein (●, $n=6$). Mean data represented in the saphenous vein were calculated from results of He *et al.* (1988). Other values are mean±s.e.mean.

U46619 at concentrations of 0.1, 0.3 and 1.0 nM caused contractions of 1.1±0.5% (21 rings), 1.6±0.5% (21 rings) and 2.4±0.5% (21 rings) F_{max} respectively. No significant effects of U46619 upon the contraction curves to 5-HT, sumatriptan and methysergide were observed (Figure 3a). In endothelium-denuded rings, the 5-HT_2 receptor antagonist, ketanserin (1 μM), depressed the maximal response to 5-HT from 20.9±5.8 to 5.1±3.1% F_{max} (Figure 3b). The concentration-contraction curves to sumatriptan and methysergide were unaffected by ketanserin treatment (Figure 3b). In the presence of ketanserin, U46619 at concentrations of 0.1, 0.3 and 1.0 nM caused contractions of 1.8±0.8% (9 rings), 1.8±0.9% (9 rings) and 3.6±1.0% (9 rings) F_{max} respectively. Under these conditions only an additive effect of U46619 upon the contraction curves to 5-HT, sumatriptan and methysergide was observed (Figure 3b).

Dog lateral saphenous vein

Effect of U46619 In the presence of ketanserin (1 μM), 5-HT (0.001–1 μM), sumatriptan (0.003–10 μM), methysergide (0.003–10 μM) and noradrenaline (0.003–30 μM) caused concentration-dependent contractions in dog isolated saphenous vein (Figure 4). The maximum responses to 5-HT and

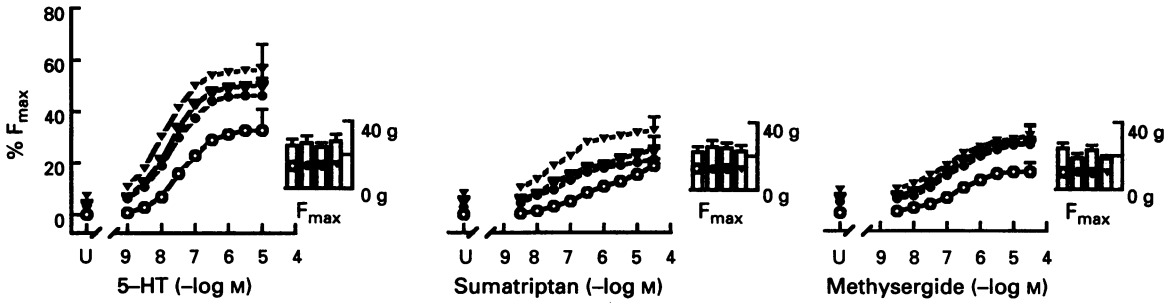

Figure 2 The effect of increasing resting active force with U46619 (U) at concentrations of 0 (○), 0.1 (●), 0.3 (▽) and 1.0 (▼) nM on the contraction curves to 5-HT ($n=6$), sumatriptan ($n=6$) and methysergide ($n=6$) in endothelium-intact rings of dog, isolated coronary artery. Inserts represent the maximum contraction to KPSS (F_{max}) in the absence and presence of U46619. Error bars (where shown) indicate s.e.mean.

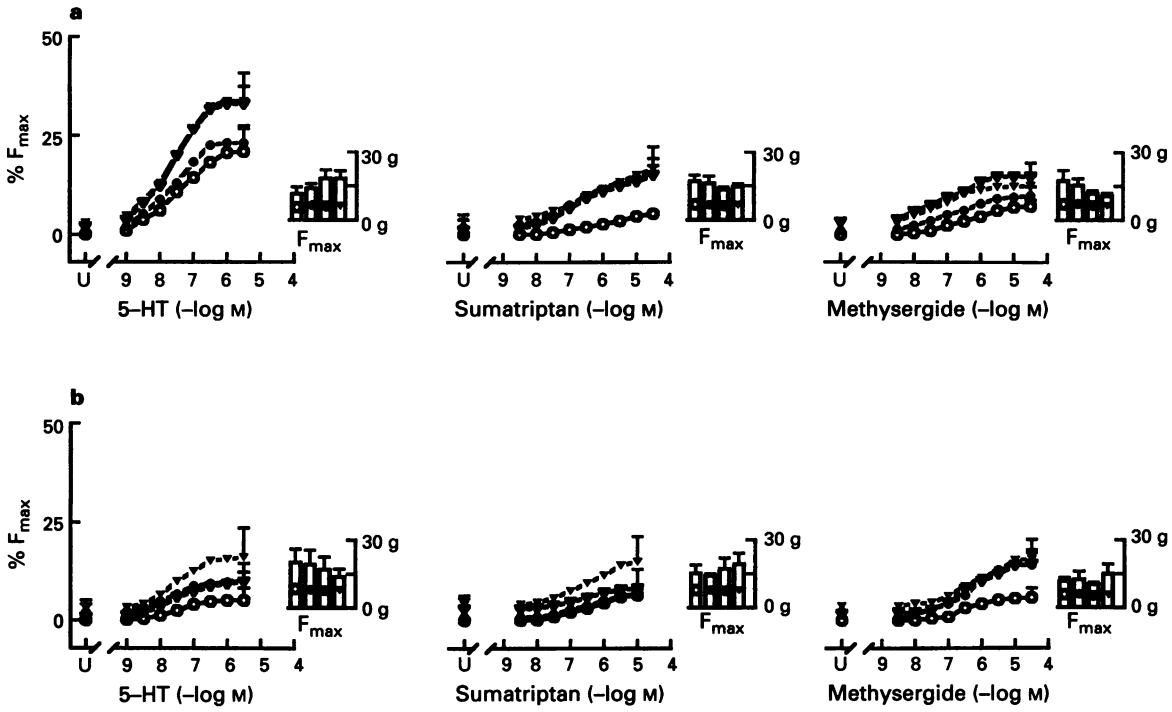

Figure 3 The effect of increasing resting active force with U46619 (U) at concentrations of 0 (○), 0.1 (●), 0.3 (▽) and 1.0 (▼) nM on the contraction curves to 5-HT, sumatriptan and methysergide in endothelium-denuded rings of dog, isolated coronary artery in the absence (a, upper panels, $n=5$), and presence (b, lower panels, $n=3$) of ketanserin (1 μM). Inserts represent the maximum contraction to KPSS (F_{max}) in the absence and presence of U46619. Error bars show s.e.mean.

sumatriptan were similar (65.8±3.4% and 66.3±6.3% F_{max} respectively) and double that of methysergide (32.3±9.7% F_{max}). Noradrenaline generated the greatest maximal response of 105.3±3.7% F_{max}. 5-HT was the most potent agonist in this tissue with a pEC_{50} value of 7.45±0.14. The pEC_{50} values for sumatriptan, methysergide and noradrenaline were 6.58±0.16, 6.08±0.18 and 6.69±0.22 respectively. U46619 at concentrations of 0.1 and 0.3 nM induced relatively small amounts of active force 0.2±0.1% (22 rings) and 0.7±0.5% (21 rings) F_{max}, whereas 1.0 nM U46619 caused a contraction of 17.4±4.1% (21 rings) F_{max}. In the presence of U46619, the agonist responses were not potentiated and the contraction curves were only raised above the tonic contraction to U46619 (Figure 4).

Functional antagonism with SNP SNP (10 μM) caused small, maintained relaxations that were taken as evidence for the presence of basal, active force (Figure 5). SNP (1–10 μM) functionally antagonized the contraction curves to 5-HT, sumatriptan and methysergide in a concentration-dependent manner, however, for clarity only the effect of 10 μM SNP is shown. In the presence of SNP and ketanserin (1 μM), the contraction curve to 5-HT was functionally antagonized such that the maximal response was reduced by 68.9% ($P<0.05$) without a signifiant change in pEC_{50} value (Table 2, Figures 5 and 6). The contraction curves to sumatriptan and methysergide were abolished by SNP (Figures 5 and 6). In comparison, SNP (10 μM) failed to antagonize functionally the contractile response curve to noradrenaline (Table 2, Figure 6). At 30 μM, however, SNP caused a significant ($P<0.05$) 10 fold rightward shift in the contraction curve to noradrenaline without depressing the maximum (Table 2).

In the presence of SNP (10 μM), U46619 in concentrations ranging from 1 to 4 nM, returned basal levels of active force to values the same or slightly greater than those prior to addition

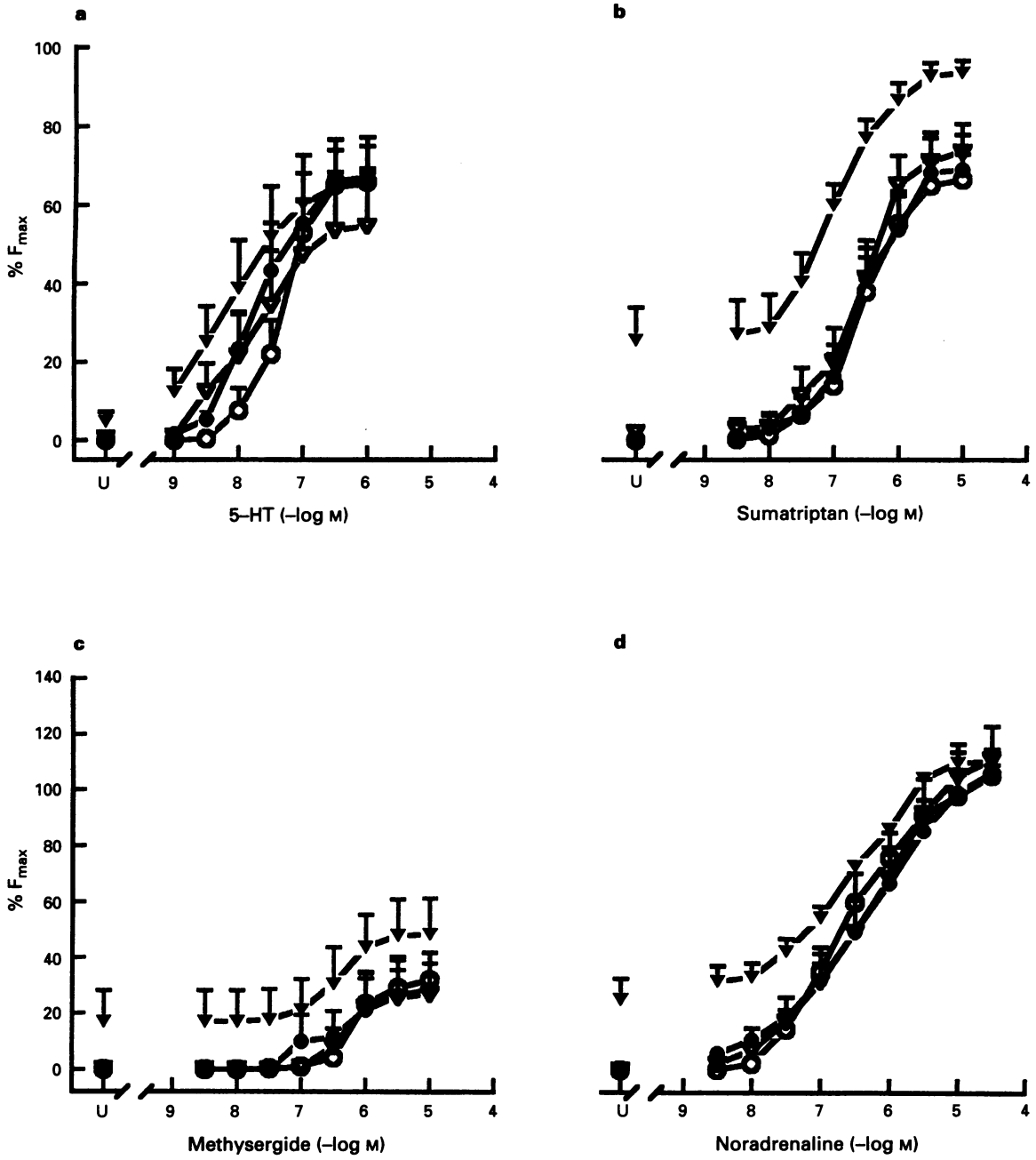

Figure 4 The effect of increasing resting active force with U46619 (U) at concentrations of 0 (○), 0.1 (●), 0.3 (▽) and 1.0 (▼) nM on the contraction curves to 5-HT (a, $n=6$), sumatriptan (b, $n=6$), methysergide (c, $n=5$) and noradrenaline (d, $n=4-5$) in dog isolated saphenous vein. Contraction curves to 5-HT, sumatriptan and methysergide were constructed in the presence of ketanserin (1 μM) and noradrenaline curves in the presence of propranolol (1 μM). Values are mean ± s.e.mean.

of SNP (Figure 5). Under these conditions, the maximal responses to 5-HT and sumatriptan and methysergide were no longer depressed and did not differ significantly from control values (Table 2, Figures 5 and 6). In the presence of 30 μM SNP, returning tone with U46619 did not alter the maximum response or sensitivity to noradrenaline (Table 2).

Discussion

This study was undertaken to investigate possible mechanisms which underlie the phenomenon of U46619-mediated enhanced reactivity to agonists such as 5-HT and sumatriptan, previously observed in the human coronary artery *in vitro* (see Cocks *et al.*, 1993). Initially, the dog coronary artery was considered comparable to the human coronary artery since it has a similar 5-HT receptor profile, containing a mixture of 5-HT$_2$ and 5-HT$_1$-like receptors on the smooth muscle which mediate contraction (see Connor *et al.*, 1989; Chester *et al.*, 1990; Cocks *et al.*, 1993) although the contraction is mediated predominantly via 5-HT$_2$ receptors. Thus, Frenken & Kaumann (1985) have shown that 5-HT contracts dog coronary arteries via ketanserin-sensitive and insensitive receptors. Also Cohen (1986) observed that ketanserin-resistant contractions to 5-HT were blocked by methiothepin, a non-selective 5-HT$_1$-like receptor antagonist. In the present study the maximum contraction to 5-HT in the absence of endothelium was reduced by approximately 90% by ketanserin without alteration to the pEC$_{50}$. Thus, contractions to 5-HT in the dog coronary artery are mediated predominantly by 5-HT$_2$ receptors with only approximately 10% of the response due to activation of 5-HT$_1$-like receptors. Sumatriptan, however, contracted dog

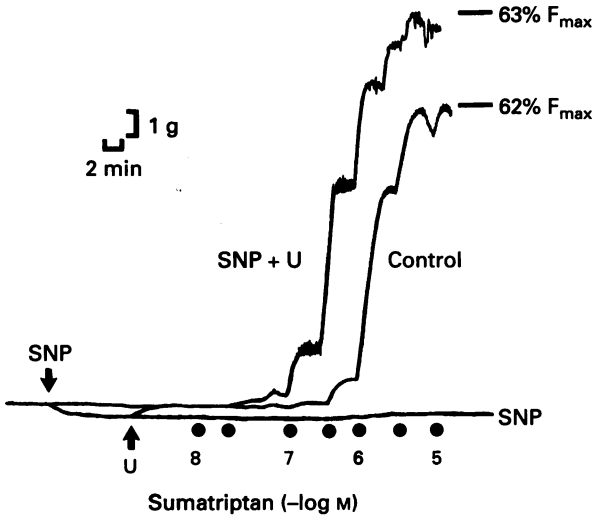

Figure 5 Tracings of original chart recordings showing isometric contractions to sumatriptan in three separate rings of dog saphenous vein. Control: (untreated); SNP: treated with sodium nitroprusside (SNP; 10 μM); SNP+U: treated with SNP and then U46619 (1.6 nM) to restore active force to approximately the pre-SNP level. Note that SNP alone abolished all contractions to sumatriptan and that the control and (SNP+U) responses are similar. % F_{max}: maximum contractions to sumatriptan expressed as a % of the maximum contraction to KPSS. Ketanserin (1 μM) was present throughout.

coronary arteries less sensitively but to the same maximum as 5-HT in vessel segments denuded of endothelium and treated with ketanserin. This indicates that sumatriptan acts as a full agonist relative to 5-HT at 5-HT$_1$-like receptors in the dog coronary artery, similar to the dog saphenous vein (Humphrey *et al.*, 1988), where nearly all the contraction to 5-HT is mediated by activation of 5-HT$_1$-like receptors (Apperley *et al.*, 1980; Feniuk *et al.*, 1985; Paiva *et al.*, 1988). Unlike the human isolated coronary artery (Cocks *et al.*, 1993), however, increasing the level of active force in both the dog coronary artery and saphenous vein with U46619 did not increase either the sensitivity or maximal response to sumatriptan. Instead, the contraction curve was raised in parallel above the new resting level of active force induced by U46619. When the experimental conditions were optimized by removing the endothelium as well as in the presence of ketanserin, there was still no enhancement by U46619 of the contractile response to sumatriptan. Similar results were obtained for 5-HT and methysergide. Therefore, in vessels which contract either poorly (dog coronary artery) or maximally (dog saphenous vein) to sumatriptan, contractions to full agonists (5-HT, sumatriptan and methysergide) at 5-HT$_1$-like receptors in the dog coronary artery and full (5-HT and sumatriptan) and partial (methysergide) 5-HT$_1$-like agonists in the dog saphenous vein, could not be potentiated by further increasing the level of basal active force. One difference between our earlier study using U46619 and 5-HT agonists in the human coronary artery where potentiation appeared to occur (Cocks *et al.*, 1993) and the present study was that the human coronary arteries were obtained from patients in end-stage heart disease or from unused donor hearts that were removed from donor patients during heart-lung bypass. Consequently, as a result of either this procedure, the disease or the drugs used to treat both recipient and donor patients, reactivity in coronary arteries removed from these human hearts may have already been reduced beyond a critical level. The data from the functional antagonism experiments in the dog saphenous vein, tentatively supports such a possibility. Here, as in the dog coronary artery, U46619 failed to potentiate contractions to sumatriptan unless resting active force was first reduced with SNP. Under these conditions, the pattern of the shifts in the contraction curves to both sumatriptan and 5-HT were remarkably similar to those for the same agonists in the human coronary artery (see Cocks *et al.*, 1993). Therefore, if U46619 acted simply to overcome any functional antagonism of vasoconstriction in both tissues, then the U46619-potentiated curves in the human coronary artery may be a fairer representation of true reactivity to both sumatriptan and 5-HT in that tissue. If so, then contractions to 5-HT in the human coronary artery are likely to be mediated equally by activation of 5-HT$_1$-like and 5-HT$_2$ receptors, since in that tissue the U46619-potentiated maximum response to sumatriptan was approximately 50% that to 5-HT in the absence of ketanserin (Cocks *et al.*, 1993). Findings by Kaumann *et al.* (1994) generally support this conclusion although they also showed that in many cases contractions to 5-HT were mediated predominantly by 5-HT$_1$-

Table 2 pEC_{50} values and maximum responses to 5-HT, sumatriptan, methysergide and noradrenaline in dog isolated saphenous vein in the absence and presence of sodium nitroprusside (SNP) and in the presence of U46619 to return basal levels of force

Agonist	*[SNP]* (μM)	n	*pEC_{50} value* (-log M)	*Maximum response* (% F_{max})	Δ *U46619* (% F_{max})
5-HT	0	6	7.37±0.16	68.1±4.6	-
	10		6.89±0.15	21.2±3.3**	-
+U46619	10		7.79±0.10	56.6±3.2	10.5±3.5
Sumatriptan	0	4	6.70±0.12	61.1±5.6	-
	10		†	1.2±0.5**	-
+U46619	10		6.69±0.08	66.3±6.8	8.1±4.9
Methysergide	0	4	6.11±0.22	39.5±9.7	-
	10		†	0.9±0.6*	-
+U46619	10		6.62±0.14	28.3±3.3	6.0±6.0
Noradrenaline	0	4	6.84±0.25	105.7±8.5	-
	10		6.27±0.07	114.4±8.1	-
	30		5.85±0.16**	107.2±4.7	-
+U46619	10		6.56±0.11	106.9±7.0	6.4±2.6
	30		6.44±0.12	110.7±1.8	4.2±1.8

Ketanserin (1 μM) was present throughout, with the exception of noradrenaline contraction curves which were constructed in the presence of propranolol (1 μM).
n=number of rings; Values are given as mean ± s.e.mean
*Mean significantly different from control ($P<0.05$, Dunnett's test); **mean significantly different from control ($P<0.001$, Dunnett's test).
†pEC_{50} value was not calculated as the maximum contraction to the agonist was less than 10% F_{max}.
ΔU46619: Increase in force to U46619 expressed as a % of the contraction to KPSS (F_{max}).

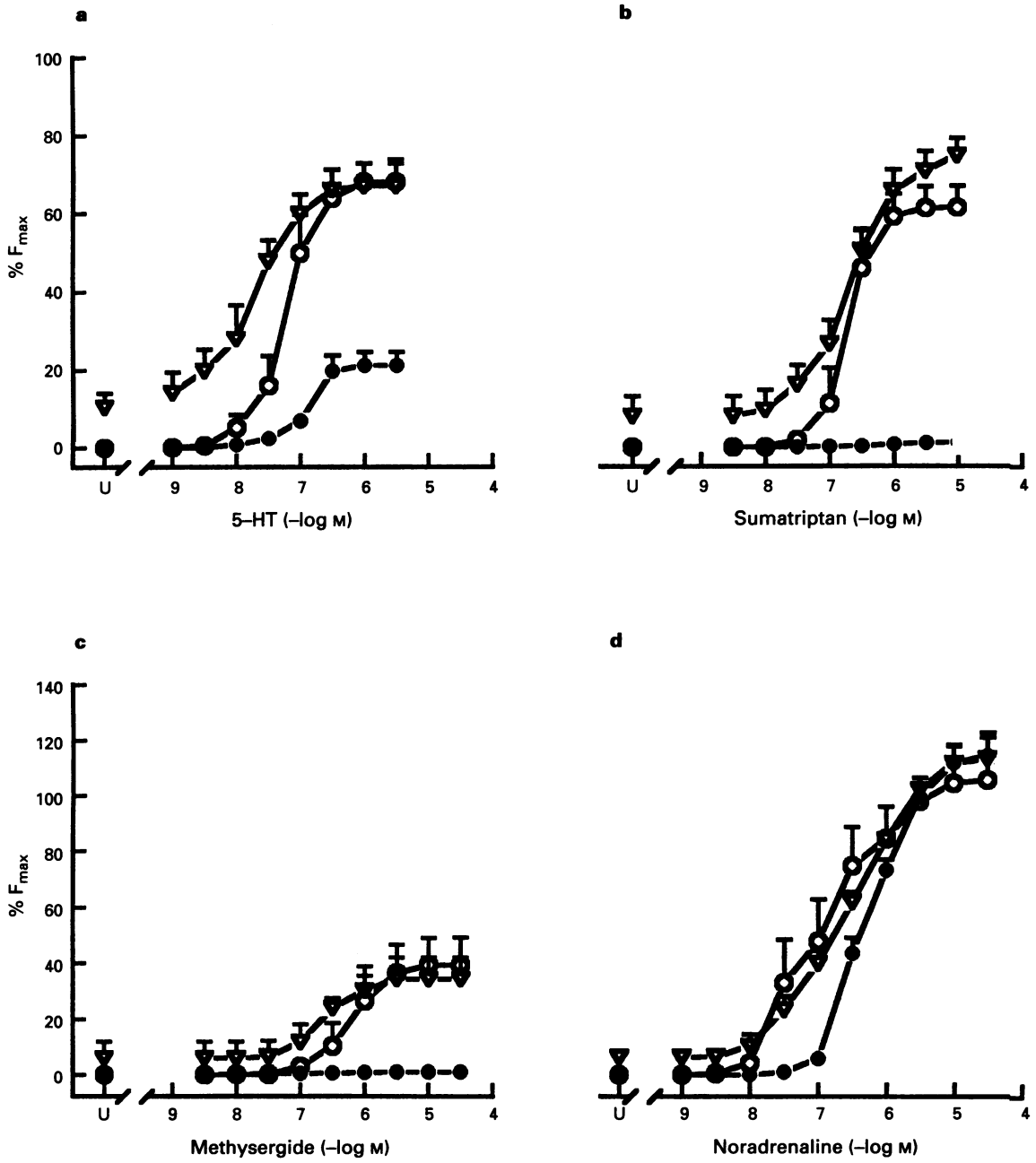

Figure 6 The effect of 10 μM sodium nitroprusside (SNP) on the contraction curves to (a) 5-HT, (b) sumatriptan, (c) methysergide and (d) noradrenaline in dog isolated saphenous vein and its reversal with titrated concentrations of U46619 (U: 1–4 nM) to restore active force to levels similar to those prior to addition of SNP (n=4–6). (○) Controls; (●) SNP; (▽) SNP plus U46619. Contraction curves to 5-HT, sumatriptan and methysergide were constructed in the presence of ketanserin (1 μM) and those to noradrenaline in the presence of propranolol (1μM). Values are mean ± s.e.mean.

like receptors, not 5-HT$_2$ receptors. These findings have important clinical implications considering the possible involvement of 5-HT and other platelet-derived autocoids in coronary vasospasm (Golino & Maseri, 1994) and the use of selective and potent 5-HT$_1$-like agonists (e.g. sumatriptan) for the treatment of migraine (Humphrey & Feniuk, 1991).

MacLennan & Martin (1992) also reported U46619-mediated enhanced reactivity to 5-HT via 5-HT$_1$-like receptors in the rabbit isolated femoral artery with a concentration of U46619 that caused approximately 50% of the maximum contraction to KCl-depolarization. Although they did not show any concentration-dependent effects of U46619 on reactivity to 5-HT, it may have been similar to our findings here in the dog coronary artery and saphenous vein in that beyond a particular level of active force, no further potentiation can occur.

The ability of a receptor-linked agonist to overcome functional antagonism depends on its receptor density, affinity of the agonist for the receptor as well as the efficiency and type of signal transduction system involved. Thus, in the present study, SNP completely abolished the contraction curves to sumatriptan and methysergide, whilst only suppressing that to the 7 fold more potent 5-HT$_1$-like receptor agonist, 5-HT (in the presence of ketanserin) by approximately 70%. By contrast, the same concentration of SNP had no effect on the contraction curve to noradrenaline, the most efficient contracting agent in this tissue (see also He *et al.*, 1988).

In vivo, arteries are subjected to numerous receptor-linked vasoactive modulators, the balance of which is partial vasoconstriction and vascular tone. Thus, the vasculature is sensitively set to constrict further or dilate, thereby allowing precise regulation of blood pressure and flow. In endotoxic circulatory

shock, this control is lost, due predominantly to excess vasodilator stimulation. This is analogous to the *in vitro* paradigm with SNP-induced functional antagonism reported here since endotoxic shock is believed to be largely due to over-production of nitric oxide (see Moncada *et al.*, 1991). Thus, our data may indicate that combination of at least two vasoconstrictors that act at different receptors with high affinity and efficient coupling to either similar or different signal transduction systems could be more efficacious than single or sequential drug therapy for the treatment of endotoxic shock.

In conclusion, the dog saphenous vein may be a useful model of how 5-HT$_1$-like agonists like sumatriptan contract human coronary arteries and thus how contractility can be artificially masked by functional antagonism.

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