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Influence of the endothelium and nitric oxide on the contractile responses evoked by 5-HT_{1D} receptor agonists in the rabbit isolated saphenous vein

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1 We investigated whether contractile responses evoked by 5-HT_{1D} receptor agonists were influenced by the endothelium (E) and nitric oxide (NO) in the rabbit isolated saphenous vein.

2 Saphenous vein rings were set up for isometric tension recording in oxygenated (5% CO_2 in O_2) Krebs solution (pH 7.4) containing (10^{-6} M): idazoxan (1), indomethacin (10), ketanserin (0.1), prazosin (10), and N^{ω} nitro-L-arginine methyl ester (L-NAME; 0 or 10), a NO synthase inhibitor. In some experiments, the E was removed mechanically.

3 5-Hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT) and sumatriptan (Sum) contracted rabbit saphenous vein rings in the potency order (pD_2 range) of 5-CT(7.2-7.6)>5-HT(6.2-7.1)>Sum(5.0-5.8), irrespective of the presence or absence of the E or L-NAME ($n=9-37$ per group) indicating that the potencies of the 3 agonists were not significantly affected by either the E or L-NAME.

4 Efficacy, as assessed by the maximal contractile response (E_{max}), was significantly greater for Sum compared to 5-HT and 5-CT with intact E irrespective of the presence (77 ± 3, 62 ± 3, and 50 ± 3 mN respectively; $P<0.05$ Sum versus 5-HT and 5-CT) or absence (26 ± 3, 14 ± 4, and 13 ± 2 mN respectively; $P<0.05$ Sum versus 5-HT and 5-CT) of L-NAME. In E-denuded rings, the E_{max} values were all higher than in E-intact rings and did not differ between the 3 agonists (36 ± 4, 37 ± 4, and 36 ± 5 mN for Sum, 5-HT and 5-CT, respectively; $P>0.5$ between the 3 agonists) indicating that an endothelium-derived relaxing factor (EDRF) counteracted the constrictor activities of the 5-HT_{1D} receptor agonists and raising the possibility that a component of the Sum-induced contractile responses was E-dependent. Without E, the presence of L-NAME did not significantly affect the E_{max} values of the 3 agonists (41 ± 4, 41 ± 5, and 41 ± 4 mN for Sum, 5-HT, and 5-CT respectively; $P>0.5$ between the 3 agonists) indicating that the NO synthase inhibited was of endothelial origin.

5 Potentiation of the E_{max} of the 3 agonists by L-NAME was significantly albeit partially reversed by L-arginine (10^{-2} M) indicating that NO synthase was indeed inhibited by L-NAME. Furthermore, in the presence of E, potentiation of E_{max} of the 3 agonists by L-NAME was mimicked by methylene blue (10^{-5} M) providing further evidence that NO was involved in the attenuation by the E of the contractile responses induced by the 5-HT_{1D} receptor agonists.

6 In the presence of an intact E and L-NAME, contractile responses elicited by 5-HT and Sum were competitively antagonized by the non-selective 5-HT_{1D} receptor antagonist, methiothepin (pA_2: 9.4 and 8.8; slopes: 0.66 and 0.81, respectively) and the highly selective 5-HT_{1D} receptor antagonist, GR 127935 (pA_2: 9.0 in each case; slopes: 1.04 and 0.93, respectively) indicating that contractions were mediated through activation of a single population of 5-HT_{1D} receptors. Contractile responses elicited by 5-CT were also competitively antagonized by methiothepin and GR 127935, but non parallel rightward shifts of the concentration-response curves were observed suggestive of the involvement of additional but as yet unidentified receptors in mediating the 5-CT-induced responses.

7 In conclusion, the efficacy, but not the potency, of 5-HT, 5-CT and Sum in evoking 5-HT_{1D} receptor-mediated contractile responses are subject to a substantial inhibitory influence of the E and of an EDRF (probably NO).

Keywords: Sumatriptan; 5-carboxamidotryptamine; 5-hydroxytryptamine; 5-HT_{1D} receptor; nitric oxide; rabbit saphenous vein

Introduction

Our understanding of the physiological and pharmacological significance of vasoconstrictor 5-HT_{1D}, previously 5-HT_1-like, receptors has progressed with the identification and development of sumatriptan (Sum), a selective agonist for 5-HT_{1D} receptors (Humphrey *et al.*, 1988), which has demonstrated efficacy as an anti-migraine agent (Subcutaneous Sumatriptan International Study Group, 1991). According to the vascular hypothesis of migraine, the therapeutic efficacy of Sum may be due to constriction of cerebral blood vessels following activation of post-junctional 5-HT_{1D} receptors (Humphrey & Feniuk, 1991; Ferrari & Saxena, 1993; Medhurst *et al.*, 1993). 5-HT_{1D} receptors mediate vasoconstriction of both arterial and venous vessels from a variety of species including human, bovine, canine and rabbit (for a review, see Hoyer *et al.*, 1994).

Vascular endothelial cells are known to synthesize nitric oxide (NO), a potent vasodilator (Palmer *et al.*, 1988; Moncada & Higgs, 1993). Evidence for an inhibitory role of the endothelium (E) and NO in the constrictor responses to Sum has recently been proposed *in vitro* in isolated pulmonary arteries (MacLean *et al.*, 1994; McCulloch *et al.*, 1994; Sweeney *et al.*, 1995) and *in vivo* in the renal vasculature of the anesthetized dog (Whiting & Cambridge, 1995). These results raised the possibility that 5-HT_{1D} receptor-mediated contractile responses could be influenced by the E and NO.

The rabbit isolated saphenous vein is a widely employed experimental model to characterize the pharmacological

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properties of 5-HT$_{1D}$ receptor agonists, in particular in determining their potency and efficacy (Martin & MacLennan, 1990; Van Heuven-Nolsen *et al.*, 1990; Razzaque *et al.*, 1995). To our knowledge, no information is currently available regarding the influence of the E and/or of NO on the 5-HT$_{1D}$ receptor-mediated constrictor responses in this experimental model.

The present study was therefore conducted to determine whether 5-HT$_{1D}$ receptor-mediated contractile responses in rabbit isolated saphenous vein preparations were influenced by the E and NO. Evidence was provided that 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT) and Sum-mediated contractile responses were mediated by 5-HT$_{1D}$ receptors in the present experiments using the non selective, and highly selective 5-HT$_{1D}$ receptor antagonists methiothepin and GR 127935, respectively (Clitherow *et al.*, 1994; Skingle *et al.*, 1994).

Methods

Male New Zealand white rabbits (ESD, France) weighing 2.2–3.1 kg were killed by an overdose of intravenous sodium pentobarbitone (Sanofi Laboratories, France). Right and left lateral saphenous veins were cleaned of surrounding adipose and connective tissue *in situ* under a binocular microscope. In some cases, the E was mechanically removed by inserting a catheter of approximately equal diameter into the vessel lumen. The veins were then excised and placed in cold oxygenated Krebs-bicarbonate buffer solution, and cut into 4 rings of approximately 5 mm in length. The buffer solution used for preparing the vascular rings and the organ bath studies had the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, D(+)-glucose 10. In adddition the solution contained (M): idazoxan (10^{-6}), indomethacin (10^{-5}), ketanserin (10^{-7}), prazosin (10^{-5}) and N^{ω}-nitro-L-arginine methyl ester (L-NAME; 0 or 10^{-5}). Each ring was suspended between two stainless steel wire hooks and mounted in an organ bath filled with 20 ml Krebs-bicarbonate solution maintained at 37°C and continuously gassed with 95% O_2 and 5% CO_2. Changes in isometric force were measured by means of a transducer (Statham) connected to an amplifier (Gould Instruments, France) and a computerized data acquisition system (Acq*Knowledge*, BIOPAC Systems Inc., Goleta, CA, U.S.A). Following tension adjustments for stress relaxation and a 15 min stabilization period, tissues were successively challenged with a submaximal concentration of KCl (50 mM) and 5-HT (10^{-6} M) to assess the functional integrity of the rings. Vessels which failed to produce contractions in response to both KCl and 5-HT were excluded. The integrity of the E was assessed functionally by measuring the extent of E-dependent relaxation following application of acetylcholine (ACh, 10^{-6} M) during the tonic component of contraction evoked by 5-HT.

Agonist potency and efficacy

After recovery from these initial challenges, cumulative concentration-effect curves to 5-HT, 5-CT (both 10^{-9} to 3.2 10^{-5} M) or Sum (10^{-9} to 10^{-4} M) were constructed in the presence or absence of the E and/or L-NAME. One concentration-effect curve was carried out per ring. In additional experiments, performed in the presence of a functional E, cumulative concentration-effect curves for the three agonists were constructed in the presence of either L-NAME plus L-arginine (10^{-2} M) or methylene blue (10^{-5} M).

Antagonism studies

Tissues were exposed to methiothepin (10^{-9} to 10^{-7} M) or GR 127935 (10^{-9} to 3.2×10^{-8} M) for 15 min, starting after washout from the initial 5-HT challenge, and before the con-

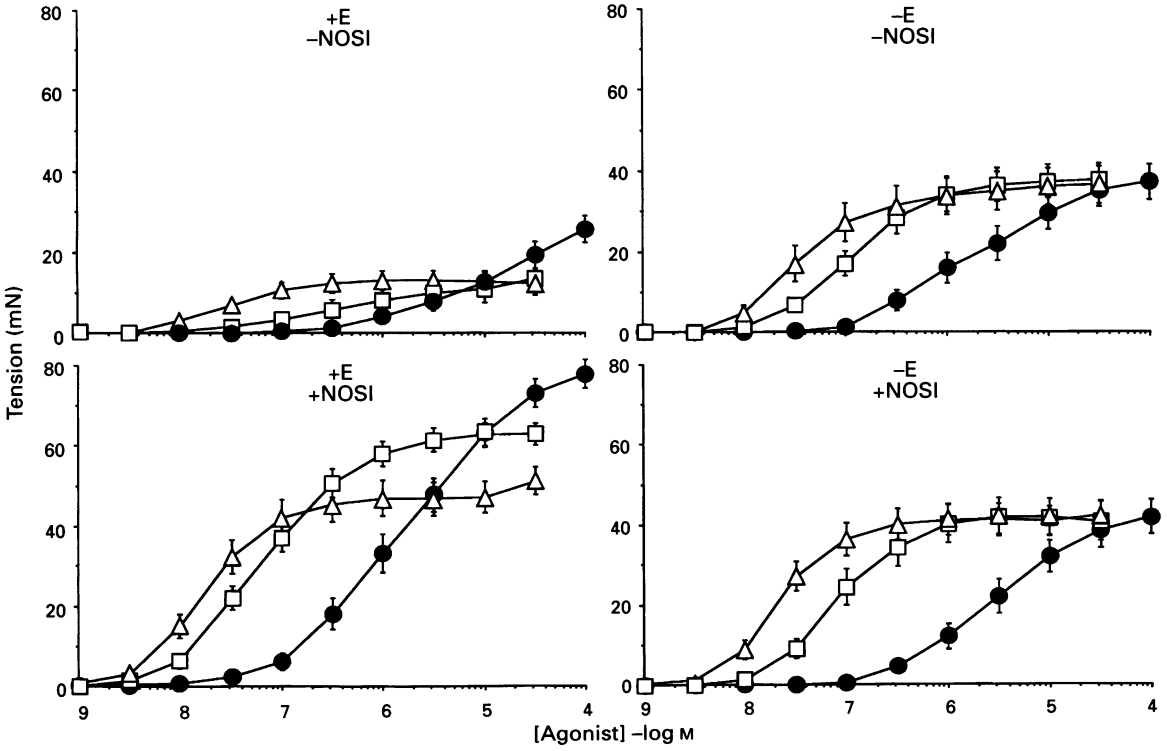

Figure 1 Influence of the endothelium (E) and of nitric oxide synthesis inhibition (NOSI), as obtained with L-NAME (10^{-5} M), on the contractile responses of rabbit saphenous vein rings evoked by 5-HT (□), 5-CT (△) and Sum (●). All 3 agonists contracted rabbit saphenous vein rings in the potency order of 5-CT > 5-HT > Sum, irrespective of the presence or absence of the E or NOSI. Significant differences in efficacy, as assessed by the maximal contractile response, were found in the presence of the E irrespective of the presence or absence of NOSI. Values are mean ± s.e.mean of 9 to 37 rings.

struction of concentration-effect curves to the 5-HT$_{1D}$ receptor agonists. Concentration-effect curves to 5-HT, 5-CT (both 10^{-9} to 10^{-4} M) or Sum (10^{-9} to 10^{-3} M) were constructed in the presence of the E and L-NAME and of either methiothepin or GR 127935.

Drugs

The following drugs were used: 5-hydroxytryptamine creatinine sulphate, idazoxan hydrochloride, indomethacin, L-arginine hydrochloride, methylene blue trihydrate, N$^{\omega}$-nitro-L-arginine methyl ester, and prazosin hydrochloride (all from Sigma Chemicals, St Louis, MO, U.S.A.), ketanserin tartrate, and methiothepin mesylate (Research Biochemicals Inc., Natick, MA, U.S.A.), 5-carboxamidotryptamine hydrochloride, and sumatriptan hydrochloride (Department of Analytical Chemistry, Centre de Recherche Pierre Fabre, Castres, France), GR 127935 hydrochloride (N-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2(-methy-4(5-methyl-1,2,4)-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide). (Division of Medicinal Chemistry IV, Centre de Recherche Pierre Fabre, Castres, France).

Calculations, logistic curve fitting and statistical analysis

Data are expressed as means ± s.e.mean for n observations. One way analysis of variance followed by Dunnett's test was used to assess significance between groups (StatView, Abacus Concepts Inc., Berkeley, CA, U.S.A.). Concentration-response curve fitting was performed using the non-linear least-square algorithm of Marquardt (1963). $pD_2 = -\log EC_{50}$ where EC_{50} refers to the geometric mean agonist concentration (with 95% confidence intervals in parentheses) inducing 50% of its maximal effect. The Hill coefficient (n_H) refers to the slope of the linear portion of a concentration-response curve. The antagonist potencies of methiothepin and GR 127935 against 5-HT and Sum were calculated according to the method of Arunlakshana & Schild (1959) using EC_{50} values obtained in the presence and absence of antagonists to give pA_2 values. The slope of the Schild regression was also determined. $P = 0.05$ was considered the minimum level of significance.

Results

Contractile responses evoked by 5-HT, 5-CT and Sum in control conditions

In the absence of nitric oxide synthase inhibition (NOSI) and in the presence of a functional E, the 3 agonists contracted rabbit isolated saphenous vein rings in the following rank order of potency (range of pD_2 values): 5-CT (7.3–7.5) > 5-HT (5.9–6.5) > Sum (4.8–5.1; Figure 1, Table 1). The maximum developed tension (E_{max}) was statistically significantly greater for Sum compared to both 5-HT and 5-CT (Table 1). We next investigated whether the E exerted an inhibitory influence on the contractile responses evoked by 5-HT$_{1D}$ agonists.

Influence of the E on the contractile responses evoked by 5-HT$_{1D}$ agonists

Successful removal of the E was confirmed functionally by the statistically significant reduction of the E-dependent relaxation to 10^{-6} M ACh (-3 ± 2 versus $-45 \pm 6\%$ in E-denuded and intact rings, respectively; $n = 45$ and 36 rings respectively; $P < 0.05$). In the absence of NOSI, the 3 agonists contracted rabbit saphenous vein rings with the same rank order of potency observed in the presence of a functional E (i.e. 5-CT (7.1–7.3) > 5-HT (6.7–6.9) > Sum (5.5–5.8); Figure 1, Table 1). Mechanical removal of the E led to a statistically significant increase in E_{max} for the 3 agonists, but the differences in E_{max} between 5-HT, 5-CT and Sum were abolished (Table 1). We

Table 1 Potencies and efficacies of 5-HD$_{1D}$ receptor agonists in contracting rabbit isolated saphenous vein rings

Experimental conditions			*5-Hydroxytryptamine*				*5-Carboxyamidotryptamine*				*Sumatriptan*				*Comparison of E_{max}*		
E	*L-NAME*	n	pD_2 (95% CL)	n_H (95% CL)	E_{max}(mN) ± s.e. mean	n	pD_2 (95% CL)	n_H (95% CL)	E_{max}(mN) ± s.e. mean	n	pD_2 (95% CL)	n_H (95% CL)	E_{max}(mN) ± s.e. mean	5-HT vs 5-CT	5-HT vs Sum	5-CT vs Sum
+	–	9	6.2 (5.9–6.5)	0.74 (0.72–0.76)	14 ± 4	12	7.4 (7.3–7.5)	0.99 (0.87–1.08)	13 ± 2	17	5.0 (4.8–5.1)	1.00 (0.92–1.14)	26[§] ± 3	NS	*	*
–	–	20	6.8 (6.7–6.9)	1.09 (1.07–1.11)	37[a,b] ± 4	19	7.2 (7.1–7.3)	0.98 (0.88–1.12)	36[a,b] ± 5	16	5.6 (5.5–5.8)	0.89 (0.86–0.97)	36[a,b] ± 4	NS	NS	NS
+	+	37	7.1 (7.0–7.2)	0.91 (0.90–0.94)	62[a] ± 3	25	7.5 (7.4–7.6)	0.79 (0.69–0.91)	50[a] ± 3	24	5.8 (5.7–5.9)	0.83 (0.81–0.87)	77[a] ± 3	*	*	*
–	+	18	7.0 (6.9–7.1)	1.26 (1.25–1.27)	41[a,b] ± 5	13	7.6 (7.5–7.7)	1.23 (1.13–1.33)	41[a] ± 4	14	5.5 (5.4–5.6)	0.99 (0.96–1.04)	41[a,b] ± 4	NS	NS	NS

n, number of rings; E, endothelium; L-NAME, N$^{\omega}$-nitro-L-arginine methyl ester; $pD_2 = -\log EC_{50}$ where EC_{50} refers to the geometric mean agonist concentration inducing 50% of its maximal effect; n_H, Hill coefficient; CL, confidence limits; E_{max}, maximal contractile response. *$P < 0.05$ comparison of E_{max} between agonists. Comparison of E_{max} between treatments: [a], $P < 0.05$ versus + E-L-NAME; [b], $P < 0.05$ – E-L-NAME or – E + L-NAME versus + E + L-NAME. All other comparisons did not reach statistical significance (NS). [§]pD_2 and E_{max} values were calculated by considering the tension induced by 0.1 mM sumatriptan as the maximum.

next determined whether contractile responses evoked by 5-HT$_{1D}$ agonists were similarly influenced by NO.

Influence of NOSI on the contractile responses evoked by 5-HT$_{1D}$ agonists

During NOSI, all 3 agonists contracted rabbit saphenous vein rings with the same rank order of potency as observed in the absence of NOSI, and irrespective of the presence or absence of E (i.e. 5-CT > 5-HT > Sum; Figure 1, Table 1). In the presence of a functional E, NOSI was associated with a statistically significant increase in E_{max} in the same proportion (3 to 4 fold) for the 3 agonists, the E_{max} for Sum again being statistically significantly greater than that produced by both 5-HT and 5-CT (Table 1). In contrast, in the absence of a functional E, NOSI did not alter the E_{max} for the 3 agonists (Table 1). Furthermore, removal of the E, in the presence of NOSI, was associated with lower E_{max} values for Sum and 5-HT but not for 5-CT. To verify that the effects of L-NAME were mediated by NOSI, we determined whether they could be (i) reversed by L-arginine and (ii) mimicked by methylene blue.

Influence of L-arginine on the contractile responses evoked by 5-HT$_{1D}$ agonists in the presence of L-NAME

Addition of a 1000 fold higher concentration of L-arginine (10^{-2} M), compared to L-NAME, in the Krebs solution significantly but only partially reversed the enhancement of the E_{max} for the 3 agonists (Figure 2). The E_{max} represented, in these conditions, 36±8, 44±5 and 67±6% for 5-HT, 5-CT and Sum respectively (all $P<0.05$ versus NOSI alone), of the E_{max} attained in the presence of NOSI alone. In the presence of L-arginine, the 3 agonists contracted rabbit saphenous vein rings in the same rank order of potency (i.e. 5-CT > 5-HT > Sum) as that observed in the absence of L-arginine (i.e. +E+NOSI), and pD_2 values were within the same range as those observed in the presence of E without NOSI.

Influence of methylene blue on the contractile responses evoked by 5-HT$_{1D}$ agonists in the absence of L-NAME

Methylene blue (10^{-5} M) enhanced (by 3–4 fold) the maximal contractions evoked by the three 5-HT$_{1D}$ agonists in E-intact rabbit saphenous vein rings in the absence of L-NAME (Figure 2). Under these conditions, the E_{max} represented 48±9, 60±17 and 86±6% for 5-HT, 5-CT and Sum respectively, of the E_{max} achieved in the presence of NOSI compared to 12±3, 17±4 and 26±10% in the absence of methylene blue (all $P<0.05$ versus absence of NOSI; Figure 2). In the presence of methylene blue, the 3 agonists contracted rabbit saphenous vein rings in the same rank order of potency (i.e. 5-CT > 5-HT > Sum) and range of pD_2 values as those observed in the absence of methylene blue (i.e. +E−NOSI). We next investigated whether the contractile responses induced by 5-HT$_{1D}$ agonists were mediated by 5-HT$_{1D}$ receptors.

Antagonism of the contractile responses evoked by 5-HT$_{1D}$ agonists by methiothepin and GR 127935

Concentration-effect curves to 5-HT, 5-CT or Sum were constructed in the presence of the E and L-NAME and of either methiothepin or GR 127935. Methiothepin (10^{-9} to 10^{-7} M) inhibited 5-HT, 5-CT and Sum induced-contractions, causing a rightward displacement of the concentration-effect curves, without depressing the maximum responses, consistent with competitive antagonism (Figure 3). Methiothepin displaced the concentration-effect curves of 5-HT and Sum in a parallel, rightward manner whereas it elicited a non parallel rightward shift of the 5-CT curves at concentrations higher than 10^{-9} M. Schild regression analysis yielded pA_2 values of 9.4 (9.1–10.1), and 8.8 (8.7–8.9) for 5-HT, and Sum respectively. The slopes of the linear Schild regression were 0.66 (0.58–0.67) and 0.81 (0.76–0.87) for 5-HT and Sum, respectively. Similarly, GR 127935 (10^{-9} to 3.2 10^{-8} M) inhibited 5-HT, 5-CT and Sum-induced-contractions, causing a rightward displacement of the concentration-effect curves, without depressing the maximum responses, again consistent with competitive antagonism (Figure 4). As observed with methiothepin, GR 127935 displaced the concentration-effect curves of 5-HT and Sum in a parallel manner whereas it elicited a non parallel shift of the 5-CT curve at concentrations higher than 10^{-9} M. Schild regression analysis yielded pA_2 values of 9.0 (8.7–9.7), and 9.0 (8.9–9.1) for 5-HT, and Sum respectively. The slopes of the linear Schild regression were 1.04 (0.80–1.22) and 0.93 (0.80–1.10) for 5-HT and Sum, respectively.

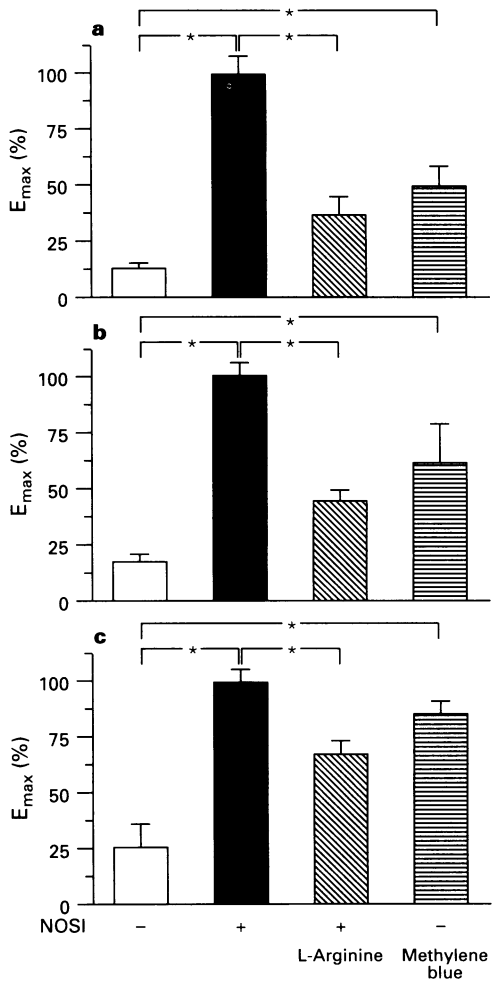

Figure 2 Influence of L-arginine (10^{-2} M) and methylene blue (10^{-5} M) on the maximal contractile responses of rabbit saphenous vein rings evoked by 5-HT (a), 5-CT (b) and Sum (c) in the presence of the endothelium. In presence of L-arginine, the maximal contractile response for each agonist was statistically significantly reduced as compared to control conditions (i.e., +NOSI; hatched columns). In the presence of methylene blue, the maximal contractile response for each agonist was enhanced as compared to control conditions (i.e., −NOSI; horizontally hatched columns). Values are mean±s.e.mean of 4–31 rings and are expressed as percentage of the maximal response induced by each agonist in the presence of NOSI.

Discussion

The present study was designed to assess whether contractile responses evoked by 5-HT$_{1D}$ receptor agonists were influenced by the E and NO in the rabbit isolated saphenous vein. The results indicate that the efficacy but not the potency of 5-HT,

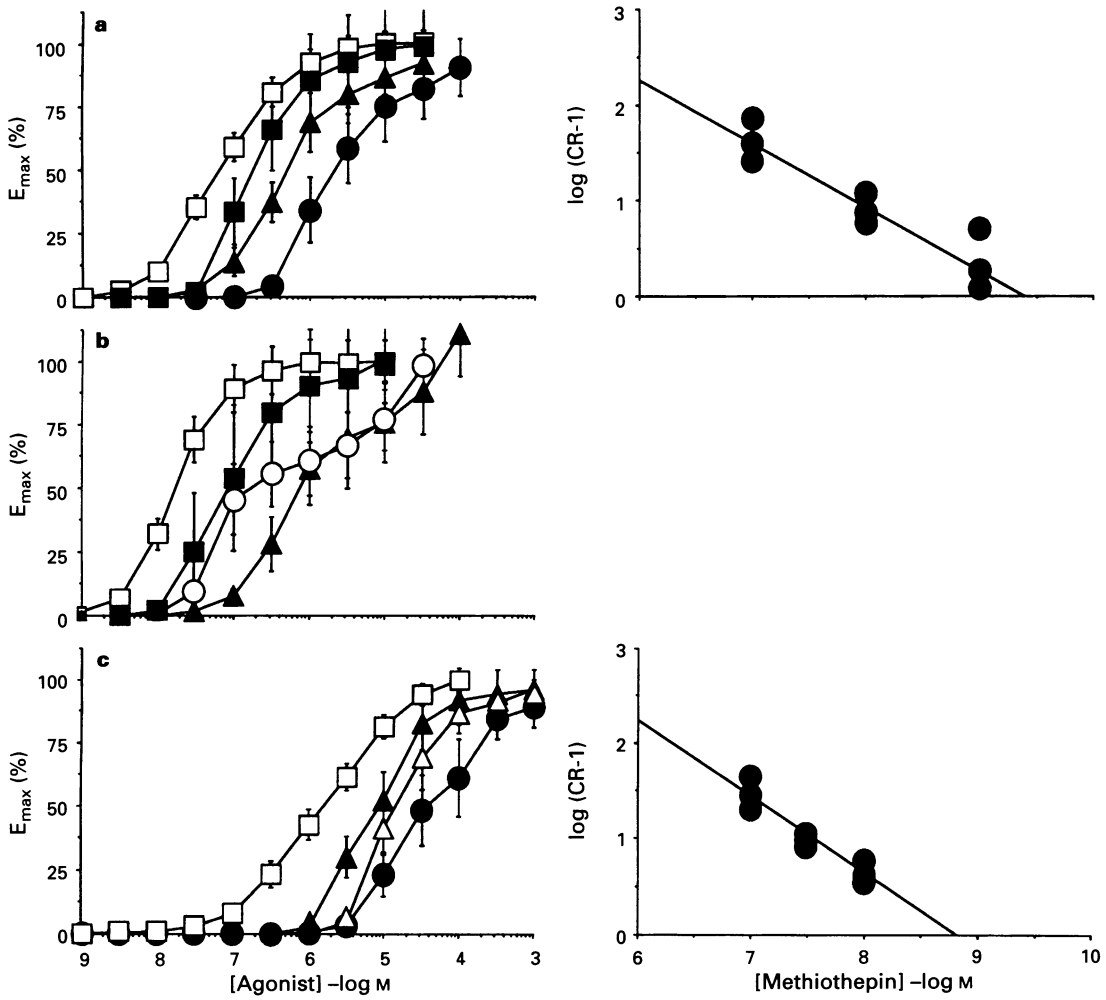

Figure 3 5-HT (a), 5-CT (b) and Sum (c)-induced contractions in rabbit isolated saphenous vein rings (left panels) in the absence (□) and presence of methiothepin at concentrations of 10^{-9} M (■), 3.2×10^{-9} M (○), 10^{-8} M (▲), 3.2×10^{-8} M (△) or 10^{-7} M (●). Values are mean ± s.e.mean of 24 to 38 and 5 to 12 rings in the absence and presence of methiothepin respectively. The right panels show the Schild regression plots for 5-HT and Sum derived from agonist concentration-ratios (CR) for each of the concentrations of methiothepin.

5-CT and Sum in evoking 5-HT$_{1D}$ receptor-mediated contractile responses are under an inhibitory control of the E and an endothelium-derived relaxing factor (EDRF).

Influence of E and NOSI on 5-HT$_{1D}$ receptor agonist potencies

The three agonists studied contracted rabbit isolated saphenous vein rings in the order of potency 5-CT > 5-HT > Sum, irrespective of the presence or absence of the E and/or NOSI. These results suggest that the potency and thus affinity of the 3 agonists at the 5-HT$_{1D}$ receptor is not altered by either the E or NOSI. Several investigators have reported the same range and order of agonist potencies at the 5-HT$_{1D}$-like receptor in rabbit (Martin & MacLennan, 1990; Van Heuven-Nolsen *et al.*, 1990; Razzaque *et al.*, 1995), dog (Feniuk *et al.*, 1985; Humphrey *et al.*, 1988), and human saphenous vein (Glusa & Müller-Schweinitzer, 1993) irrespective of the presence (Van Heuven-Nolsen *et al.*, 1990; Glusa & Müller-Schweinitzer, 1993) or absence (Martin & MacLennan, 1990) of a functional E.

5-HT$_{1D}$ receptor agonist efficacies

Under basal conditions (i.e. +E/−L-NAME) the magnitude of the contractile response evoked by 5-HT (i.e. 10–20 mN) was within the same range as that reported by Van Heuven-Nolsen *et al.* (1990) under comparable experimental conditions. In addition, the possibility of both α_1- and α_2-adrenoceptor-mediated contractions was excluded by the presence of prazosin (10^{-5} M) and idazoxan (10^{-6} M) respectively. In preliminary experiments, tyramine (10^{-5} M) produced no contractile responses under these conditions (data not shown) thus excluding a contribution from indirect sympathomimetic activity. Sum produced greater maximum responses than those evoked by 5-HT and 5-CT; these differences were observed irrespective of NOSI and were abolished by removal of the E. These results suggest that a component of the responses evoked by Sum, compared to those evoked by 5-HT and 5-CT, is dependent upon an intact E. Although we have no evidence to indicate the precise underlying mechanism, we are tempted to speculate that this could be linked to the release of an endothelium-derived contracting factor (EDCF). Situations associated with vascular endothelial cell dysfunction may result in increased responsiveness to 5-HT$_{1D}$ receptor agonists, in particular Sum.

Influence of E and NOSI on 5-HT$_{1D}$ receptor agonist efficacies

In the absence of NOSI, mechanical removal of the E led to a statistically significant increase of the maximal contractile responses for 5-CT, 5-HT and Sum and abolished the differences between them. These results raise the possibility that the con-

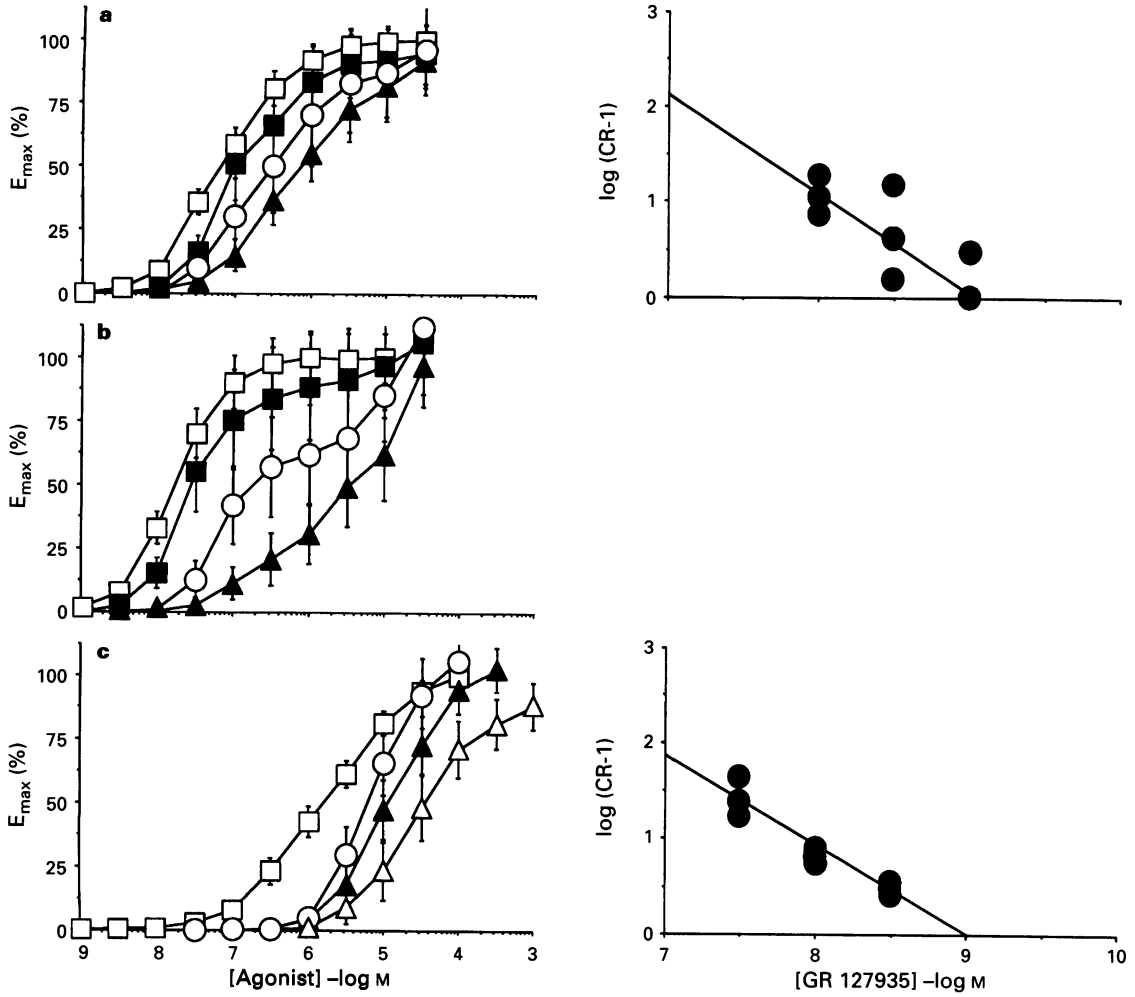

Figure 4 5-HT (a), 5-CT (b) and Sum (c)-induced contractions in rabbit isolated saphenous vein rings (left panels) in the absence (□) and presence of GR 127935 at concentrations of 10^{-9} M (■), 3.2×10^{-9} M (○), 10^{-8} M (▲), or 3.2×10^{-8} M (△). Values are mean ± s.e.mean of 24 to 31 and 5 to 18 rings in the absence and presence of GR 127935 respectively. The right panels show the corresponding Schild regression plots for 5-HT and Sum derived from agonist concentration-ratios (CR) for each of the concentrations of GR 127935.

strictor activities of the 5-HT$_{1D}$ receptor agonists are counteracted by spontaneously or agonist-induced release of EDRF, or both. At the present time, we cannot exclude any of these possibilities. In the absence of E, NOSI did not affect the contractile responses of the 3 agonists, thus demonstrating that the NOS inhibited was mainly of endothelial origin. In addition, removal of the E, in the presence of NOSI, was associated with lower maximal responses for Sum and 5-HT but not for 5-CT. It may be speculated that the 3 agonists induced the release of EDCF(s) in the order of potency Sum > 5-HT > > 5-CT. Further studies are required to address this issue.

In order to verify that the effects of L-NAME were indeed mediated by NOSI, we determined whether they could be (i) reversed by L-arginine and (ii) mimicked by methylene blue.

(i) Enhancement by NOSI of the maximal contractile responses to 5-HT$_{1D}$ receptor agonists, in the presence of a functional E, could be reversed, at least in part, by L-arginine, the physiological NO precursor (Palmer *et al.*, 1988). The reversibility of these responses by L-arginine, although partial, indicates that NO synthase was indeed inhibited by L-NAME. L-Arginine has been reported to reverse, at least partially, the effects of NOSI, as obtained by L-NAME, in a number of *in vivo* as well as *in vitro* preparations (Moncada & Higgs, 1993).

(ii) In the present study, methylene blue statistically significantly enhanced the contractions induced by the 3 agonists in vessels with a functional E, thus mimicking the effects of L-NAME. These observations strongly support the notion that E-derived NO is involved in the attenuation of the 5-HT$_{1D}$ receptor-mediated contractions in rabbit isolated saphenous veins. Interestingly, similar findings were reported by Sweeney *et al.* (1995) who showed that increases in intracellular cyclic GMP in response to Sum in the bovine isolated pulmonary artery were E-dependent and mediated by the release of NO. Furthermore, the presence of 5-HT$_{1D}$ receptors on endothelial cells has recently been demonstrated (Schoeffter *et al.*, 1995).

Our results suggest that the efficacy, but not the potency of 5-HT, 5-CT and Sum in evoking contractile responses of rabbit saphenous vein rings are largely subject to an inhibitory influence of the E and of an EDRF which is probably NO. However, it remains to be established whether the E-derived NO was released spontaneously or by the 5-HT$_{1D}$ agonists, or both.

Involvement of 5-HT$_{1D}$ receptors in the contractile responses evoked by 5-HT, 5-CT and Sum

Methiothepin, a non-selective 5-HT$_{1D}$ receptor antagonist elicited parallel shifts to the right of the 5-HT and Sum concentration-effect curves, suggesting competitive interaction with a single receptor population even though Schild analyses gave slopes of near unity for Sum (0.81) but 0.66 for 5-HT. Our

results are consistent with those of Van Heuven-Nolsen *et al.* (1990) and Humphrey *et al.* (1988) in rabbit and dog saphenous veins. Similarly, the novel and selective 5-HT$_{1D}$ receptor antagonist, GR 127935 (Clitherow *et al.*, 1994; Skingle *et al.*, 1994) surmountably antagonized the contractile responses evoked by 5-HT and Sum, again suggesting interaction with a single receptor population. This is corroborated by the Schild analyses which gave slopes near unity (1.04 and 0.93, respectively). In contrast to our findings, GR 127935, was shown to antagonize non-competitively the 5-HT and Sum-induced contractions of either dog (Clitherow *et al.*, 1994) or rabbit (Razzaque *et al.*, 1995) saphenous veins. However, the involvement of 5-HT$_{2A/C}$ receptor-mediated contractions of the rabbit isolated saphenous vein cannot be excluded in the study performed by Razzaque *et al.* (1995) due to the absence of an appropriate antagonist. Interestingly, Feniuk *et al.* (1985) have shown that a small fraction of the 5-HT-induced contraction of the dog isolated saphenous vein was due to activation of 5-HT$_2$ receptors. In our hands, the possibility of 5-HT$_{2A/C}$ mediated contractions could be excluded by the presence of ketanserin (10^{-7} M) in the Krebs solution. This was confirmed in preliminary experiments with the 5-HT$_{2A/C}$ receptor agonist, DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane)] which failed to contract the rabbit saphenous vein in the presence of ketanserin (10^{-7} M) but which elicited contractile responses in the absence of ketanserin (data not shown).

Both methiothepin and GR 127935 elicited rightward non parallel displacement of the concentration-effect curves to 5-CT, thus indicating a probable interaction of 5-CT with more than one receptor population in this preparation. Further studies should determine which receptors are involved in mediating the 5-CT-induced responses. Although GR 127935 has been reported to exert intrinsic activity at cloned human and porcine 5-HT$_{1D}$ receptors (Pauwels & Colpaert, 1995; De Vries *et al.*, 1995; Walsh *et al.*, 1995), we observed no such activity in the rabbit saphenous vein rings (data not shown), despite the experimental conditions which enhanced agonist efficacy (i.e. NOSI).

Our results suggest that contractile responses evoked by 5-HT and Sum of rabbit isolated saphenous veins are mediated through activation of a single population of 5-HT$_{1D}$ receptors whereas the contractile responses to 5-CT are not mediated exclusively through activation of the same receptor population in this preparation.

The present findings demonstrate that in the rabbit isolated saphenous vein, the E and an EDRF (probably NO) exert a substantial inhibitory influence upon the efficacy of 5-HT$_{1D}$ receptor agonists in producing contraction whereas agonist potency remains unaffected.

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