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Characterisation of the contractile activity of eletriptan at the canine vascular 5-HT_{1B} receptor

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Abstract

The functional activity of eletriptan ((*R*)-3-(1-methyl-2-pyrrolidinylmethyl)-5-[2-(phenylsulphonyl)ethyl]-1*H*-indole) at the contractile serotonin (5-hydroxytryptamine; 5-HT) '1B-like' receptor in dog isolated saphenous vein and basilar artery was investigated. Eletriptan, like 5-HT and sumatriptan potently contracted saphenous vein (pEC_{50}: 6.3, 6.9 and 6.1, respectively) and basilar artery (pEC_{50} 7.2, 7.5 and 6.8, respectively). The maximum responses evoked by eletriptan was, unlike sumatriptan, significantly lower than that to 5-HT (intrinsic activity saphenous vein: eletriptan 0.57, 5-HT 1.0, sumatriptan 0.85; basilar artery: eletriptan 0.77, 5-HT 0.98, sumatriptan 0.89). Contractions evoked by eletriptan were antagonised by the 5-$HT_{1B/1D}$ receptor antagonist GR125743 (*N*-[4-methoxy-3-(4-methyl piperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)benzamide) with pA_2 values of 9.1 in saphenous vein and 9.4 in basilar artery. Affinity estimates (pK_A) for 5-HT and sumatriptan determined from receptor alkylation studies in saphenous vein were 6.6 and 6.3, respectively, compared to the apparent equilibrium dissociation constant (pK_B) for eletriptan of 6.8. The rank order of relative intrinsic efficacies (ε) was 5-HT > sumatriptan > eletriptan. Thus, eletriptan required greater receptor occupancy (4.4-fold) to evoke an equivalent contraction to 5-HT and sumatriptan in dog isolated saphenous vein. These data demonstrate that eletriptan is a potent partial agonist at the canine vascular 5-HT_{1B} receptor.

Keywords: Eletriptan; Sumatriptan; 5-HT_{1B} receptor; 5-HT_{1D} receptor; Partial agonist; Efficacy, intrinsic; Migraine

1. Introduction

There is evidence that the 5-HT_{1B} receptor located on craniovascular smooth muscle (Hamel et al., 1993) and 5-HT_{1B} and/or 5-HT_{1D} receptors located on the Vth cranial (trigeminal) nerve (Bouchelet et al., 1996) are important therapeutic targets for the acute treatment of migraine. All of the 5-HT_1 receptor agonists currently in clinical use and also those undergoing clinical evaluation exhibit high affinity at the human recombinant 5-HT_{1B} and 5-HT_{1D} receptors (Peroutka et al., 1993; Connor and Beattie, 1996; Saxena and Ferrari, 1996). Thus, their effectiveness in migraine is generally attributed to constriction of blood vessels in the cranial circulation via post-synaptic 5-HT_{1B} receptors (Humphrey and Feniuk, 1991) and blockade of neurogenic dural inflammation via pre-synaptic 5-$HT_{1B/1D}$ receptors (Moskowitz, 1992).

In functional studies, this class of agent exhibits agonist activity at contractile 5-$HT_{1B/1D}$ receptors in rabbit, canine, and human vascular smooth muscle (Connor and Feniuk, 1989; MacLennan and Martin, 1990; Jansen et al., 1992), and at the putative neuronal 5-$HT_{1B/1D}$ receptor(s) located on perivascular trigeminal afferents (Saito et al., 1988; Buzzi and Moskowitz, 1990). Contractile responses of canine vascular tissues, such as the saphenous vein and basilar artery, to serotonergic agonists have frequently been used to identify potent vasoconstrictor agents and predict anti-migraine activity of compounds. This paper describes the functional activity of eletriptan ((*R*)-3-(1-methyl-2-pyrrolidinylmethyl)-5-[2-(phenylsulphonyl) ethyl]-1*H*-indole), an effective acute treatment of migraine (Jackson, 1996), at the contractile 5-HT_{1B} receptor in dog isolated saphenous vein and basilar artery. In order to confirm that the eletriptan-evoked responses in these preparations were mediated via activation of 5-$HT_{1B/1D}$ receptors, the effects of the potent and selective 5-$HT_{1B/1D}$ receptor antagonist, GR125743 (*N*-[4-methoxy-3-(4-methyl piperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)benzamide)

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on these responses were studied. GR125743 is reported to have over 100-fold selectivity for 5-$HT_{1B/1D}$ receptors compared to other 5-HT receptor subtypes (Scopes et al., 1994), thus making it a useful tool with which to confirm that the receptor mediating eletriptan-evoked contractions in the dog isolated basilar artery and the saphenous vein are indeed identical. Also in this study, the apparent equilibrium dissociation constant (pK_P) for eletriptan in the saphenous vein was determined and compared to affinity estimates (pK_A) determined for 5-HT and sumatriptan from receptor alkylation studies using benextramine. From these K_P and K_A estimates, the receptor occupancy–response relationships of eletriptan, sumatriptan, and 5-HT in saphenous vein were defined, from which intrinsic efficacies (ε) relative to 5-HT were determined.

2. Methods

2.1. Tissue preparation

Saphenous veins were removed from anaesthetised Pfizer beagle dogs (10–18 kg, either sex) and a length of vein was cleared of connective tissue and cut into rings 2–3 mm in length. Basilar arteries were removed from Pfizer beagle dogs (10–18 kg, either sex) killed by an intravenous overdose of sodium pentobarbitone. A section of brainstem with an attached length of basilar artery was removed and dissected free of the brainstem and the endothelium removed by perfusing with 0.5 ml Triton X-100 in distilled water over 1 min followed by a 1 ml wash through with Krebs solution. The artery was then cut into rings 2–3 mm in length. Rings of tissue were suspended between parallel stainless steel wires and mounted in 15 ml organ baths containing Krebs solution gassed with 95% O_2 and 5% CO_2 at 37°C, under a resting tension of 0.5 g (saphenous vein) or 1.0 g (basilar artery). The composition of the Krebs solution used was as follows (mM): NaCl (118.4), KCl (4.7), $NaHCO_3$ (25.0), $MgSO_4$ (1.2), KH_2PO_4 (1.2), glucose (11.1), $CaCl_2$ (1.25). In addition, the Krebs contained: the muscarinic receptor antagonist, atropine (1 μM), the histamine H_1 receptor antagonist, mepyramine (1 μM), the mixed 5-$HT_{2/7}$ receptor antagonist, mesulergine (1 μM), the alpha$_1$-adrenoceptor antagonist prazosin (1 μM), the inhibitor of neuronal uptake$_1$, imipramine (1 μM) and the inhibitor of extra-neuronal uptake$_2$, corticosterone (10 μM).

The tension in each preparation was measured using isometric force-displacement transducers and displayed on a Grass 79D polygraph. Tissues were equilibrated with the irreversible monoamine oxidase inhibitor, pargyline (100 mM) for 15 min, after which they were washed with fresh Krebs solution, and the tissue load was re-adjusted back to the original resting tension.

2.2. Determination of agonist and antagonist potency

In order to assess tissue contractility, saphenous vein tissues were sequentially exposed to KCl (45 mM) twice at 20 min intervals, while basilar artery tissues were sequentially exposed to KCl (45 mM) and then 5-HT (10 μM) at 20 min, with wash-out after each contraction had reached a plateau. After a further 30 min, a cumulative concentration–response curve to 5-HT (1 nM–10 μM) was prepared in all tissues (curve 1). Tissues were washed after the maximum response to 5-HT had been established, and after further equilibration for at least 30 min, a second cumulative concentration–response curve to 5-HT (control) or test agonist (1 nM–10 μM) was prepared (curve 2).

The potency of GR 125743 was determined as described above, except that following the concentration–response curve to 5-HT or eletriptan (curve 1), the tissues were washed for at least 1.5 h prior to repeating the concentration–response curve to 5-HT or eletriptan in the presence of a single concentration of GR125743 (1–30 nM) or vehicle.

2.3. Determination of K_P for eletriptan in saphenous vein

The apparent equilibrium dissociation constant (K_P) of eletriptan was estimated by the method of Stephenson (1956). A cumulative concentration–response curve to 5-HT was prepared in all tissues (curve 1). After wash-out, eletriptan (1 μM) or vehicle were equilibrated with tissues for 30 min, and then a second cumulative concentration–response curve to 5-HT was constructed (curve 2). Linear regression analysis of a plot of equiactive concentrations of 5-HT in the absence and presence of eletriptan was used to derive a slope value, and K_P was calculated by:

$$K_P = \frac{[P]\text{slope}}{1 - \text{slope}}(1 - \varepsilon_P/\varepsilon_A)$$

where $[P]$ is the concentration of eletriptan, and ε_P and ε_A are intrinsic efficacies of a eletriptan and 5-HT, respectively. Since the estimate of K_P is affected by the relative efficacies of the agonists, it is assumed that $\varepsilon_A \gg \varepsilon_P$ (Kenakin, 1987).

2.4. Determination of K_A for 5-HT and sumatriptan in saphenous vein

The apparent equilibrium dissociation constants (K_A) for 5-HT and sumatriptan were estimated by the receptor alkylation method of Furchgott and Bursztyn (1967). A cumulative concentration–response curve to either 5-HT or sumatriptan (1 nM–10 μM) was constructed (curve 1). Preparations were then washed and equilibrated for 60 min with either vehicle, or the 5-HT receptor alkylating agent benextramine to produce an irreversible fractional inactiva-

tion of tissue receptors. Since benextramine had a variable effect on the tissue maxima in tissues from different animals, three different concentrations of benextramine (10 μM, 30 μM and 100 μM) were routinely administered to separate tissues from the same animal. After washout, a cumulative concentration–response curve to 5-HT or sumatriptan was repeated. To reduce errors in K_A, a tissue was used for analysis if the agonist-evoked maxima following benextramine treatment was $\leq 50\%$ of that evoked in curve 1 (Kenakin, 1987). Regression analysis of a double-reciprocal plot of mean equiactive concentrations of 5-HT or sumatriptan, before and after receptor alkylation, yielded slope and intercept values allowing the estimation of K_A by:

$$K_A = \frac{\text{slope} - 1}{\text{intercept}}$$

2.5. Determination of intrinsic efficacy (ε) relative to 5-HT in saphenous vein

An estimate of relative ε was determined by the method of Furchgott and Bursztyn (1967). Responses to each agonist were plotted as functions of the logarithms of the respective receptor occupancies. Receptor occupancies (ρ) were determined over the agonist concentration range using the calculated agonist-equilibrium dissociation constants by:

$$\rho = \frac{[A]}{[A] + K_A}$$

where $[A]$ is the agonist concentration.

The lateral displacement between the occupancy–response curves on the abscissa was a measure of the logarithm of relative ε. The slopes of the curves for eletriptan and sumatriptan were constrained to the observed slope for 5-HT to ensure that the linear portions of all curves were parallel for measurement of relative ε (Kenakin, 1987).

2.6. Data analysis

Agonist potencies are expressed as an EC_{50} value, the concentration of agonist required to produce 50% of the maximum response attainable for that particular agonist. EC_{50} values were derived using the logistic curve-fitting program 'ORIGIN'. pEC_{50} values where quoted represent the negative $\log_{10}$ of the mean EC_{50} value, with 95% confidence limits shown in parentheses, for n separate experiments. pA_2 values for GR125743 against eletriptan and 5-HT were derived across tissues on a per experiment basis from Schild plots. The intrinsic activity of an agonist was determined by expressing the maximum tissue response evoked by agonist in curve 2, as a fraction of the maximum tissue response produced to 5-HT in curve 1, in that particular tissue. An agonist will be referred to as full agonist if the intrinsic activity is not significantly different from that for 5-HT (unity), or a partial agonist if the intrinsic activity value is significantly less than 1.0 and greater than 0. To adjust for time-dependent changes in tissue sensitivity and maximum effect between first and second curves to 5-HT (or to consecutive curves to sumatriptan in receptor alkylation studies, see above), correction factors were derived by calculating agonist concentration-ratios (EC_{50} of curve 2 divided by EC_{50} of curve 1) and maximum response ratios (maximum of curve 2 divided by maximum of curve 1) in respective control tissues. Correction factors were used in all experiments, except in GR125743 experiments where 5-HT control curves did not differ significantly.

The differences between the mean intrinsic activity of eletriptan and sumatriptan compared to 5-HT were determined by use of a one-sample Student's *t*-test. Mean pEC_{50} values for 5-HT, eletriptan and sumatriptan and mean pA_2 values for GR125743 were compared using a Student's *t*-test for unpaired samples. A *P*-value of less than 0.05 was considered to indicate a significant difference between the responses being compared.

2.7. Drugs used

The following drugs were used in this study: atropine sulphate, imipramine hydrochloride, 5-hydroxytryptamine hydrochloride (5-HT), mepyramine maleate, pargyline hydrochloride, benextramine tetrahydrachloride, corticosterone 21-sulphate (Sigma, Poole, Dorset, UK); mesulergine hydrochloride (RBI Natick, MA, USA); Prazosin hydrochloride, eletriptan ((*R*)-3-(1-methyl-2-pyrrolidinylmethyl)-5-[2-(phenylsulphonyl)ethyl]-1*H*-indole), sumatriptan and GR125743 (*N*-[4-methoxy-3-(4-methyl piperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)benzamide) were synthesised at Pfizer Central Research, UK.

Imipramine, mepyramine and mesulergine were dissolved in 100% v/v dimethyl sulphoxide and diluted in distilled H_2O. Prazosin hydrochloride was dissolved in lactic acid and diluted in distilled H_2O. All other drugs were dissolved in distilled H_2O.

3. Results

3.1. Agonist potency and intrinsic activity in the dog isolated basilar artery

Eletriptan and sumatriptan produced concentration-dependent contractions of the dog isolated basilar artery (pEC_{50}s ($\pm$S.E.M.) of 7.2 ± 0.03 and 6.8 ± 0.06). Both compounds were significantly less potent than 5-HT ($P < 0.05$) and in addition, sumatriptan was significantly less potent than eletriptan ($P < 0.05$) in evoking these contractile responses. Fig. 1 shows that, unlike sumatriptan, the

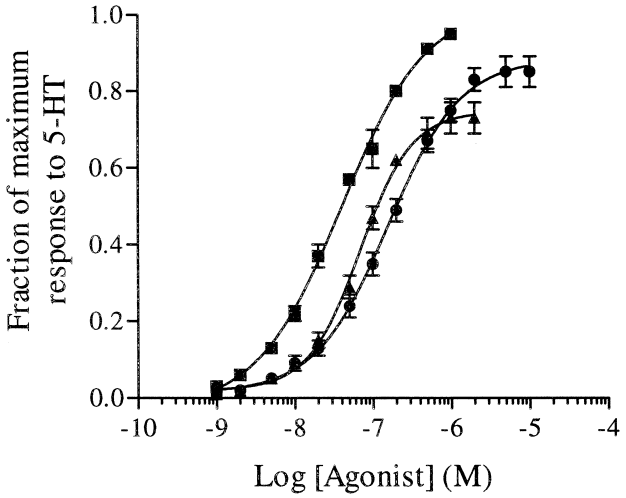

Fig. 1. Cumulative concentration–response curves to 5-HT (■), eletriptan (▲) and sumatriptan (●) in dog isolated basilar artery. Data are expressed on the ordinate as a fraction of the maximum response of the first concentration–response curve to 5-HT, and represent the mean of between 6–7 separate experiments, with the S.E.M. represented by the vertical bars.

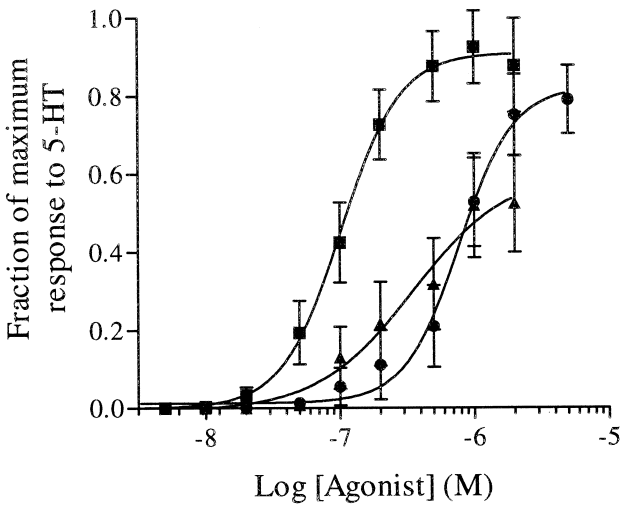

Fig. 2. Cumulative concentration–response curves to 5-HT (■), eletriptan (▲), and sumatriptan (●) in dog isolated saphenous vein. Data are expressed on the ordinate as a fraction of the maximum response of the first concentration–response curve to 5-HT, and represent the mean of between 8–10 separate experiments, with the S.E.M. represented by the vertical bars.

intrinsic activity of eletriptan in this preparation was significantly lower than that to 5-HT (eletriptan 0.77, $P < 0.05$ compared to 5-HT 0.98, sumatriptan 0.89, $P > 0.05$ compared to 5-HT 0.98). A summary of agonist potency and intrinsic activity estimates is shown in Table 1.

3.2. Agonist potency and intrinsic activity in the dog isolated saphenous vein

Eletriptan and sumatriptan also evoked potent and concentration-dependent contractions of the dog isolated saphenous vein (pEC_{50}s ($\pm$S.E.M.) of 6.3 ± 0.16 and 6.1 ± 0.13). Fig. 2 shows that, consistent with its effects in the basilar artery, sumatriptan appeared to be less potent than eletriptan in evoking the contractile response, although the difference was not statistically significant. Eletriptan produced a significantly lower maximum response than that to 5-HT (eletriptan 0.57, $P < 0.05$ compared to 5-HT 1, sumatriptan 0.85, $P > 0.05$ compared to 5-HT 1). A summary of agonist potency and intrinsic activity estimates is shown in Table 1.

3.3. Effect of GR125743 on 5-HT and eletriptan-evoked contractions of the dog isolated saphenous vein and basilar artery

A slight, but apparently concentration-related contraction was observed when GR125743 was added to basilar artery (data not shown), consistent with the suggestion that this compound is a low efficacy agonist (Newman-Tancredi et al., 1997). Typically, this contraction was approximately 10% of 5-HT maximum at 30 nM GR125743, but on one occasion was nearly 50%. In general, the tissues relaxed before the second agonist curve was obtained, though some remained slightly above original baseline. The contraction was negligible in saphenous vein.

Table 1
Comparison of drug potency and intrinsic activity values in dog isolated saphenous vein and basilar artery

Saphenous vein				Basilar artery		
Agonist	pEC_{50}	Intrinsic activity	*n*	pEC_{50}	Intrinsic activity	*n*
5-HT	6.9 ± 0.16	1	10	7.5 ± 0.07	0.98	7
Eletriptan	$6.3^{a} \pm 0.16$	0.57^{b}	8	$7.2^{a} \pm 0.03$	0.77^{b}	7
Sumatriptan	6.1 ± 0.13	0.85	8	$6.8^{a} \pm 0.06$	0.89	6

pEC_{50} values represent the negative $\log arithm_{10}$ of the molar concentration of agonist which produced 50% of the maximum contraction produced to that particular agonist. Intrinsic activity of an agonist was determined by expressing the maximum tissue response evoked by agonist in curve 2, as a fraction of the maximum response produced to 5-HT in curve 1, in that particular tissue. Data are expressed as the mean $\pm$ S.E.M. for *n* separate experiments.

[a] $P < 0.05$ when compared to 5-HT or eletriptan using a Student's *t*-test for unpaired data.

[b] $P < 0.05$ when compared to 5-HT using a one-sample Student's *t*-test.

Table 2
pA_2 values derived from Schild plots of antagonism of 5-HT and eletriptan-induced contractions in the dog isolated saphenous vein and basilar artery by GR125743

	5-HT	Slope	*n*	Eletriptan	Slope	*n*
Saphenous vein	9.1 ± 0.19	0.85 ± 0.11	6	9.1 ± 0.17	1.04 ± 0.15	6
Basilar artery	9.9 ± 0.22^{a}	0.93 ± 0.10	7	$9.4 + 0.10$	0.97 ± 0.11	6

Data are expressed as mean $\pm$ S.E.M.

[a] $P < 0.05$ when compared to saphenous vein using Student's *t*-test for unpaired data.

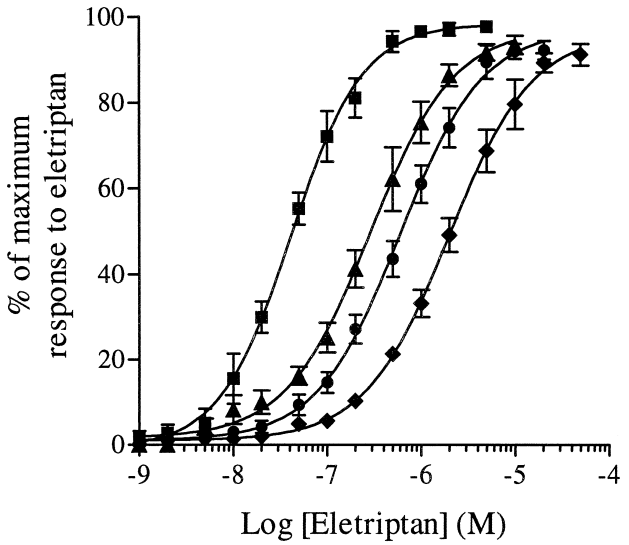

Fig. 3. Cumulative concentration–response curves to eletriptan in dog isolated basilar artery in the absence (■) and presence of 3 nM (▲), 10 nM (●) and 30 nM (◆) GR125743. Data are expressed on the ordinate as percent maximum response of the first concentration–response curve to eletriptan, and represent the mean of five experiments, with the S.E.M. represented by the vertical bars.

GR125743 caused a concentration-related rightward shift in the concentration–response curve to eletriptan and 5-HT in both tissues examined with pA_2 values (±S.E.M.) of 9.1 ± 0.19 and 9.1 ± 0.17 for 5-HT and eletriptan, respectively in saphenous vein and 9.9 ± 0.22 and 9.4 ± 0.10, respectively in basilar artery (Table 2). GR125743 did not suppress the maximum response to 5-HT or eletriptan and slopes of Schild plots were not significantly different from unity, indicating that the compound was acting as a surmountable antagonist. Although pA_2 values for GR125743 antagonism of 5-HT were significantly higher in basilar artery than saphenous vein ($P < 0.05$), there was no statistically significant difference with pA_2 values for GR125743 antagonism of eletriptan between the two preparations, consistent with the suggestion that eletriptan is acting on the same 5-HT receptor subtype in both tissues. The concentration–response curves to eletriptan in the presence of GR125743 (3–30 nM) in basilar artery is shown in Fig. 3 and the Schild plots are shown in Fig. 4.

3.4. Estimation of K_P

Eletriptan (1 μM) pre-treatment of tissues evoked an initial contraction, which subsequently stabilised, over a

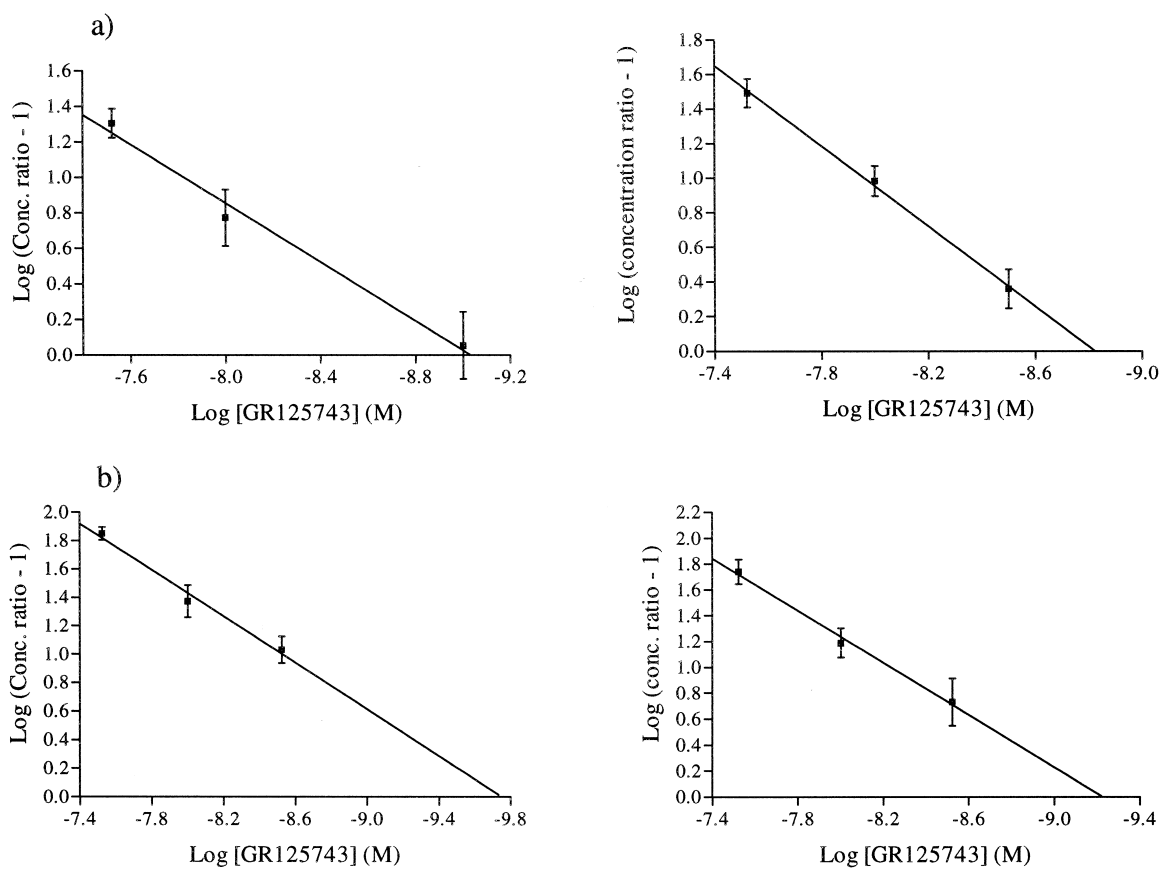

Fig. 4. Schild plots showing antagonism of 5-HT and eletriptan-induced contractions in the dog isolated saphenous vein (a) and basilar artery (b) by GR125743. Each point represents the mean of between 5–9 separate experiments, with the S.E.M. represented by the vertical bars. The gradient of the best-fit straight line was determined by linear regression.

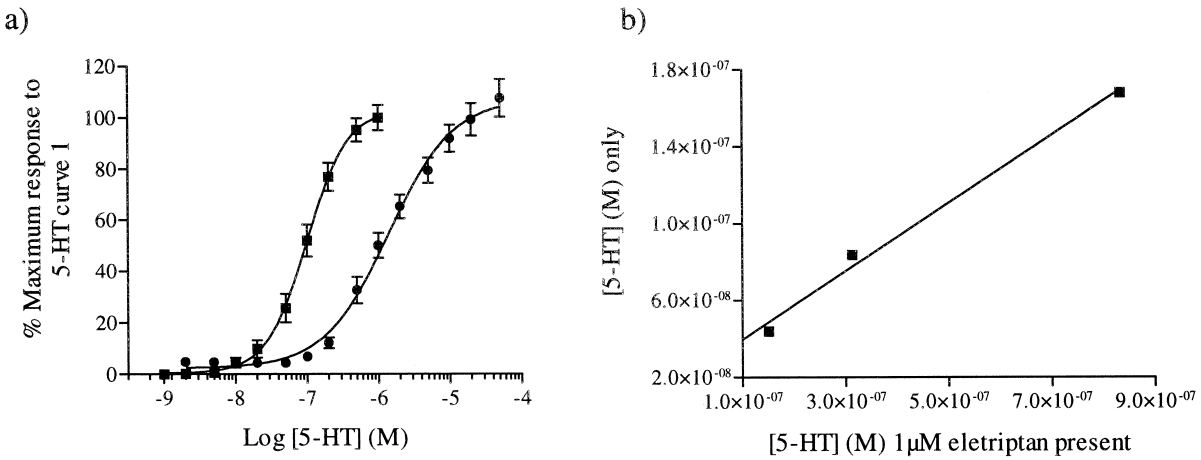

Fig. 5. (a) Cumulative concentration–response curves to 5-HT in the absence (■) and presence (●) of eletriptan (1 μM) in dog isolated saphenous vein. Data on the ordinate are expressed as percent maximum response of the first concentration–response curve to 5-HT. Values represent the mean of 7–10 separate experiments, with the S.E.M. represented by the vertical bars. (b) Representative plot of equiactive concentrations of 5-HT in the absence and presence of eletriptan (1 μM) at 20, 50 and 80% of maximal response in dog isolated saphenous vein. The slope of the plot was determined by linear regression analysis and used to estimate K_P for eletriptan for each separate experiment.

30-min period, to approximately 5–10% of the maximum response observed to 5-HT in curve 1. Subsequently, eletriptan produced a rightward displacement of the concentration–response curve to 5-HT without reducing the maximum response (Fig. 5a). From a plot of equiactive concentrations of 5-HT in the absence and presence of eletriptan (Fig. 5b), the dissociation constant for eletriptan was determined (mean ± S.E.M., K_P 6.8 ± 0.1, $n = 10$).

3.5. Estimation of K_A

Treatment with benextramine (10, 30 or 100 μM) produced a rightward displacement and suppression of the maxima of concentration–response curves to both 5-HT and sumatriptan (Fig. 6a and b). Using linear regression analysis of a double-reciprocal plot of mean equiactive concentrations of 5-HT or sumatriptan, before and after receptor alkylation, the apparent equilibrium dissociation constants for 5-HT and sumatriptan were determined (mean ± S.E.M., pK_A values for 5-HT and sumatriptan, 6.6 ± 0.2, $n = 10$, and 6.3 ± 0.2, $n = 10$, respectively).

3.6. Estimation of relative ε

Agonist affinity estimates were used to establish the receptor–occupancy response relationships of eletriptan and sumatriptan compared with 5-HT (Fig. 7). The lateral displacement between curves demonstrated a rank order of relative ε of 5-HT > sumatriptan > eletriptan. Thus, to evoke an equivalent contraction to 5-HT in dog isolated

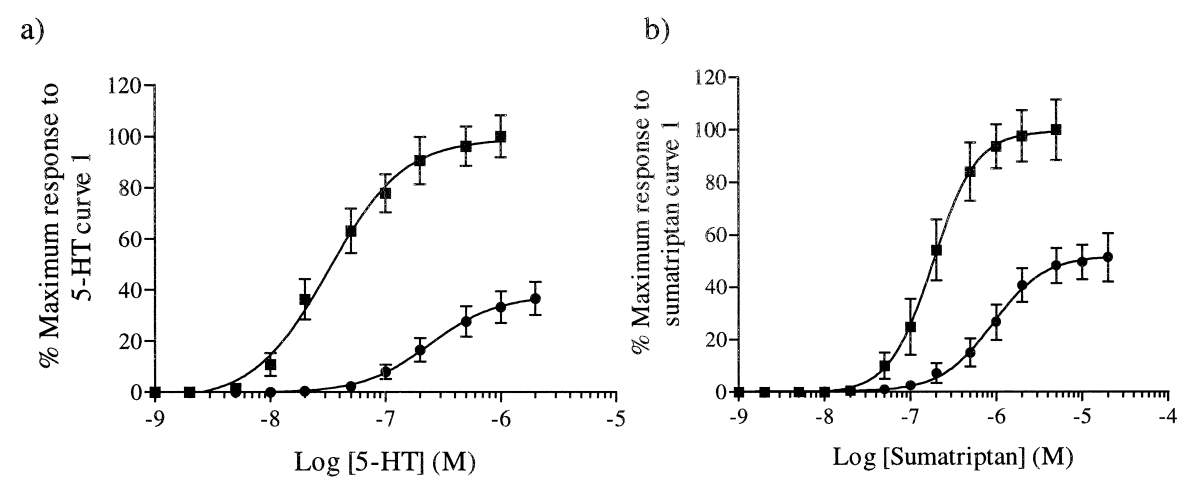

Fig. 6. Cumulative concentration–response curves to (a) 5-HT and (b) sumatriptan in the absence (■) and presence (●) of benextramine (10, 30 or 100 μM) in dog isolated saphenous vein. Data n the ordinate are expressed as percent maximum response of the first concentration–response curve to 5-HT or sumatriptan. Values represent the mean of 7–10 separate experiments, with the S.E.M. represented by the vertical bars.

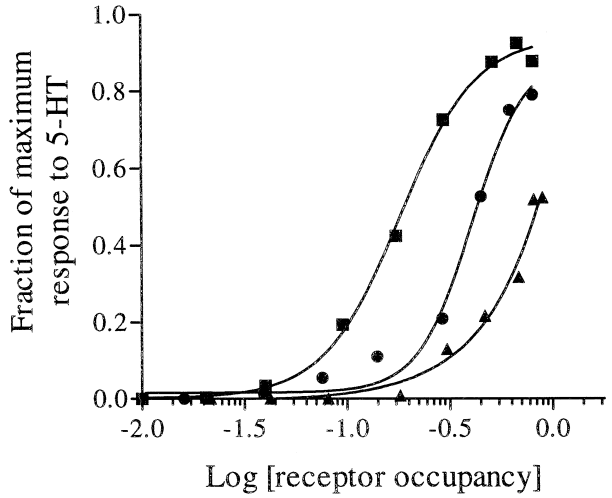

Fig. 7. Receptor occupancy–response curves for 5-HT (■), eletriptan (▲) and sumatriptan (●) in dog isolated saphenous vein. Data on the ordinate are expressed as a fraction of the maximum response of the first concentration–response curve to 5-HT. Data on the abscissa are expressed as receptor occupancies (ρ) which were determined over the agonist concentration range as described in Section 2. Values represent the mean of 8–10 separate experiments.

saphenous vein, eletriptan and sumatriptan required a 4.4- and 2.2-fold greater receptor occupancy, respectively.

4. Discussion

Recent clinical trials indicate that eletriptan is an effective acute treatment of migraine (Jackson, 1996). In the present study, we have characterised the pharmacological activity of eletriptan at the contractile 5-HT_{1B} receptor in dog isolated saphenous vein and basilar artery, since activity at this receptor may reflect activity at the contractile receptor in human vasculature. In addition, a key objective of this study was to investigate the nature of eletriptan's agonist activity by determining relative intrinsic efficacy at the vascular 5-HT_{1B} receptor expressed in dog saphenous vein.

Eletriptan was demonstrated to be a partial agonist in both dog isolated saphenous vein and basilar artery since its intrinsic activity was, unlike sumatriptan, significantly less than that for 5-HT. However, whilst intrinsic activity is a standard method for quantifying the ability of a drug to produce a response in a given tissue, it cannot be used to quantify agonist activity relating specifically to a drug at a given receptor. Thus, the magnitude of the maximal response is both drug and tissue related, since the efficiency of stimulus-coupling and the density of receptor expression greatly affect the tissue maxima to a given agonist (Kenakin, 1987). Therefore, studies were performed to characterise the agonist activity of eletriptan in a way that is independent of tissue to tissue differences. Using well characterised experimental paradigms (Stephenson, 1956; Furchgott and Bursztyn, 1967), functional estimates of agonist dissociation constants were derived for eletriptan, sumatriptan and 5-HT in the saphenous vein. By use of the K_P and K_A estimates, the receptor occupancy–response relationships of eletriptan, sumatriptan, and 5-HT in dog isolated saphenous vein were defined, from which relative intrinsic efficacies were measured. Thus, the rank order of intrinsic efficacy relative to 5-HT was: 5-HT > sumatriptan > eletriptan. These data indicate that at the canine vascular 5-HT_{1B} receptor, eletriptan is a partial agonist exhibiting a lower relative efficacy to 5-HT or sumatriptan since occupancy of a greater fraction of the available receptors is required to evoke an equivalent tissue response. Studies to investigate whether eletriptan is a partial agonist at human vascular tissues and recombinant human 5-HT_{1B} and 5-HT_{1D} receptors are ongoing.

GR125743 has recently been shown to competitively antagonise sumatriptan-evoked contractile responses in human middle meningeal artery with a pA_2 of 9.1 (Razzaque et al., 1997). In our studies, the contractions evoked by 5-HT and eletriptan in both saphenous vein and basilar artery were shown to be antagonised by the selective 5-$HT_{1B/1D}$ receptor antagonist GR125743 consistent with the suggestion that the contractions are mediated by the same receptor in both tissues. The greater intrinsic activity of eletriptan in the basilar artery may be related to a bigger receptor reserve in this tissue compared to saphenous vein. This may also explain the small contractile response evoked by GR125743 in the basilar artery which was not observed in the saphenous vein.

Radioligand binding studies have revealed that eletriptan has high affinity for the human recombinant 5-HT_{1B} and 5-HT_{1D} receptors (Napier et al., 1999). There is some evidence that contraction of dog isolated saphenous vein is mediated via the 5-HT_{1B} receptor (Sgard et al., 1995; Branchek et al., 1996). The relative potency of eletriptan and sumatriptan to cause contraction of dog isolated saphenous vein and basilar artery correlates closely with their affinity at the human recombinant 5-HT_{1B} receptor, where eletriptan has 3–5 fold higher affinity than sumatriptan (Napier et al., 1999). However, whilst drug receptor theory predicts that K_A and K_i values should be equal, it was evident on comparison of absolute values that the functional affinity estimates derived from isolated tissue studies were more than 10-fold lower than those derived from binding experiments using the human recombinant 5-HT_{1B} receptor. Assuming that the 5-HT_{1B} subtype is the receptor which mediates contractions of the saphenous vein, the possibility exists that a species difference between the human and canine receptors might contribute to the observed disparity between K_A and K_i. However, work by Branchek et al. indicate that there is no obvious difference in agonist affinities at the human and canine 5-HT_{1B} receptor (Branchek et al., 1996). Similar differences between functional and binding affinity estimates have been

observed within the same species. For example, Kaumann et al. using human isolated cerebral vessels reported an affinity estimate for sumatriptan (pK_P 6.1, Kaumann et al., 1994) which is 20-fold lower than the binding affinity at the human recombinant 5-HT_{1B} subtype estimated in our binding studies (pK_i 7.4, Napier et al., 1999). Similar observations have also been observed for 5-HT and sumatriptan in rabbit saphenous vein and at rabbit recombinant 5-HT_{1B} and 5-HT_{1D} receptors (MacLennan and Martin, 1990; Harwood et al., 1995). Thus, the observed disparity may not be due to differences in species, but instead, the product of methodological differences relating to the use of a functional paradigm in an intact tissue compared with a binding paradigm using membranes prepared from recombinant expression systems. These observed differences in the affinity of drugs for the native receptor and the recombinant counterpart are perhaps not surprising, and reinforce the importance of using combinations of these systems when characterising drugs.

These studies demonstrate that eletriptan is a potent partial agonist at the 5-HT_{1B} receptor mediating vasoconstriction in dog isolated saphenous vein and basilar artery. This pharmacological profile is consistent with clinical findings that eletriptan is an effective treatment for migraine in man.

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