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Evidence for different 5-$HT_{1B/1D}$ receptors mediating vasoconstriction of equine digital arteries and veins

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Abstract

5-hydroxytryptamine (5-HT) is a potent vasoconstrictor of equine digital arteries and veins which may play a role in the ischaemic disease, laminitis. The present investigation compared the properties of 5-$HT_{1B/1D}$ receptors in arteries with those in veins using isolated rings of equine digital blood vessels. The 5-$HT_{1B/1D}$ receptor-selective agonists, anpirtoline and sumatriptan were 17.9 and 10 times more potent and produced 4.1 and 5.6 times greater maximum contractions, respectively, in veins when compared to arteries. Other agonists tested were of equal potency and produced the same maximum responses in veins and arteries. Propranolol competitively inhibited 5-$HT_{1B/1D}$ receptor mediated responses in arteries, with a pK_B of 6.7, but had no significant effects on responses in veins at 1 μM. Metergoline competitively inhibited 5-$HT_{1B/1D}$ receptor mediated responses in veins, with a pK_B of 8.1, but had no significant effect in arteries at 0.1 μM. These data suggest that 5-$HT_{1B/1D}$ receptors mediating vasoconstriction in equine digital arteries are pharmacologically different to those found in digital veins.

Keywords: 5-$HT_{1B/1D}$ receptor; Digital blood vessel, equine; Laminitis, equine

1. Introduction

Acute laminitis is a common vascular problem of the horse which has a number of similarities with Raynaud's Phenomenon in man (Hood et al., 1990). It is characterised by decreased perfusion of the sensitive laminae of the hoof causing ischaemia, which is followed by reperfusion injury (Flaherty and Weisfeldt, 1988) resulting in the typical signs of hoof pain and bounding digital pulses. Acute laminitis has a systemically mediated aetiology, occurring most commonly as a sequel to gastrointestinal disturbances and sepsis (Hood et al., 1993). Although the pathophysiology of this condition remains unclear, haemodynamic studies suggest that increased vascular resistance occurs preferentially on the venous side of the laminar capillary bed (Allen et al., 1990). It seems likely that the extensive arteriovenous anastomoses found in the digital circulation also contribute to laminar ischaemia (Robinson, 1990). The precise mechanisms which disturb the digital haemodynamics and lead to preferential venoconstriction are still a subject of speculation (Hunt, 1991) and no single agent has been identified as the specific vasoactive mediator triggering acute laminitis.

Given the observed link between gastrointestinal disturbances and acute laminitis and the possible importance of platelets in the pathophysiology of this and other ischaemic diseases, one vasoactive mediator which may be involved in the haemodynamic disturbances leading to laminitis is 5-hydroxytryptamine (5-HT). Enterochromaffin cells of the alimentary tract and platelets are major sources of 5-HT. This amine has also been implicated in the pathophysiology of the human digital ischaemic disease, Raynaud's phenomenon (Biondi et al., 1988).

The saphenous vein from a number of species has been used to study vascular 5-HT receptors, particularly those of the 5-$HT_{1B/1D}$ type (see Martin, 1994). 5-HT is a potent vasoconstrictor of equine digital blood vessels (Baxter et al., 1989) which are much more sensitive to 5-HT than other equine peripheral arteries (Bailey and Elliott, 1998). Equine digital arteries and veins appear to possess both 5-$HT_{1B/1D}$ and 5-HT_2 receptors located on the vascular

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smooth muscle (Weller et al., 1994), both of which mediate vasoconstriction. The purpose of the present study was to investigate the pharmacological profile of 5-$HT_{1B/1D}$ receptors mediating vasconstriction of equine digital blood vessels and to make a direct comparison between digital arteries and veins in functional studies. To this end, the potency and efficacy of a series of 5-HT receptor agonists were ascertained for both arteries and veins and the effects of 5-HT receptor antagonists on the responses to these agonists were analysed. These results have been presented in abstract form (Bailey and Elliott, 1996a,b).

2. Materials and methods

2.1. Animals and tissues

Equine digital arteries and digital veins were isolated from the hindlimbs of mixed breed healthy adult horses obtained within 15 min of death from an abattoir. The vessels were flushed with modified Krebs Henseleit solution (Krebs solution) and transported to the laboratory in ice-cold Krebs solution. Rings of digital artery and vein 3–4 mm in length were cut and the intimal surface of the vessel rubbed to remove the endothelium. This technique for removal of the endothelium has been previously validated by staining formalinised strips of artery and vein with silver nitrate to look for the presence of endothelial cells microscopically and also by performing functional tests for agonist-induced vasorelaxation on pre-contracted vessel rings (Elliott et al., 1994).

2.2. Isometric tension recording

The vessel rings were placed in organ baths in Krebs solution maintained at 30°C, gassed with 95% O_2 and 5% CO_2 and mounted between two parallel wires, one of which was connected to an HSE 30 isometric force transducer. This signal from the force transducer was fed via an HSE bridge amplifier to a Linseis 650 dual channel chart recorder. Ten vessel rings could be examined simultaneously within the course of any one experiment. For each experiment, an initial 60 min equilibration period was allowed, during which time the vessels were incubated with 0.1 mM benextramine to inhibit α-adrenoceptors and 0.5 mM pargyline to prevent 5-HT metabolism by monoamine oxidase. Both these inhibitors were removed at the end of a 30 min incubation period by thorough washing of the tissue with drug-free Krebs solution. Preliminary experiments showed that neither of these drug treatments caused a significant difference in the concentration response relationships seen to 5-HT in both arteries and veins or to sumatriptan in veins, but benextramine caused complete inhibition of responses of the tissues to noradrenaline (0.3 μM). In each experiment, a response to Krebs solution where the sodium had been exchanged for potassium on an equimolar basis (depolarising Krebs solution) was obtained for each vessel ring. In tissues where 5-HT was to be used as the agonist, ketanserin (10 nM to 0.1 μM) was added to the Krebs solution 30 min prior to stimulation of the tissue with 5-HT. In all subsequent experiments where 5-HT was used as the agonist, ketanserin (0.1 μM) was added to the organ bath 30 min prior to construction of the cumulative concentration response curves.

2.3. Effects of 5-HT receptor agonists

A direct comparison was made between the responses of arteries and veins by constructing cumulative concentration response curves to 5-HT (0.1 nM to 0.1 mM; in the presence of 0.1 μM ketanserin), 5-carboxamidotryptamine (5-CT; 0.1 nM to 0.1 mM), sumatriptan (0.1 nM to 30 μM), 6-chloro-2-[piperidyl-4-thiol]-pyridine hydrochloride (anpirtoline; 0.1 nM to 30 μM) and (±)-8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT; 10 nM to 0.1 mM). Only one cumulative concentration response curve was obtained per vessel ring and each agonist was tested on adjacent rings of digital artery and digital vein from the same horse on each experimental day ($n = 6$ per agonist). The increase in tension obtained with each concentration of agonist was measured, expressed as a percentage of the response to depolarising Krebs solution obtained in the same tissue and plotted against $\log_{10}$ agonist concentration.

2.4. Effects of 5-HT receptor antagonists

Antagonists were used to characterise the 5-$HT_{1B/1D}$ receptors further. Tissues were incubated for 30 min with each antagonist prior to construction of the cumulative concentration response curve to the relevant agonist. The effects of ketanserin (10 nM) on responses of arteries to 5-CT and veins to 5-CT and sumatriptan were examined. The responses of arteries and veins to 5-HT (in the presence of 0.1 μM ketanserin) and 5-CT and of veins to sumatriptan were studied in the presence of yohimbine (0.1 to 1 μM). The effects of methiothepin (0.1 μM) on responses of arteries and veins to 5-CT were investigated. Methiothepin (0.01 to 0.3 μM) was also tested against responses to sumatriptan in the veins. Propranolol (1 μM) was investigated for its inhibitory effects on responses of arteries and veins to 5-HT (in the presence of 0.1 μM ketanserin), 5-CT and anpirtoline and of veins to sumatriptan. Finally, the effects of metergoline (0.01 and 0.1 μM) were studied on the responses of arteries to 5-CT and veins to 5-CT and sumatriptan. In each case, the concentration range examined for 5-CT was 0.1 nM to 0.3 μM which encompassed the first phase of the cumulative concentra-

tion response curve to 5-CT only. In the case of 5-HT (in the presence of 0.1 μM ketanserin), experiments performed in the presence of antagonists were conducted using the concentration range 0.1 nM to 10 μM which took the responses well beyond the plateau of the biphasic curve.

2.5. Statistical analysis of data

Cumulative concentration response curves were fitted by computerised non-linear iterative procedure to either a one or a two process logistic equation (see below), depending on which fitted the data most closely. Full details of the software programmes used are given by Barlow (1983). The two equations used were:

$$E = E_{\max} A^{n_H} / \left(A^{n_H} + EC_{50}^{n_H} \right) \quad (1)$$

$$E = \left[E_{\max(1)} A_{(1)}^{n_{H(1)}} / \left(A_{(1)}^{n_{H(1)}} + EC_{50(1)} \right) \right] + \left[E_{\max(2)} A_{(2)}^{n_{H(2)}} / \left(A_{(2)}^{n_{H(2)}} + EC_{50(2)} \right) \right] \quad (2)$$

The appropriateness of the equation was assessed by comparing the sum of squares of the residuals, the scatter

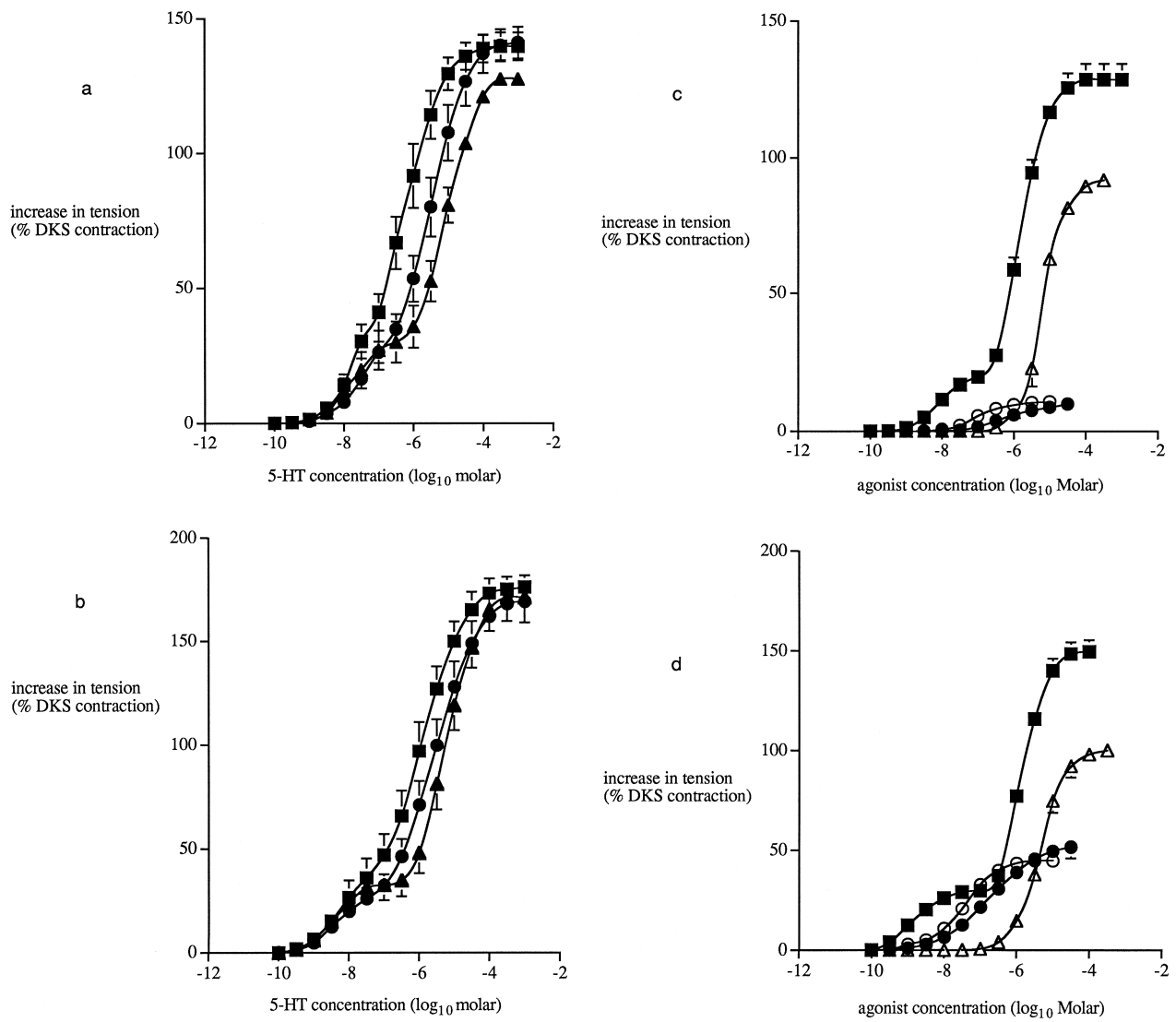

Fig. 1. (a and b) Cumulative concentration response curves showing the effect of ketanserin on the contraction of equine digital artery (a) and vein (b) segments to 5-HT, at ketanserin concentrations of 10^{-8} M (■), 3×10^{-8} M (●) and 10^{-7} M (▲). Each point represents the mean value ± S.E.M. obtained from blood vessel segments from six horses. Each individual response curve was fitted to a two site logistic equation and the curve shown was drawn by interpolation through each mean value. (c and d) Cumulative concentration response curves showing contraction of equine digital artery (c) and vein (d) segments to the agonists 5-carboxamidotryptamine (5-CT; ■), sumatriptan (●) and anpirtoline (○) and 8-OH-DPAT (△). Each point represents the mean value ± S.E.M. obtained from blood vessel segments from six horses. Each individual response curve was fitted to either a one or two site logistic equation and the curve shown was drawn by interpolation through each mean value. Mean values of EC_{50}, E_{max} and n_H are shown in Table 1. DKS—depolarising Krebs solution.

Table 1
Concentration response curve parameters for 5-HT receptor agonists evaluated on equine digital arteries and veins

Agonist	EC_{50} (M) Geometric mean (95% CL)		E_{max} (percent response to depolarising Krebs solution) mean ± S.E.M.		n_H mean ± S.E.M.	
	Arteries	Veins	Arteries	Veins	Arteries	Veins
5-CT (1st phase)	$7.5\ (5.4–9.5) \times 10^{-9}$	$1.5\ (1.0–2.0) \times 10^{-9}$	20.8 ± 1.5	26.8 ± 5.2	0.94 ± 0.2	1.02 ± 0.1
5-HT (1st phase)[a]	$1.9\ (0.4–3.3) \times 10^{-8}$	$5.4\ (2.4–8.5) \times 10^{-9}$	29.7 ± 7.2	29.3 ± 5.8	0.93 ± 0.1	1.32 ± 0.1
Anpirtoline	$6.8\ (5.1–8.5) \times 10^{-7}$	$3.8\ (2.8–4.9) \times 10^{-8}$[b]	10.3 ± 0.5	42.6 ± 2.9[c]	1.05 ± 0.1	1.03 ± 0.1
Sumatriptan	$4.9\ (1.8–8.4) \times 10^{-7}$	$4.9\ (1.0–8.8) \times 10^{-8}$[b]	8.7 ± 1.4	48.6 ± 6.2[c]	0.92 ± 0.1	0.97 ± 0.1
5-CT (2nd phase)	$1.9\ (1.4–2.4) \times 10^{-6}$	$1.5\ (1.1–2.0) \times 10^{-6}$	106.3 ± 7.8	132.4 ± 6.8	1.05 ± 0.1	1.09 ± 0.1
5-HT (2nd phase)[a]	$9.5\ (4.7–14.5) \times 10^{-6}$	$6.2\ (3.0–9.3) \times 10^{-6}$	100.0 ± 7.2	133.1 ± 12.9	1.09 ± 0.1	1.20 ± 0.1
8-OH-DPAT	$6.5\ (0.5–10.1) \times 10^{-6}$	$9.3\ (6.2–12.6) \times 10^{-6}$	93.9 ± 1.4	104.2 ± 3.3	1.01 ± 0.1	1.04 ± 0.1

Cumulative concentration response curves to each agonist were constructed in adjacent segments of equine digital artery and vein from the same animal (one vessel ring used per agonist; see Fig. 1).
The best fit values of EC_{50}, E_{max} and n_H were obtained by computerised non-linear curve fitting to either Eq. (1) (Anpirtoline, sumatriptan and 8-OH-DPAT) or Eq. (2) (5-HT and 5-CT).
[a]5-HT concentration response curves were obtained in the presence of 0.1 μM ketanserin to produce a biphasic curve. The geometric or arithmetic mean of those values obtained from vessel rings from six animals are presented.
[b]Denotes $P < 0.05$ and [c]denotes $P < 0.01$ vs. values obtained in arterial tissue for the same agonist by a paired Student's *t*-test.
CL—confidence limits.

Table 2
Effects of 5-HT receptor antagonists on the responses of equine digital arteries to 5-HT receptor activation

Antagonist (μM)	Agonist	*n*	Concentration ratio geometric mean (95% CL)	E_{max} (percent control) mean ± S.E.M.	Apparent pK_B value geometric mean (95% CL)
Ketanserin (0.01)	5-CT (1st phase)[a]	6	0.95 (0.80–1.10)	99.1 ± 5.4	NA
MDL 72222 (10)	5-HT (1st phase)[b]	6	0.66 (0.15–1.17)	111.2 ± 12.6	NA
Yohimbine (0.1)	5-HT (1st phase)[b]	4	2.46 (0.96–3.96)	99.8 ± 17.2	7.03 (6.64–7.42)
(0.3)		4	5.66 (3.31–8.01)[c]	89.3 ± 21.3	7.14 (6.95–7.33)
(1.0)		4	8.87 (3.17–14.6)[c]	72.6 ± 14.8	6.87 (6.51–7.23)
Yohimbine (1.0)	5-CT (1st phase)[a]	6	5.72 (2.46–8.98)[c]	119.8 ± 15.8	6.71 (6.49–6.93)
Methiothepin (0.1)	5-CT (1st phase)[a]	6	3.00 (2.57–3.43)[c]	115.5 ± 14.2	7.30 (7.21–7.39)
Propranolol (0.3)	5-HT (1st phase)[b]	6	3.96 (4.86–8.76)	106.8 ± 12.6	7.12 (6.96–7.28)
(1.0)		6	8.98 (3.38–14.6)[c]	82.0 ± 9.6	6.87 (6.53–7.21)
(3.0)		6	14.12 (8.92–19.3)[c]	104.8 ± 16.5	6.56 (6.47–6.79)
Propranolol (1.0)	5-CT (1st phase)[a]	6	4.43 (1.93–6.93)[c]	115.3 ± 9.18	6.56 (6.32–6.80)
Propranolol (1.0)	Anpirtoline	6	6.45 (4.71–8.19)[c]	110.4 ± 10.3	6.73 (6.60–6.86)
Metergoline (0.01)	5-CT (1st phase)[a]	4	1.50 (1.31–1.69)	109.6 ± 9.3	NA
(0.1)		6	0.96 (0.72–1.20)	105.2 ± 6.8	NA

Cumulative concentration response curves to the agonists indicated were constructed in the presence and absence of the antagonists shown.
Control concentration response curves were constructed using adjacent vessel rings from the same animal within the same experiment.
Concentration ratios have been calculated from the EC_{50} obtained in the presence and absence of each antagonist with vessel rings from the same horse. The geometric mean concentration ratio from *n* animals is presented in the Table.
The E_{max} value obtained in the presence of the antagonist has been expressed as a percentage of the E_{max} value obtained in the appropriate control vessel ring and the mean ± S.E.M. value is presented in the Table.
Apparent pK_B values have been calculated where appropriate from each concentration ratio and the geometric mean value from *n* animals is presented.
[a]5-CT concentration response curves were constructed obtained to 0.1 nM to 0.3 μM giving the first phase of the concentration response curve which was fitted to a one site logistic equation.
[b]5-HT concentration response curves were constructed in the presence of 0.1 μM ketanserin, fitted to a two site logistic equation and the data from the first phase are presented.
[c]Denotes a significant ($P < 0.05$) change in EC_{50} or E_{max} value induced by the antagonist when tested by One-way ANOVA followed by Dunnett's test or by a paired Student's *t*-test.
NA—not applicable.
NC—non-competitive.
CL—confidence limits.

of the points about the line (expressed as the coefficient of variation) and the occurrence of consecutive points lying above or below the line. The equation giving the lowest sum of squares of the residuals and coefficient of variation and the least number of consecutive points above or below the fitted line was judged to be the most appropriate. The best fit values for EC_{50}, E_{max} and Hill coefficient (n_H) obtained from each vessel segment were used to calculate the geometric mean (and 95% confidence intervals; EC_{50}) and the arithmetic mean ± S.E.M. (E_{max} and n_H). EC_{50}, E_{max} and n_H values were compared between arteries and veins using a paired *t*-test. The effects of the antagonists on the EC_{50}, E_{max} and n_H were compared to the values obtained in the absence of the antagonists using One-way ANOVA followed by Dunnett's comparison (if more than one concentration of antagonist or more than one antagonist was compared to the same control curve) or a paired *t*-test. In all cases, statistical significance was accepted at $P < 0.05$. If no change in the E_{max} or n_H occurred, the apparent pK_B value for the antagonist was calculated using the equation:

$$pK_B = \log_{10}(\text{concentration ratio} - 1/[\text{antagonist}])$$

The concentration ratio was calculated from the EC_{50} obtained in the presence of the antagonist divided by the EC_{50} obtained in the absence of the antagonist. A concentration ratio and pK_B value was obtained for vessels from each animal and the geometric mean (and 95% confidence limits) are presented in each case. With the biphasic curves produced by 5-HT (in the presence of 0.1 μM ketanserin) when studying the effects of antagonists, the parameters for the process occurring at higher concentrations of 5-HT were not known accurately but this did not affect the estimates for the processes occurring at lower concentrations ($E_{max(1)}$, $EC_{50(1)}$ and $n_{H(1)}$). Concentration ratios and

Table 3
Effects of 5-HT receptor antagonists on the responses of equine digital veins to 5-HT receptor activation

Antagonist (μM)	Agonist	*n*	Concentration ratio geometric mean (95% CL)	E_{max} (percent control) mean ± S.E.M.	Apparent pK_B value geometric mean (95% CL)
Ketanserin (0.01)	5-CT (1st phase)[a]	6	0.71 (0.45–0.97)	102.7 ± 4.0	NA
Ketanserin (0.01)	Sumatriptan	4	0.92 (0.74–1.10)	105.9 ± 9.9	NA
MDL 72222 (10)	5-HT (1st phase)[b]	6	1.31 (0.62–2.00)	108.8 ± 12.7	NA
Yohimbine (0.1)	5-HT (1st phase)[b]	4	1.95 (1.25–2.65)	78.1 ± 23.4	NA
(0.3)		4	3.61 (2.65–4.57)[c]	64.4 ± 12.0[c]	NC
(1.0)		4	10.23 (5.07–15.4)[c]	65.8 ± 14.7[c]	NC
Yohimbine (1.0)	5-CT (1st phase)[a]	6	4.15 (1.95–6.35)[c]	83.4 ± 7.8[c]	NC
Yohimbine (0.1)	Sumatriptan	4	1.42 (0.66–2.18)	113.9 ± 13.2	NA
(1.0)		4	5.95 (4.55–7.35)[c]	94.3 ± 9.6	6.82 (6.60–7.00)
Methiothepin (0.1)	5-CT (1st phase)[a]	6	5.48 (3.93–7.03)[c]	57.0 ± 8.7[c]	NC
Methiothepin (0.01)	Sumatriptan	4	2.32 (0.48–3.69)	84.7 ± 12.5	7.79 (7.14–8.44)
(0.1)		4	9.75 (4.35–15.2)[c]	78.5 ± 9.2	7.93 (7.72–8.14)
(0.3)		4	18.30 (2.39–36.4)[c]	93.9 ± 11.2	7.78 (7.41–8.15)
Propranolol (1.0)	5-HT (1st phase)[b]	6	2.01 (0.21–3.81)	92.6 ± 15.6	NA
Propranolol (1.0)	5-CT (1st phase)[a]	6	0.76 (0.44–1.08)	95.6 ± 9.6	NA
Propranolol (1.0)	Sumatriptan	4	0.93 (0.25–1.61)	116.7 ± 14.2	NA
Propranolol (1.0)	Anpirtoline	6	1.10 (0.74–1.46)	88.8 ± 4.3	NA
Metergoline (0.01)	5-CT (1st phase)[a]	4	3.61 (3.08–4.14)[c]	97.0 ± 15.2	8.19 (8.06–8.32)
Metergoline (0.01)	Sumatriptan	6	2.92 (2.14–3.70)	104.6 ± 11.6	8.38 (8.18–8.58)
(0.1)		6	9.47 (0.67–18.3)[c]	101.4 ± 8.4	7.89 (7.54–8.24)

Cumulative concentration response curves to the agonist indicated were constructed in the presence and absence of the antagonists shown.
Control concentration response curves were constructed using adjacent vessel rings from the same animal within the same experiment.
Concentration ratios have been calculated from the EC_{50} obtained in the presence and absence of each antagonist with vessel rings from the same horse. The geometric mean concentration ratio from *n* animals is presented in the Table.
The E_{max} value obtained in the presence of the antagonist has been expressed as a percentage of the E_{max} value obtained in the appropriate control vessel ring and the mean ± S.E.M. value is presented in the Table.
[a]5-CT concentration response curves were constructed obtained to 0.1 nM to 0.3 μM giving the first phase of the concentration response curve which was fitted to a one site logistic equation.
[b]5-HT concentration response curves were constructed in the presence of 0.1 μM ketanserin, fitted to a two site logistic equation and the data from the first phase are presented.
Apparent pK_B values have been calculated where appropriate from each concentration ratio and the geometric mean value from *n* animals is presented.
[c]Denotes a significant ($P < 0.05$) change in EC_{50} or E_{max} value induced by the antagonist when tested by One-way ANOVA followed by Dunnett's test or by a paired Student's *t*-test.
NC—non-competitive inhibition.
NA—not applicable.
Cl—confidence limits.

pK_B values could be calculated from the $EC_{50(1)}$ values obtained in the presence and absence of antagonist provided there was no change in the $E_{max(1)}$ and $n_{H(1)}$ values by using the Gaddum–Schild equation as described above.

2.6. Drugs and solutions

The modified Krebs Henseleit solution had the following composition (mM): NaCl 118, KCl 4.57, $CaCl_2$ 1.27, KH_2PO_4 1.19, $MgSO_4$ 1.19, $NaHCO_3$ 25 and glucose 5.55. 5-hydroxytryptamine creatinine sulphate, (±)-8-hydroxy-2-(di-*n*-propylamino) tetralin hydrobromide (8-OH-DPAT), pargyline hydrochloride and yohimbine hydrochloride were obtained from Sigma, Poole, Dorset. 5-carboxamidotryptamine maleate, 6-chloro-2-[piperidyl-4-thiol]-pyridine hydrochloride (anpirtoline), methiothepin mesylate, metergoline, 1α *H*,3α,5α *H*-tropan-3yl-3,5-dichlorobenzoate (MDL 72222), ketanserin tartrate, benextramine hydrochloride and *S*(−)-propranolol hydrochloride were purchased from Research Biochemicals International, Natick, MA, USA. Sumatriptan (3-[2-dimethylamino]-ethyl-*N*-methyl-1 *H*-indole-5-methane sulphonamid) was a generous gift from Glaxo, Greenford, UK. All other reagents were of analytical grade where possible.

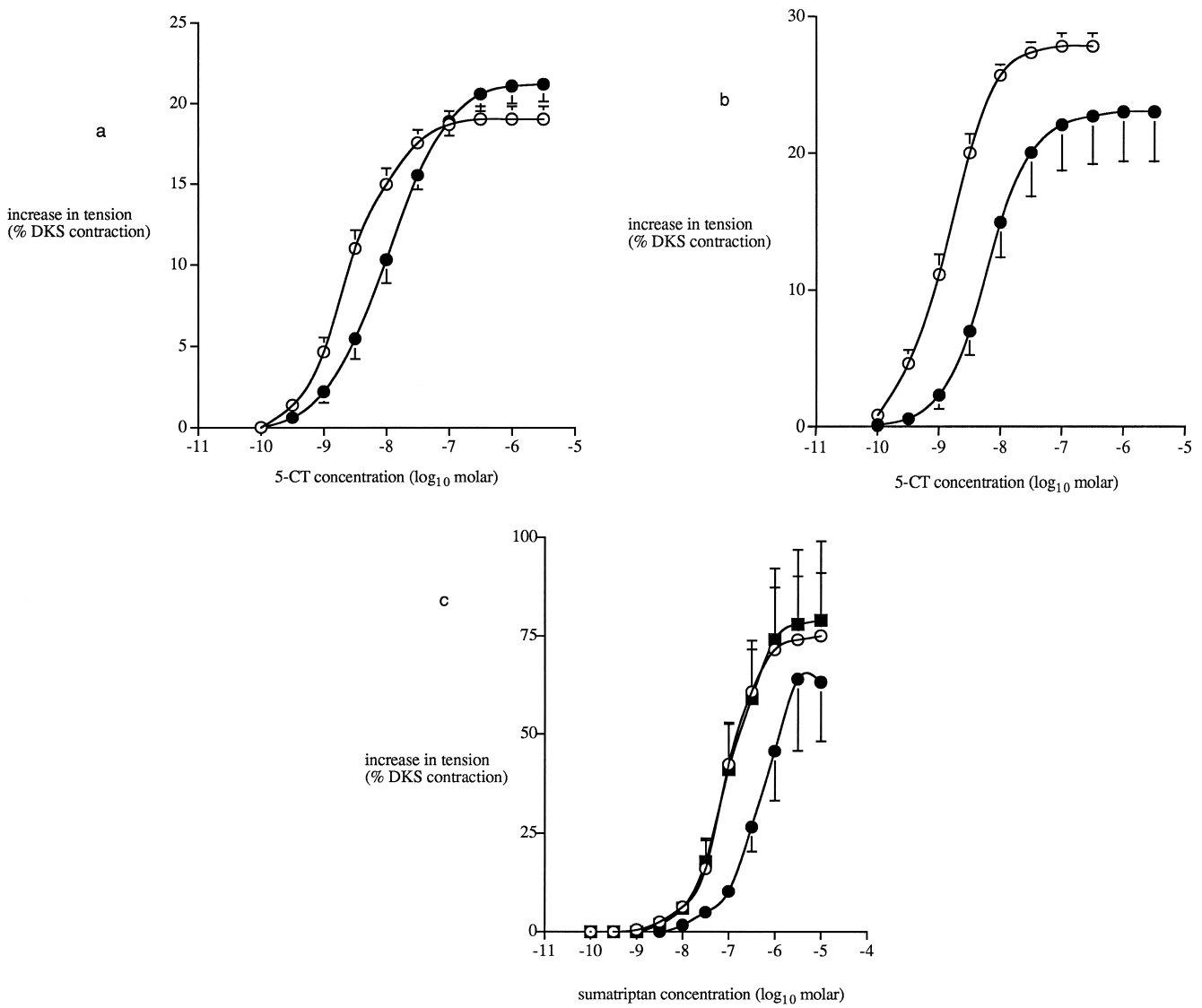

Fig. 2. Cumulative concentration response curves showing the effect of yohimbine on the contractile response to 5-carboxamidotryptamine of segments of equine digital artery (a) and vein (b), and on the responses to sumatriptan of digital veins (c). Responses were obtained to 5-CT or sumatriptan in the absence (○; control) or in the presence of yohimbine (■ 0.3 μM; ● 1 μM). Only one concentration response curve was obtained for each vessel ring and adjacent rings from the same horse were examined in the absence or presence of antagonist within each experiment. Each point represents the mean value ± S.E.M. obtained from blood vessel segments from six horses. Each individual response curve was fitted to a one site logistic equation and the curve shown was drawn by interpolation through each mean value. Mean values of EC_{50}, E_{max} and n_H are shown in Tables 2 and 3. DKS—depolarising Krebs solution.

All drugs, with the exception of metergoline, were dissolved initially in distilled water or 0.01 M hydrochloric acid and diluted in 0.9% w/v saline. Metergoline was dissolved in ethanol initially and diluted in 0.9% w/v saline. All solutions were freshly prepared on the day of the experiment.

3. Results

3.1. Agonist studies

In the presence of increasing concentrations of ketanserin, the cumulative concentration response curves of both digital arteries and veins to 5-HT were biphasic (see Fig. 1a and b). In arteries, the $EC_{50(1)}$ values (95% confidence limits) were 14.1 (6–22), 21.1 (14–28) and 18.7 (4–33) nM and the $EC_{50(2)}$ values were 0.8 (0.58–1.03), 2.9 (2.3–3.4) and 9.46 (4.7–14.2) μM in the presence of 10, 30 and 100 nM ketanserin, respectively. In veins, the $EC_{50(1)}$ values (95% confidence limits) were 6.99 (4.8–9.1), 6.28 (3.8–8.8) and 5.43 (2.4 to 8.5) nM and the $EC_{50(2)}$ values were 1.1 (0.9–1.2), 1.88 (1.4–2.3) and 6.14 (3.0–9.3) μM in the presence of 10, 30 and 100 nM ketanserin, respectively. No significant changes in the E_{max} or n_H values were found to occur in either of the two phases of the cumulative concentration response curve with increasing concentrations of ketanserin.

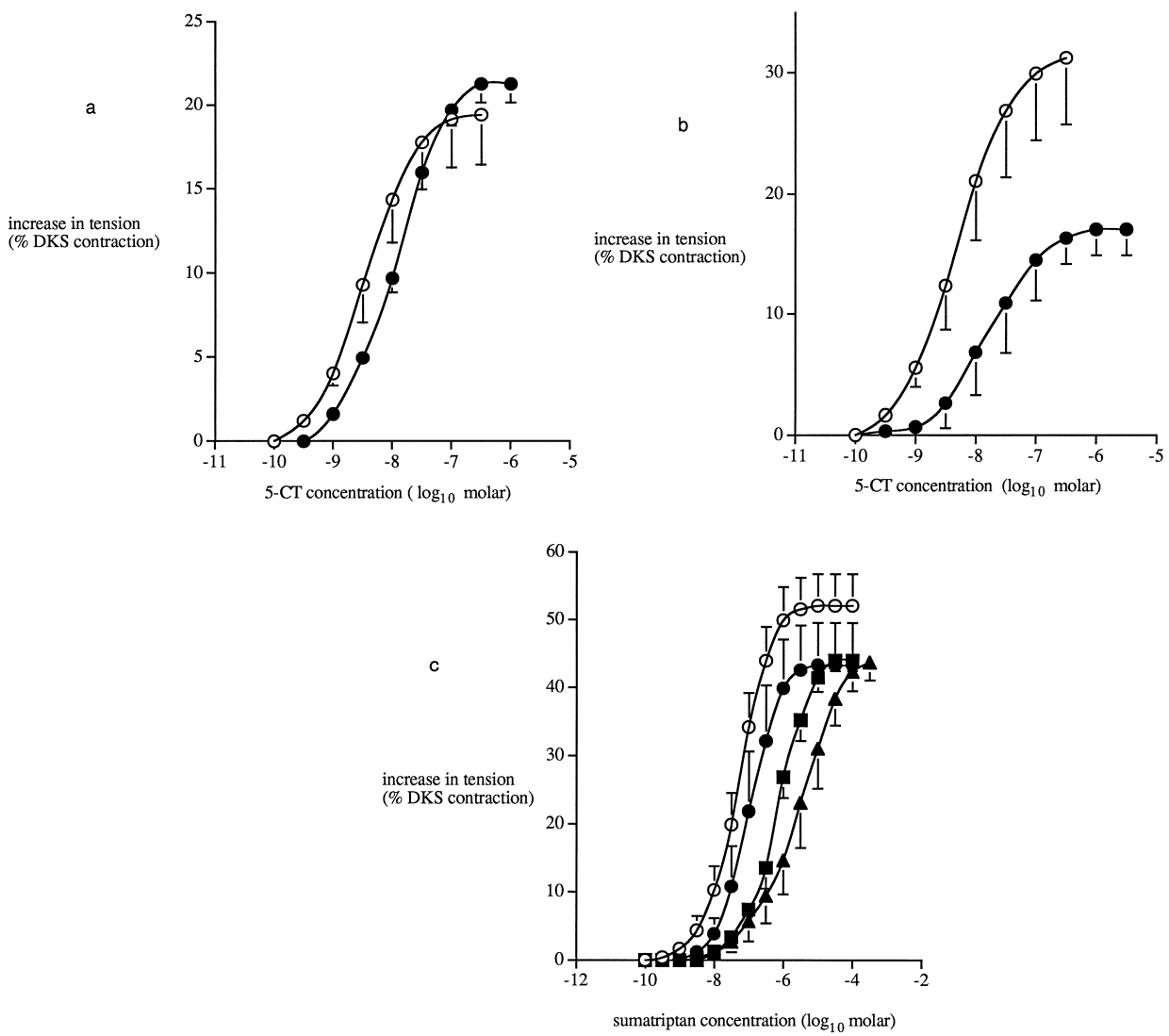

Fig. 3. Cumulative concentration response curves showing the effect of methiothepin on the contractile response to 5-carboxamidotryptamine of segments of equine digital artery (a) and vein (b), and on the responses to sumatriptan of digital veins (c). Responses were obtained to 5-CT or sumatriptan in the absence (○; control) or in the presence of methiothepin (● 10 nM; ■ 30 nM; ▲ 0.1 μM). Only one concentration response curve was obtained for each vessel ring and adjacent rings from the same horse were examined in the absence or presence of antagonist within each experiment. Each point represents the mean value ± S.E.M. obtained from blood vessel segments from six horses. Each individual response curve was fitted to a one site logistic equation and the curve shown was drawn by interpolation through each mean value. Mean values of EC_{50}, E_{max} and n_H are shown in Tables 2 and 3. DKS—depolarising Krebs solution.

The EC_{50}, E_{max} and n_H values for the series of agonists tested are presented in Table 1 and the cumulative concentration response curves are shown in Fig. 1c and d. As with 5-HT (in the presence of 0.1 μM ketanserin), 5-CT also produced cumulative concentration response curves which were best fitted to the Eq. (2) whereas the other agonist cumulative concentration response curves fitted best to Eq. (1). Anpirtoline and sumatriptan were 17.9 and 10 times more potent on digital veins compared to digital arteries and produced 5.6 and 4.1 times higher maximum responses in veins compared to arteries. All the other agonists tested proved to be equipotent and produced the same maximum responses in arteries and veins.

3.2. Antagonist studies

The concentration ratios calculated for the effects of various 5-HT receptor antagonists tested against 5-HT (in the presence of 0.1 μM ketanserin), 5-CT, or anpirtoline in arteries and veins and sumatriptan in veins are presented in Table 2 (arteries) and Table 3 (veins).

Ketanserin (10 nM) had no significant effect on responses to low concentrations of 5-CT (0.1 nM to 0.3 μM)

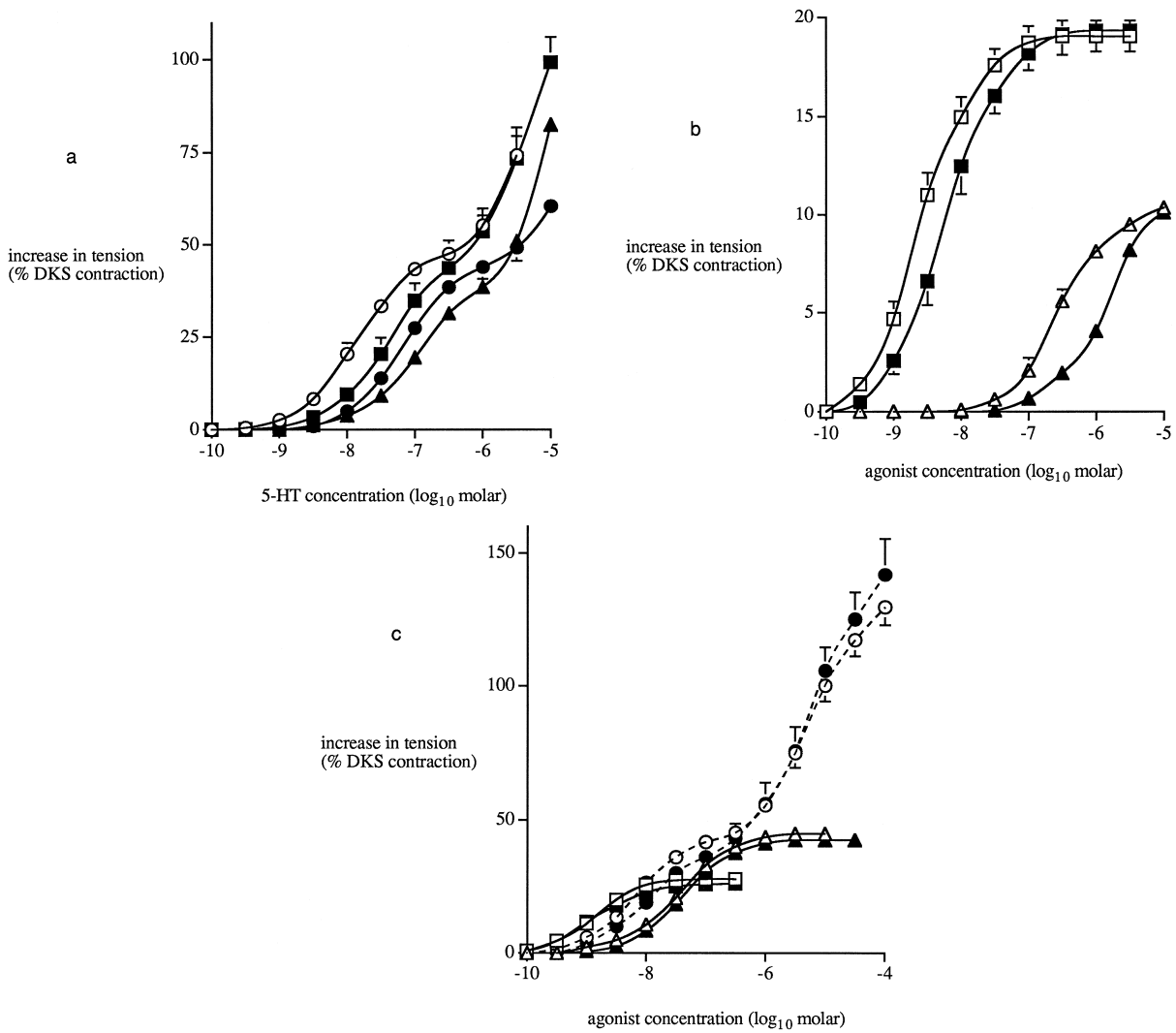

Fig. 4. Cumulative concentration response curves showing the effect of propranolol on the contractile responses to 5-HT in the presence of 0.1 μM ketanserin (a) and to 5-carboxamidotryptamine and anpirtoline (b) of equine digital arteries and on the responses to the same three agonists of digital veins (c). Responses were obtained to 5-HT in the absence (○; control) or in the presence of propranolol (■ 0.3 μM; ● 1 μM; ▲ 3 μM). Responses to 5-CT and anpirtoline were also obtained in the absence (□ and △, respectively) or in the presence of propranolol (■ and ▲, respectively; 1 μM). Only one concentration response curve was obtained for each vessel ring and adjacent rings from the same horse were examined in the absence or presence of antagonist within each experiment. Each point represents the mean value ± S.E.M. obtained from blood vessel segments from six horses. Each individual response curve was fitted to a one site logistic equation and the curve shown was drawn by interpolation through each mean value. Mean values of EC_{50}, E_{max} and n_H are shown in Tables 2 and 3. DKS—depolarising Krebs solution.

in the arteries and veins and to sumatriptan in the veins. MDL 72222 (10 μM) caused no significant inhibition of cumulative concentration response curves to 5-HT (obtained in the presence of 0.1 μM ketanserin) in either arteries or veins.

Yohimbine caused concentration dependent inhibition of the first phases of the cumulative concentration response curves to 5-HT (obtained in the presence of 0.1 μM ketanserin) in both arteries and veins (see Tables 2 and 3). In the arteries, the effect of yohimbine appeared competitive at 0.1, 0.3 and 1 μM giving apparent pK_B values of 7.03, 7.14 and 6.87. At 1 μM, yohimbine caused competitive antagonism of the responses to low concentrations of 5-CT in arteries (see Fig. 2) giving an apparent pK_B value of 6.71. Venous responses to 5-HT (in the presence of 0.1 μM ketanserin) and to low concentrations of 5-CT were non-competitively antagonised by yohimbine which suppressed the maximum response of the first phase of the cumulative concentration response curves to both these agonists. Yohimbine, however, caused concentration dependent and competitive inhibition of the venous responses to sumatriptan giving apparent pK_B value of 6.82.

Methiothepin (0.1 μM) competitively antagonised arterial responses to low concentrations of 5-CT giving an apparent pK_B value of 7.30 (Fig. 3a and Table 2). In venous tissue, methiothepin caused non-competitive antagonism of 5-CT responses but concentration dependent and competitive antagonism of responses to sumatriptan giving

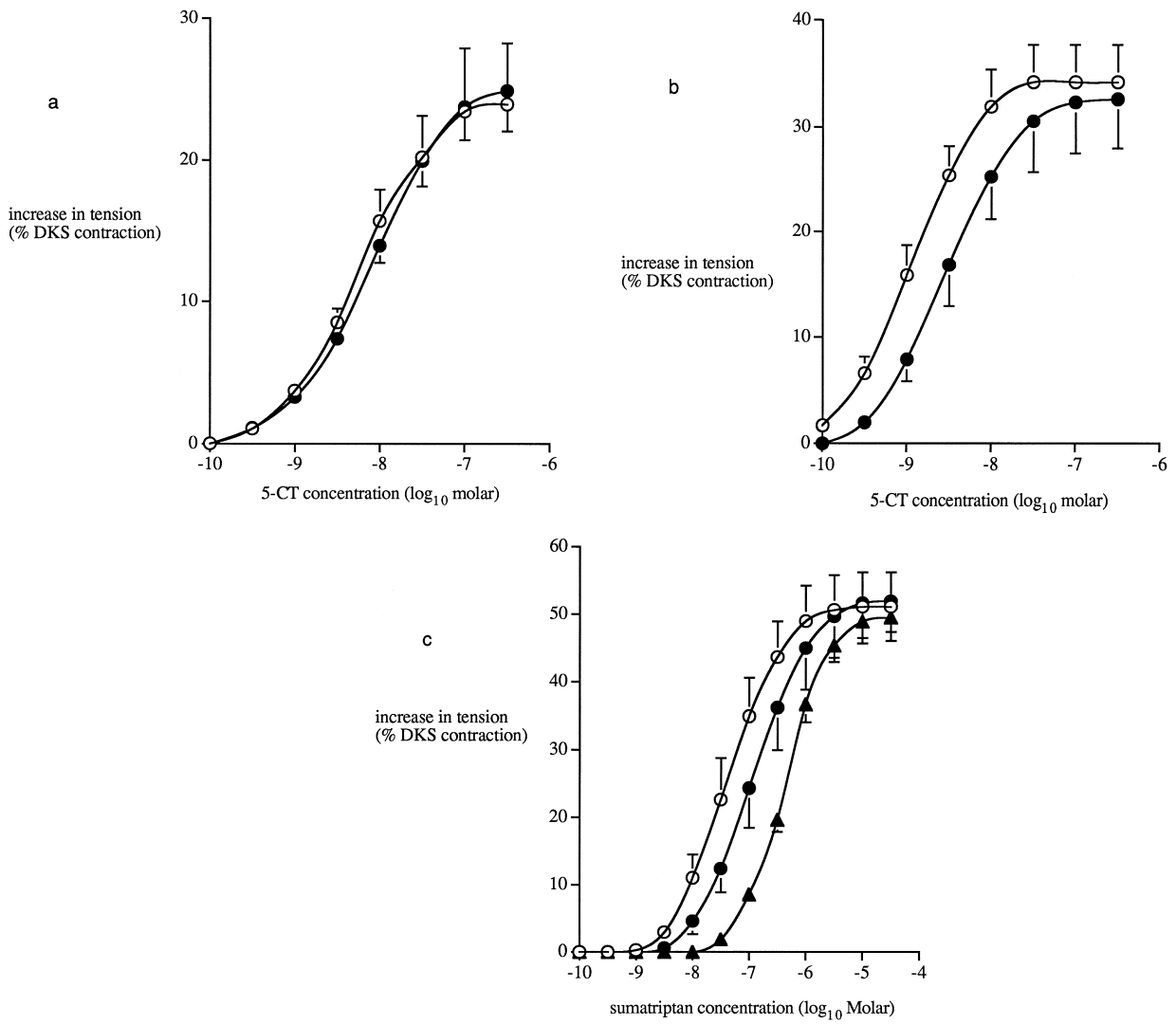

Fig. 5. Cumulative concentration response curves showing the effect of metergoline on the contractile responses to 5-carboxamidotryptamine of segments of equine digital artery (a) and vein (b) and on the responses to sumatriptan of digital veins (c). Responses were obtained to these agonists in the absence (○; control) or in the presence of metergoline (• 10 nM; ▲ 0.1 μM). Only one concentration response curve was obtained for each vessel ring and adjacent rings from the same horse were examined in the absence or presence of antagonist within each experiment. Each point represents the mean value ± S.E.M. obtained from blood vessel segments from six horses. Each individual response curve was fitted to a one site logistic equation and the curve shown was drawn by interpolation through each mean value. Mean values of EC_{50}, E_{max} and n_H are shown in Tables 2 and 3. DKS—depolarising Krebs solution.

pK_B values of 7.79, 7.93 and 7.78 at 0.01, 0.1 and 0.3 μM, respectively (Fig. 3b and c and Table 3).

Propranolol (0.3 to 3 μM) caused concentration dependent and apparently competitive antagonism of the first phase of the arterial responses to 5-HT (in the presence of 0.1 μM ketanserin; see Fig. 4a and Table 2) giving apparent pK_B values of 7.12, 6.87 and 6.56, respectively. At 1 μM, propranolol also appeared to competitively antagonise arterial responses to 5-CT and anpirtoline (Fig. 4b and Table 2; pK_B values of 6.56 and 6.73) but had no significant effect on venous responses to 5-HT (in the presence of 0.1 μM ketanserin), sumatriptan, anpirtoline or 5-CT (see Fig. 4c and Table 3).

Metergoline (0.01 and 0.1 μM) had no significant effect on arterial responses to low concentrations of 5-CT (Fig. 5a and Table 2). In venous tissue, however, the responses to 5-CT appeared to be competitively inhibited by 0.01 μM metergoline, giving an apparent pK_B value of 8.19 (Fig. 5b and Table 3). Metergoline (0.01 and 0.1 μM) also caused concentration dependent and apparently competitive inhibition of responses to sumatriptan giving apparent pK_B values of 8.38 and 7.89, respectively (Fig. 5c and Table 3).

4. Discussion

Our previous work had shown that equine digital veins possess both 5-$HT_{1B/1D}$ and 5-HT_2 receptors mediating vasoconstriction (Weller et al., 1994; Soydan and Elliott, 1995) and the present study utilised 5-HT receptor agonists and antagonists to investigate further the nature of the 5-$HT_{1B/1D}$ receptors and compare directly the properties of these receptors found on digital veins with those found on digital arteries.

4.1. Effects of ketanserin

Ketanserin, the 5-HT_{2A} receptor-selective antagonist, was used at a concentration of 0.1 μM to produce a biphasic cumulative concentration response curve for both digital arteries and digital veins to 5-HT. Fitting the cumulative concentration response curve data to a two process equation enabled potency data to be obtained for 5-HT at the 5-$HT_{1B/1D}$ receptor. Biphasic cumulative concentration response curves could be discerned in the presence 10 and 30 nM ketaserin and the EC_{50}, E_{max} or n_H of the first phase of the 5-HT cumulative concentration response curve did not change with increasing concentrations of ketanserin, suggesting that the 5-HT_1 receptor mediating vasoconstriction in digital arteries and digital veins was resistant to inhibition by submicromolar concentrations of ketanserin. These findings concur with those from vasoconstrictor 5-$HT_{1B/1D}$ receptors of other vascular preparations (Humphrey et al., 1988; Parsons, 1991; Bax et al., 1992) but differ from findings in rabbit saphenous vein, where submicromolar concentrations of ketanserin were found to inhibit vasoconstrictor responses mediated via 5-$HT_{1B/1D}$ receptors (see Martin and MacLennan, 1990).

4.2. Responses to 5-carboxamidotryptamine

5-CT produced biphasic concentration response curves in both digital arteries and digital veins in the absence of ketanserin, consistent with previous observations that this drug is selective for 5-HT_1 receptors over 5-HT_2 receptors. Nevertheless, 5-CT is non-selective for different 5-HT_1 receptor sub-types (see Hoyer et al., 1994). The first phases of the 5-CT cumulative concentration response curve in both digital arteries and digital veins were sufficiently distinct that responses analysed over the concentration range 0.1 nM to 0.3 μM could be fitted to a one site logistic equation and the effects of antagonists on this first phase of the 5-CT cumulative concentration response curve could be reliably studied. Responses to 5-CT over this concentration range were not blocked by submicromolar concentrations of ketanserin in either digital arteries or digital veins, a property which is consistent with the conclusion that 5-$HT_{1B/1D}$ receptors mediated the responses seen.

The lack of inhibition of ketanserin-resistant 5-HT responses in either digital arteries or digital veins by a high concentration of the 5-HT_3 receptor-selective antagonist, MDL 72222 (Fozard, 1984) suggested that 5-HT_3 receptors were not involved in mediating the responses seen. 5-HT_4 and 5-HT_7 receptor mRNA has been reported to be present in some blood vessels (Ullmer et al., 1995) but functionally, these receptors have been shown to be linked to activation of adenylate cyclase in cardiac tissue (Kaumann et al., 1990) and to vasorelaxation (Cocks and Arnold, 1992; Leung et al., 1996). The low potency of the 5-HT_{1A} receptor-selective agonist 8-OH-DPAT (Gozlan et al., 1983) found in the present study would suggest that 5-HT_{1A} receptors were not involved in the vasoconstrictor response of digital arteries and veins.

4.3. Responses to sumatriptan and anpirtoline

Sumatriptan, an agonist with some selectivity for 5-HT_{1D} receptors (Peroutka and McCarthy, 1989), caused concentration dependent constriction of digital veins and proved to be 9.1 times less potent and produced a 1.45 times higher maximum response than the ketanserin-resistant response to 5-HT. Ketanserin did not block the responses to sumatriptan, a finding which is consistent with the conclusion that 5-$HT_{1B/1D}$ receptors were mediating the responses seen. The potency of sumatriptan relative to that of 5-HT in digital veins was similar to reports for these agonists acting at 5-$HT_{1B/1D}$ receptors of other blood vessels, such as rabbit cerebral arteries (Deckert et al., 1994), dog saphenous vein (Humphrey et al., 1988) and human saphenous vein (Bax et al., 1992). By contrast, in

digital arteries sumatriptan was 25.8 times less potent and produced a maximum response which was 3.4 times lower than the ketanserin resistant responses to 5-HT.

We also examined the commercially available 5-$HT_{1B/1D}$ receptor agonist, anpirtoline, which has been demonstrated to be a potent agonist at the rodent 5-HT_{1B} receptor (Schlicker et al., 1992). The same pattern of results described above for sumatriptan was also found for anpirtoline. This compound, like sumatriptan, had significantly higher potency (17.8 times) and produced a larger maximum response (4.1 times) in digital veins when compared to digital arteries. The other three agonists tested on digital veins and digital arteries did not differ significantly in terms of their potency or the magnitude of the maximum responses obtained. These data suggest that sumatriptan and anpirtoline were much less effective agonists at the 5-$HT_{1B/1D}$ arterial receptor than the equivalent receptor on the venous side of the circulation when examined under identical conditions. It is possible that these findings could be explained in terms of a lower 5-$HT_{1B/1D}$ receptor reserve being present in digital arteries compared to digital veins or by the presence of different sub-types of 5-$HT_{1B/1D}$ receptors on the two types of digital blood vessel.

4.4. Antagonism of responses by methiothepin and yohimbine

In order that this question might be addressed, the effects of receptor antagonists were examined on the 5-HT_1 receptor mediated vasoconstriction of digital arteries and digital veins. The definitive classification of 5-$HT_{1B/1D}$ receptors mediating vasoconstriction is currently hampered by the lack of a truly selective antagonist. Potent antagonism by the mixed 5-HT_1/5-HT_2 receptor antagonist, methiothepin, is one of the properties common to all vascular 5-$HT_{1B/1D}$ receptors mediating vasoconstriction. 5-CT responses in digital arteries were blocked competitively by methiothepin, giving an apparent pK_B of 7.3. Methiothepin also competitively antagonised the responses of sumatriptan in digital veins with an apparent pK_B value of 7.83. These pK_B values are similar to those reported 5-$HT_{1B/1D}$ receptors in dog and human saphenous veins (Humphrey et al., 1988; Bax et al., 1992). Antagonism of 5-CT responses in digital veins by methiothepin proved to be non-surmountable so it was not possible to make direct comparison of methiothepin's affinity between arteries and veins using the same agonist.

Yohimbine produced concentration dependent competitive antagonism of ketanserin resistant 5-HT responses in digital arteries, giving mean apparent pK_B value of 7.01. Antagonism of 5-CT responses in digital arteries also appeared competitive and an apparent pK_B value of 6.70 was obtained. Once again, antagonism by yohimbine of the responses of digital veins to the same agonists proved to be non-surmountable so a direct comparison was not possible. Nevertheless, yohimbine did block responses of digital veins to sumatriptan in a competitive manner and an apparent pK_B value of 6.82 was obtained. These pK_B values obtained for yohimbine are in close agreement with the values reported for 5-$HT_{1B/1D}$ mediated constriction of rabbit cerebral arteries (Deckert et al., 1994) and with the reported affinity of yohimbine for 5-HT_{1D} receptors in neuronal tissue (Waeber et al., 1988).

The mechanism by which methiothepin and yohimbine caused non-surmountable inhibition of 5-HT and 5-CT responses in digital veins remains to be determined but is not an unusual finding for antagonists used to classify 5-HT receptors (see Leff and Martin, 1989). This phenomenon could be explained on the basis of slow dissociation kinetics of the antagonist producing this effect. However, if 5-HT, 5-CT and sumatriptan all activate the same receptor in digital veins, it seems more likely that methiothepin and yohimbine have some additional effect on post-receptor mechanisms in digital veins and that lower efficacy of 5-HT and 5-CT at the 5-HT_1 receptor of digital veins, when compared to sumatriptan, has led to the non-surmountable antagonism being seen with these two agonists. The same effect was not evident in digital artery, possibly because 5-HT and 5-CT had greater receptor reserve in this tissue.

4.5. Antagonism of responses by propranolol and metergoline

S(−)-propranolol, the β-adrenoceptor antagonist which has affinity for the rodent neuronal 5-HT_{1B} receptor and 5-HT_{1A} receptors (see Hoyer et al., 1994), blocked the responses of the digital artery to 5-HT_1 receptor stimulation. Competitive antagonism was seen for propranolol vs. 5-HT (in the presence of 0.1 μM ketanserin), 5-CT and anpirtoline, giving rise to pK_B values ranging from 6.56 to 7.12. The calculated affinity of propranolol for digital artery 5-$HT_{1B/1D}$ receptors is similar to its affinity for the rodent neuronal 5-HT_{1B} receptor in binding and functional studies (Hoyer et al., 1985; Engel et al., 1986). No similar significant inhibitory effects of propranolol at 1 μM were seen when this antagonist was used against 5-HT, 5-CT, anpirtoline and sumatriptan in equine digital vein. It would have been useful to have examined higher concentrations of propranolol to determine more precisely the difference in affinity of this antagonist for arterial vs. venous 5-HT_1 receptors in the present study. Unfortunately, at concentrations of 10 μM and above, generalised suppression of vasoconstrictor responses of both digital arteries and digital veins occurred (data not shown) making the affinity of propranolol for digital vein 5-HT_1 receptors impossible to determine.

Metergoline, the 5-HT_{1D}/5-HT_{2C} receptor antagonist demonstrated concentration dependent and competitive antagonism of responses of digital veins to sumatriptan giving rise to a pK_B of 8.14. Competitive antagonism of responses of digital veins to 5-CT was also observed and a

pK_B of 8.19 found. Metergoline has been shown to inhibit 5-HT_1 receptor mediated vasoconstriction of human saphenous vein (Bax et al., 1992) with a pK_B value of 7.3. By contrast, in the present study, arterial responses to 5-CT were completely resistant to metergoline at the highest concentration tested (0.1 μM). There is evidence that at concentrations of 1 μM and above, metergoline causes non-specific antagonism of vasoconstrictor agents (Perren et al., 1991), thus higher concentrations of metergoline were not tested in the present study.

5. Conclusions

The data from the present study provides evidence that 5-$HT_{1B/1D}$ receptors mediating vasoconstriction of the digital blood vessels of the horse are different on the arterial and venous sides of the circulation. Agonist and antagonist data suggest that the digital venous receptor is a classical vascular 5-$HT_{1B/1D}$ receptor being potently and effectively stimulated by sumatriptan, resistant to inhibition by propranolol and sensitive to inhibition by metergoline. The non-surmountable nature of methiothepin and yohimbine as antagonists of responses to 5-HT and 5-CT in digital veins made these antagonists less useful in the characterisation of the venous receptor. The arterial receptor differed from the venous receptor in terms of the low potency and efficacy of sumatriptan and anpirtoline relative to 5-HT and 5-CT as agonists. Sensitivity to competitive inhibition by propranolol and resistance to inhibition by metergoline showed that the differences in potency and efficacy of sumatriptan and anpirtoline were not due to low receptor reserve in arterial tissue compared to venous tissue, but occurred because a different receptor sub-type mediated the ketanserin-resistant effects of 5-CT and 5-HT. Neither propranolol nor metergoline were ideal receptor antagonists for these functional studies nevertheless, our data suggests these potency ratios were greater than 10 for both antagonists. Further studies are necessary using newly discovered compounds with selectivity for 5-HT_{1B} or 5-HT_{1D} receptors to characterise the arterial and venous receptors more clearly.

It is interesting to note that the human 5-HT_{1B} receptor has high affinity for metergoline and sumatriptan and low affinity for propranolol in its wild form but that replacement of a single amino acid (threonine at residue 355 replaced with asparagine) resulted in a substantial decrease in affinity for metergoline and sumatriptan and a dramatic increase in affinity for propranolol (Oksenberg et al., 1992). The structural differences between 5-$HT_{1B/1D}$ receptors in equine digital arteries and veins responsible for their differing pharmacological properties may, therefore, be very small.

The pharmacological differences in the 5-$HT_{1B/1D}$ receptors between arterial and venous components of the equine digital circulation may be of significance in the pathogenesis of acute equine laminitis. The high potency of 5-HT as a vasoconstrictor of equine digital blood vessels relative to other agonists, such as noradrenaline, has been known for some time (Baxter et al., 1989). If 5-HT is indeed an important mediator of the haemodynamic disturbances seen in laminitis, differences in the properties of receptors for this vasoactive amine on the arterial and venous sides of the circulation may be of importance in understanding pathophysiology of the haemodynamic disturbances, such as intense venoconstriction (Allen et al., 1990) which cause this ischaemic disease.

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