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An examination of 5-hydroxytryptamine receptors in human saphenous vein

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1 We have examined the effects of antagonists on the isometric contraction of the human saphenous vein produced by 5-hydroxytryptamine (5-HT).

2 The 5-HT_2-antagonist ketanserin (1 μM) had little effect on the lower part of the concentration-response curve to 5-HT, but markedly shifted the upper part of the curve.

3 Yohimbine caused an approximately parallel shift of the concentration-response curve to 5-HT, with a pA_2 of 5.48, much lower than its pA_2 against noradrenaline in the absence (6.36) or presence (7.06) of cocaine.

4 It is concluded that there are two components to the contractile response to 5-HT in human saphenous vein: at low concentrations 5-HT activates a yohimbine-sensitive receptor, and at higher concentrations 5-HT activates a 5-HT_2-receptor.

Introduction

Receptors for 5-hydroxytryptamine (5-HT) were subdivided into 5-HT_1 and 5-HT_2 based on the affinities of radioligands for brain binding sites (see Peroutka & Snyder, 1979) and the 5-HT_1 binding site has been further subclassified (Middlemiss & Fozard, 1983). In the periphery, 5-HT is a potent vasoconstrictor in many vascular beds, but the constriction seems to involve two distinct receptor subtypes: contractions to 5-HT in the rabbit aorta, rat aorta, rat tail artery and dog femoral artery are mediated by 5-HT_2-receptors at which ketanserin is a potent and competitive antagonist (Humphrey, 1984; Peroutka, 1984); contractions in the dog saphenous vein and human and dog basilar arteries are mediated by 5-HT_1 or 5-HT_1-like receptors (Peroutka, 1984; Feniuk *et al.*, 1985). However, no selective antagonist is available for characterization of these 5-HT_1-like receptors, and indeed the phrase 5-HT_1-like has also been used to describe the 5-HT receptors mediating vasodilatation (Marwood & Stokes, 1984), tachycardia (Saxena *et al.*, 1985) and inhibition of transmitter release (Fozard, 1984a).

The object of this study was to characterize the receptors mediating contractions to 5-HT in human saphenous vein, and to ascertain whether a homogeneous population of receptors is present. 5-HT is a potent veniconstrictor in the human saphenous vein and its effects are antagonized by yohimbine in concentrations higher than those necessary for α-adrenoceptor blockade (Müller-Schweinitzer, 1984). Thus, we examined the effects of yohimbine, the 5-HT_2-antagonists ketanserin, cyproheptadine and methysergide and the neuronal 5-HT receptor antagonist MDL 72222 (Fozard, 1984b) on the contractions produced by 5-HT.

Some of these results have been published in abstract form (Docherty & Hyland, 1985a).

Methods

Human saphenous veins were obtained from coronary artery bypass grafts of male patients aged 37–71 years. None had been treated with α-adrenoceptor agonists or antagonists or drugs which interact with 5-HT receptors. We were unable to find any predictable effects of other prior drug treatment on tissue responsiveness in comparison to tissues from patients receiving no medication. Tissues were cut spirally into strips approximately 3 mm wide and 20–30 mm long, placed between platinum electrodes in organ baths and superfused at 37°C in Krebs-Henseleit solution of the following composition (mM): NaCl 119, $NaHCO_3$ 25, (+)-glucose 11.1, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.0, ascorbic acid 0.28 and tetrasodium EDTA 0.03. In all experiments using noradrenaline, propranolol (1 μM) and in some experiments, cocaine (1 μM) was present. Tissues were attached to myograph transducers under 1 g tension for isometric tension recording.

In all experiments, after 30 min equilibration, tis-

sues were pre-exposed to the test agonist (noradrenaline or 5-HT) in a concentration of 1 μM, and the agonist was washed out. This procedure was found to improve tissue responsiveness. Bathing fluid was changed every 15 min and after a further 60 min the test agonist was administered cumulatively in 0.5 log unit additions. When the maximum contraction to the agonist was reached, the agonist was washed out, and the bathing fluid was changed every 15 min for the next 120 min, for the final 60 min of which the test antagonist or vehicle was present. The agonist was again administered cumulatively in the presence of the antagonist, until a maximum contraction was reached. Agonist potency was expressed as an EC_{50} (concentration producing 50% of maximum contraction) and effects of antagonist were examined on the EC_{50} and maximum contractile response to the agonist. When appropriate, the potency of the antagonist was expressed as a pA_2, the negative log of the concentration producing a 2 fold shift in agonist potency, by obtaining the x-intercept of the relationship between log (agonist dose-ratio -1) and log antagonist concentration (Arunlakshana & Schild, 1959). Antagonist pA_2 values were not calculated where the antagonist reduced the maximum response to the agonist or caused non-parallel shifts in the agonist concentration-response curve or where the slope of the Schild plot was significantly different from negative unity. In control experiments, an equivalent volume of distilled water replaced the antagonist.

Drugs

Cocaine hydrochloride (Cockburns); cyproheptadine hydrochloride (Sigma); desipramine hydrochloride (Sigma); 5-hydroxytryptamine hydrochloride (Sigma); ketanserin hydrochloride (gift: Janssen); MDL 72222 (1αH, 3αH, 5αH-tropan-3yl 3,5 dichlorobenzoate methane sulphonate; gift: Merrell); methysergide maleate (gift: Sandoz); noradrenaline hydrochloride (Sigma); (±)-propranolol hydrochloride (Sigma); yohimbine hydrochloride (Sigma). Drug stocks were prepared and dilutions made up in distilled water with the exception of ketanserin (stock dissolved in 20 μl of acetic acid 1 M). Distilled water was administered in control experiments.

Statistics

Values are expressed as mean and s.e.mean or mean and 95% confidence limits. Differences caused by drugs in agonist pD_2 ($-\log EC_{50}$) or maximum contraction were compared, by Student's t test for paired data, with pre-drug values, or by Student's unpaired t test, with the effects of distilled water vehicle (in control experiments, maximum contraction increased significantly in the second agonist concentration-response curve). Differences in the slope or elevation (i.e. position) of the Schild plots for yohimbine against different agonists were compared by Analysis of Covariance (see Snedecor & Cochran, 1980).

Results

5-HT contracted the human saphenous vein with an EC_{50} of 0.40 μM (95% confidence limits of 0.27–0.59 μM, $n = 29$) and a maximum contraction of 0.99 ± 0.12 g ($n = 29$). Noradrenaline (NA) contracted the human saphenous vein with an EC_{50} of 0.45 μM (0.14–1.38 μM, $n = 7$) and a maximum contraction of 0.93 ± 0.26 g ($n = 7$). In the presence of cocaine (1 μM), the EC_{50} of NA was not significantly altered (0.48 μM, 95% confidence limits of 0.16–1.41 μM, $n = 7$). In experiments in which desipramine (1 μM) was administered from 60 min before the second concentration-response curve to 5-HT, the potency of 5-HT was not significantly altered between the first and second administrations (EC_{50}s of 0.16 μM and 0.20 μM, respectively, $n = 4$).

For Figures 1 and 2, agonist concentrations producing 10–90% of maximum were interpolated from individual concentration-response curves, and graphs were constructed from the mean concentrations obtained. This eliminates distortion of curve shape that occurs when individual curves are averaged vertically rather than horizontally (see Docherty & Starke, 1981). The 5-HT_2 antagonist ketanserin (1 μM) produced a non-parallel shift in the 5-HT concentration-response curve, so that there was no shift in the effects of low concentrations of 5-HT but a marked shift in the effects of high concentrations of 5-HT (Figure 1). In contrast, yohimbine (10 μM) produced a near parallel shift in the potency of 5-HT (Figure 2). There was no shift in the potency of 5-HT in vehicle experiments.

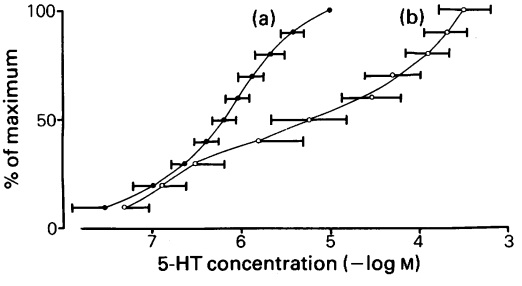

Figure 1 Mean concentration-response curves obtained to 5-hydroxytryptamine (5-HT) before (a, ●) and after (b, ○) 60 min exposure to ketanserin (1 μM) in human saphenous vein. Horizontal lines indicate s.e.mean, $n = 4$.

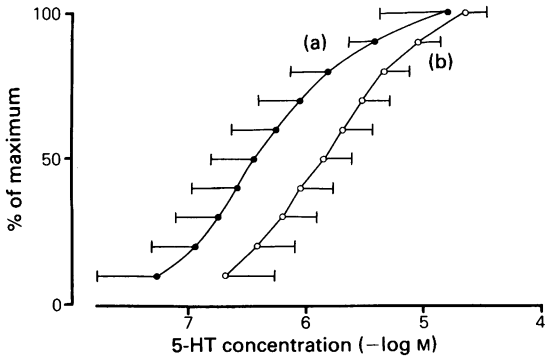

Figure 2 Mean concentration-response curves obtained to 5-hydroxytryptamine (5-HT) before (a, ●) and after (b, ○) 60 min exposure to yohimbine (10 μM) in human saphenous vein. Horizontal lines indicate s.e.mean, $n = 3$.

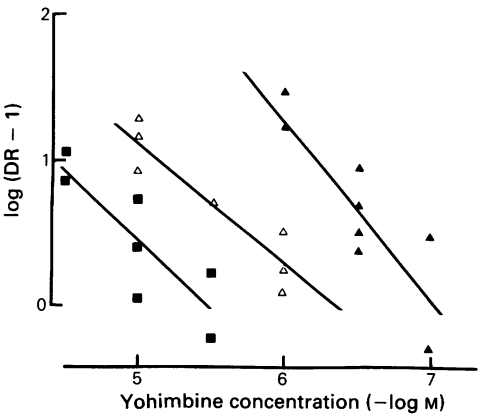

Figure 3 Schild plots of the relationship between log (agonist dose-ratio − 1) and yohimbine concentration (− log M) for the agonists 5-hydroxytryptamine (5-HT, ■), noradrenaline (NA, △) and NA in the presence of cocaine (▲). Each point is the result of an individual experiment.

In vehicle experiments, the maximum contraction to 5-HT increased in the second period to 129 ± 5% ($n = 6$) of that obtained in the first period. As compared with the effects of vehicle, yohimbine (10 μM) had no significant effect on the maximum contraction (126 ± 10%, $n = 4$), but ketanserin (1 μM) produced a small but significant reduction to 99 ± 7% ($n = 6$). The 5-HT_2-antagonist cyproheptadine (1 μM) significantly reduced the maximum contraction to 11 ± 6% ($n = 3$), but cyproheptadine 0.1 μM had variable effects (reducing the maximum contraction in 2 of 3 experiments). The 5-HT_2-antagonist methysergide (0.1 μM) reduced the maximum contraction in 2 of 3 experiments (90 ± 29%, $n = 3$) but shifted the potency of 5-HT (apparent pA_2 of 7.45 ± 0.22, $n = 3$). The neuronal 5-HT antagonist MDL 72222 (1 and 10 μM) did not significantly alter the potency of 5-HT (total of 4 experiments).

Since only yohimbine produced parallel shifts in agonist concentration-response curves without altering the maximum contraction, Schild plots were constructed for yohimbine (Figure 3). None of the slopes differed significantly from negative unity, although standard errors of slopes were admittedly large. A pA_2 value of 5.48 was obtained for yohimbine against 5-HT (Figure 3 and Table 1), and a pA_2 of 6.36 was obtained against NA (Figure 3 and Table 1).

Analysis of covariance was used to test whether the Schild plots for yohimbine against NA and 5-HT differed. There was no difference in slope for the regression lines against the two agonists ($F = 0.08$, d.f. = 1,10) but there was a significant difference in elevation ($F = 19.07$, d.f. = 1,11; $P < 0.01$). Hence, there is a significant difference between the potency of yohimbine against NA and its potency against 5-HT.

In experiments carried out in the presence of cocaine (1 μM), a pA_2 of 7.06 was obtained for yohimbine against NA (Figure 3 and Table 1).

Discussion

This investigation has revealed 2 components to the isometric contraction to 5-HT in human saphenous vein, one of which appears to be mediated by 5-HT_2-receptors. The identity of the other component is discussed below.

Table 1 pA_2 values and slope of the Schild plots for the antagonist yohimbine against 5-hydroxytryptamine (5-HT), noradrenaline (NA) and NA in the presence of cocaine (1 μM)

	Yohimbine	
Agonist	*pA_2*	*Slope*
5-HT	5.48 (4.67–7.37) $n = 7$	−0.94 ± 0.26
NA (cocaine)	7.06 (6.44–8.22) $n = 8$	−1.26 ± 0.30
NA	6.36 (5.77–7.39) $n = 7$	−0.83 ± 0.14

Values are pA_2 and 95% confidence limits with n the number of experiments, and slope ± s.e.

The contraction produced by 5-HT in the human saphenous vein does not appear to involve indirect actions by release of NA since its potency was unaffected by uptake blockade and the α-adrenoceptor antagonist yohimbine did not affect the contraction at concentrations at which it blocks α_2- and α_1-adrenoceptors. The potency of 5-HT was, however, altered in a competitive manner by yohimbine, but not by α-adrenoceptor antagonism as the pA_2 of yohimbine against 5-HT of 5.48 was much lower than pA_2s of 6.36 and 7.06 obtained for yohimbine against NA, and lower than the potency of yohimbine at inhibiting field stimulation-evoked contractions (significant reduction by yohimbine 0.01 μM; Docherty & Hyland, 1985b). The pA_2 of 6.36 obtained for yohimbine in the absence of cocaine is similar to the pA_2 of 6.56 obtained at α_1-adrenoceptors in the rabbit pulmonary artery (Weitzell *et al.*, 1979). Müller-Schweinitzer (1984), also using the human isolated saphenous vein, found that yohimbine was approximately 60 times less potent against 5-HT than against NA. The higher pA_2 of 7.06 obtained for yohimbine against NA in the presence of cocaine suggests that α_2-adrenoceptor-mediated effects become more marked when uptake blockade allows access to the junctional receptors which are α_2-receptors (Docherty & Hyland, 1985b).

Since ketanserin shifts the upper part of the concentration-response curve of 5-HT with little effect on the lower part of the curve, it seems that high concentrations of 5-HT activate 5-HT_2-receptors: the curve shape fits the theoretical curve for a two receptor system in which the antagonist acts at the receptor for which the agonist has lower affinity (see Docherty & Starke, 1981). Results obtained for cyproheptadine and methysergide are difficult to interpret due to their non-competitive effects against 5-HT-induced contractions. Müller-Schweinitzer (1984) found that the 5-HT antagonist pizotifen behaved much as ketanserin did in our experiments, both against 5-HT. Overall, it is likely that the effects of high concentrations of 5-HT are mediated by 5-HT_2-receptors in human saphenous vein.

5-HT acts potently at a yohimbine-sensitive 5-HT receptor but less potently at a ketanserin-sensitive 5-HT receptor: yohimbine appears to act with approximately equal potency against both components of the 5-HT response in causing a parallel shift. These results demonstrate that the 5-HT receptors of the human saphenous vein differ in one major respect from those of the dog saphenous vein. In the dog saphenous vein, the 5-HT receptors have been found to be predominantly '5-HT_1-like' (Feniuk *et al.*, 1985), with little evidence for 5-HT_2-receptors. Our data do not allow us to positively identify our yohimbine-sensitive, ketanserin-resistant receptor as being '5-HT_1-like', but certainly 5-HT acts with higher potency at these receptors and 5-HT has higher affinity for 5-HT_1-sites in ligand binding studies (Peroutka & Snyder, 1979). It remains to be seen whether yohimbine is a useful antagonist at these non-5-HT_2-receptors, bearing in mind its low potency and activity as a 5-HT_2-antagonist (present results and Kaumann, 1983).

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