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Differences in the effects of ketanserin and GR127935 on 5-HT-receptor mediated responses in rabbit saphenous vein and guinea-pig jugular vein

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Abstract

Ketanserin has higher affinity for 5-$HT_{1D\alpha}$ receptors compared to 5-$HT_{1D\beta}$ receptors, whereas, GR127935 (*N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2(methyl-4(-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide), a novel and selective 5-HT_{1D} receptor antagonist, has higher affinity for 5-$HT_{1D\beta}$ receptors compared to 5-$HT_{1D\alpha}$ receptors. In the present study, we compared the effects of ketanserin and GR127935 on sumatriptan-induced responses of rabbit saphenous vein and guinea-pig jugular vein. In rabbit saphenous vein, contractile responses elicited by sumatriptan were antagonised by ketanserin ($pA_2 = 6.76$) and GR127935 (apparent $pA_2 = 9.4$). In guinea-pig jugular vein, concentration-dependent relaxations evoked by sumatriptan were antagonised by ketanserin and GR127935 (apparent $pA_2 = 5.9$ and 10, respectively). Ketanserin but not GR127935, inhibited Ca^{2+}-induced contraction of depolarised strips of guinea-pig ileum longitudinal muscle/myenteric plexus, however, in rabbit saphenous vein and guinea-pig jugular vein, 5-HT receptor mediated responses were insensitive to nifedipine (Ca^{2+} channel blocker), eliminating the possibility that the inhibitory effects of ketanserin and GR127935 were due to the blockade of voltage-operated Ca^{2+} channels. Thus, antagonism by ketanserin and GR127935 confirms the presence of 5-HT_{1D} receptors in rabbit saphenous vein and guinea-pig jugular vein. The differential effects of ketanserin and GR127935 on responses elicited by sumatriptan in rabbit saphenous vein and guinea-pig jugular vein may reflect either species differences in 5-HT_{1D} receptors or the involvement of 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor subtypes.

Keywords: Ketanserin; GR127935; Saphenous vein, rabbit; Jugular vein, guinea-pig; 5-HT_{1D} receptor

1. Introduction

Ketanserin is generally regarded as a selective 5-HT_2 receptor antagonist and indeed previous reports have shown that this antagonist has nanomolar affinity for 5-HT_2 receptors (Humphrey et al., 1982; Leff and Martin, 1986). However, higher concentrations of ketanserin, also show affinity for other receptor types (Leysen et al., 1981). Recently, it has been demonstrated that ketanserin, in the submicromolar concentration range, has affinity for human 5-HT_{1D} receptors showing higher affinity for 5-$HT_{1D\alpha}$ receptor subtype compared to the 5-$HT_{1D\beta}$ receptor subtype (i.e. pK_D values of 7.0–7.5 and <5 for 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors, respectively; Kaumann et al., 1993). For human cerebral and coronary arteries, the EC_{50} values for the contractile responses elicited by a series of 5-HT_{1D} (also known as 5-HT_{1like}) receptor agonists correspond to the EC_{50} values obtained for the same compounds in the activation of adenylate cyclase mediated via 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors expressed in cell lines. However, pharmacological studies have shown that the contractile responses in human cerebral and coronary arteries evoked by 5-HT_{1D} receptor agonists are relatively insensitive to blockade by ketanserin and therefore, it has been suggested that the responses elicited by sumatriptan in these human blood vessels are mediated via the activation of 5-$HT_{1D\beta}$ receptors (Kaumann et al., 1993, 1994). To support this finding, Hamel et al. (1993) demonstrated the presence of mRNA encoding 5-$HT_{1D\beta}$ receptors and not 5-$HT_{1D\alpha}$ receptors, in human temporal arteries.

Until recently, no selective 5-HT_{1D} receptor antagonists have been available, however, GR127935 (*N*-[4-

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methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2(-methyl-4((5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide) has been developed and this has been shown to have high affinity and selectivity for 5-HT_{1D} receptors. Furthermore, GR127935 displays some differential affinity for the 5-HT_{1D} receptor subtypes, having approximately 10-fold higher affinity for 5-$HT_{1D\beta}$ compared to 5-$HT_{1D\alpha}$ receptors (pK_i values of 8.9 and 9.9 for 5-$HT_{1D\alpha}$ receptors and 5-$HT_{1D\beta}$ receptors, respectively; Clitherow et al., 1994), thus, serving as a useful tool for the investigation of 5-HT_{1D} receptors.

In the present study, we have compared the effects of ketanserin on sumatriptan-induced responses in two preparations containing functional 5-HT_{1D} receptors, rabbit saphenous vein (Martin et al., 1991) and guinea-pig jugular vein (Gupta, 1992). We also studied the effects of GR127935 in order to confirm that the sumatriptan evoked responses were mediated via the activation of 5-HT_{1D} receptors, in these tissues. Effects on responses to 5-HT (5-hydroxytryptamine, the putative endogenous ligand) were also investigated.

2. Materials and methods

2.1. Rabbit saphenous vein

Tissues were obtained from male New Zealand White rabbits (2–2.5 kg) as described by Martin and Maclennan (1990). Briefly, 5 mm ring segments of rabbit saphenous vein were obtained and mounted for isometric tension recording in 25 ml organ baths containing Krebs physiological salt solution (PSS) aerated with 95% O_2-5% CO_2 and maintained at 37°C, pH 7.4. A resting tension of 2 g was applied and after 60 min equilibration, the irreversible antagonists, pargyline (500 μM) which prevents the metabolism of 5-HT by monoamine oxidase and phenoxybenzamine (0.3 μM) which eliminates the indirect responses to 5-HT and sumatriptan mediated via the release of noradrenaline, were added to the baths (for 30 min) and then any excess was removed by washing. Following 30 min, 5-HT (1 μM) was then added as a reference response and washed off. Cumulative concentration-effect curves to 5-HT or sumatriptan were performed with the responses to each agonist concentration being allowed to plateau (2 min) before the addition of the next concentration. Following wash-off of the agonists, the tissues were then incubated with either ketanserin (1, 3 or 10 μM) or GR127935 (1, 3 or 10 nM, in the case of sumatriptan-treated tissues or 10, 30 or 100 nM with regards to 5-HT treated tissues) for 30 min after which a second concentration-effect curve to 5-HT or sumatriptan was performed. Appropriate vehicle control concentration-effect curves were obtained.

2.2. Guinea-pig jugular vein

The left and right jugular veins were obtained from male Dunkin-Hartley guinea-pigs (300–350 g) and cleaned of connective tissue and cut into 3–5 mm ring segments. The tissues were mounted for isometric tension recording in 25 ml organ baths containing Krebs physiological salt solution (PSS) aerated with 95% O_2-5% CO_2 and maintained at 37°C, pH 7.4. A resting load of 1 g was applied. The tissues were allowed to equilibrate for 60 min then the irreversible antagonists, pargyline (500 μM) and phenoxybenzamine (0.3 μM) were added to the baths (for 30 min) and any excess was removed by washing. Then U-46619 (9,11-dideoxy-11a,9a-epoxy-methanoprostaglandin $F_{2\alpha}$, 30 nM), a thromboxane mimetic, was added to the baths and once the contractile response to U-46619 had reached a plateau (30 min), acetylcholine (1 μM) was added to confirm that the endothelium was intact and also served as a reference response. Following wash-off (5 min) and rest (10 min) periods the protocol involved using 'matched pair' experiments where one of the pair of tissues obtained from the same animal were either incubated (30 min) with an antagonist (ketanserin, 1 or 10 μM or GR127935, 1 or 10 nM) and the other of the pair served as a control. This was necessary as relaxations induced by sumatriptan showed desensitization at high concentrations. The tissues were then contracted with a second addition of U-46619 (30 nM) and after the response had reached a plateau, cumulative concentration-effect curves to sumatriptan in both antagonist/vehicle-treated pairs, were performed simultaneously.

2.3. Guinea-pig ileum longitudinal muscle / myenteric plexus

Strips of guinea-pig ileum longitudinal muscle/myenteric plexus were obtained as previously described by Razzaque and Longmore (1993). Tissues were mounted in 3 ml organ baths containing Krebs physiological salt solution containing atropine (1 μM), mepyramine (1 μM), indomethacin (1 μM) and methysergide (0.1 μM) at 37°C, aerated with 95% 0_2-5% CO_2 (pH 7.4). An initial resting tension of 1 g was applied. Following a 60 min equilibration period, the Krebs physiological salt solution was replaced by modified Krebs physiological salt solution containing 0 mM $CaCl_2$ (i.e. nominally Ca^{2+}-free), 2.4 mM $MgSO_4$ and a high concentration of KCl (85 mM). Cumulative concentration-effect curves to $CaCl_2$ (0.03–30 mM) were then performed. The tissues were then washed with normal Krebs physiological salt solution until the tissues were again fully relaxed. Ketanserin (300 nM) or GR127935 (1 μM) was then added and following a 45

min period normal Krebs physiological salt solution was then replaced with the modified Krebs physiological salt solution (containing the appropriate antagonist) for a further 30 min, after which time a second concentration-effect curve to $CaCl_2$ was performed. Vehicle control experiments were carried out.

2.4. Analysis of data

For sumatriptan in guinea-pig jugular vein, the relaxant responses were calculated as a percentage of the acetylcholine-induced relaxation of the U-46619-elicited contraction. The contractile responses elicited by the remaining agonists in guinea-pig ileum longitudinal muscle /myenteric plexus and rabbit saphenous vein, were calculated as a percentage of the maximum contractile response achieved in the first concentration-effect curves.

In rabbit saphenous vein full Schild plots (according to Arunlakshana and Schild, 1959) for ketanserin versus 5-HT or sumatriptan, were also calculated.

In experiments using antagonists, for individual tissues, concentration-ratios were calculated and used to obtain estimates of apparent pA_2 values using the following equation:

$$\text{apparent } pA_2 = \log(\text{concentration ratio} - 1) - \log(\text{antagonist concentration})$$

In experiments where non-competitive antagonism was evident (i.e. where there was a marked depression of the maximum response), estimates of the apparent pA_2 values were calculated using the EC_{50} values (for control and antagonist-treated curves) calculated relative to the reduced maximum of the second antagonist treated concentration-effect curve.

The negative logarithm of the molar concentration of an agonist eliciting half maximal response is represented by pEC_{50} values for both rabbit saphenous vein and guinea-pig jugular vein. Concentration-effect curves were fitted to the mean data by least squares non-linear analysis of regression using the equation:

$$Y = Y\max/1 + (EC_{50}/[\text{agonist}])^{nH}$$

where EC_{50} is as described above and nH is the Hill coefficient.

2.5. Drugs and solutions

Krebs physiological salt solution had the following composition (mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25.3, KH_2PO_4 1.19, $MgSO_4$ 1.19, $CaCl_2$ 2.5 and glucose 11.1. Modified Krebs physiological salt solution had the following composition (mM): NaCl 118, KCl 84.7, $NaHCO_3$ 25.3, KH_2PO_4 1.19, $MgSO_4$ 2.38, $CaCl_2$ 0 and glucose 11.1). The following substances were used (in brackets the concentration of the stock solutions and solvents have been quoted): 5-hydroxytryptamine (5-HT) creatinine sulphate complex (10 mM: H_2O), prostaglandin $F_{2\alpha}$ (10 mM: ethanol), U-46619 (9,11-dideoxy-11a,9a-epoxy-methanoprostaglandin $F_{2\alpha}$, 1 μM: ethanol), acetylcholine chloride (10 mM: H_2O), isoprenaline (10 mM: H_2O), nifedipine (10 mM: dimethyl sulphoxide), pargyline hydrochloride (0.1 M: H_2O), atropine sulphate (0.1 M: H_2O), mepyramine maleate (0.1 M: methanol), indomethacin (0.1 M: ethanol), all purchased from Sigma. Ketanserin (+)-tartrate (10 mM: dimethyl sulphoxide), (±)-methiothepin maleate (10 mM: dimethyl sulphoxide), phenoxybenzamine hydrochloride (10 mM: ethanol), methysergide maleate (10 mM: methanol), all purchased from RBI. Dimethyl sulphoxide was purchased from Fluka and sumatriptan (0.1 M: dimethyl sulphoxide) and GR127935 (0.1 M: dimethyl sulphoxide) were synthesized in MSD Laboratories. Nifedipine was light-protected.

3. Results

3.1. Rabbit saphenous vein

Effect of ketanserin on 5-HT and sumatriptan-induced contractions

The pEC_{50} values for 5-HT and sumatriptan were 7.6 ± 0.08 ($n = 19$) and 6.2 ± 0.04 ($n = 21$), respectively. Ketanserin caused a concentration-dependent antagonism of both 5-HT and sumatriptan-induced contractions causing a parallel displacement of the concentration-effect curves, without depressing the maximum responses (Fig. 1). The concentration-ratios obtained for 5-HT and sumatriptan in the presence of ketanserin (1 μM) were 5.06 ± 0.3 ($n = 4$) and 6.52 ± 1.5 ($n = 8$), respectively. Full Schild plots for ketanserin (1, 3 and 10 μM) versus 5-HT and sumatriptan gave pA_2 values (with 95% confidence limits) of 6.75 (6.68–6.82; 13 data points) and 6.76 (6.58–6.93; 29 data points), respectively (Fig. 2). The slope of the Schild plots for both 5–HT and sumatriptan in the presence of ketanserin was 0.8 (confidence limits for 5-HT = 0.6–1.0 and sumatriptan = 0.5–1.0), which is not significantly different from unity, consistent with competitive antagonism. Vehicle-treated concentration-effect curves to 5-HT and sumatriptan produced a concentration ratio of < 2, indicating no change in sensitivity to these agonists with time.

Effect of GR127935 on 5-HT and sumatriptan-induced contractions

GR127935 inhibited both sumatriptan and 5-HT elicited responses in a concentration-dependent manner, accompanied by a marked reduction in the maximum response (Fig. 3). GR127935 had a more marked

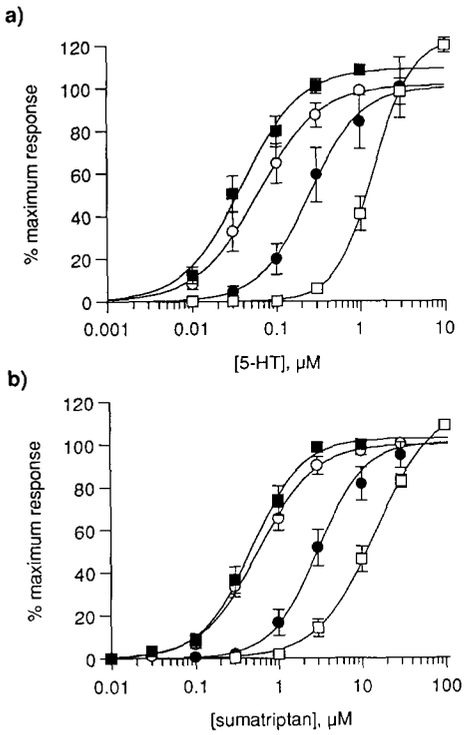

Fig. 1. Concentration-effect curves to (a) 5-HT and (b) sumatriptan in the absence (open circle) and presence of ketanserin (1 μM, filled circle and 10 μM, open square) or vehicle (filled square) in rabbit saphenous vein. Each point, expressed as a percentage of the maximum contractile response obtained in the initial curve, is the mean value $\pm$ S.E.M. (n = 4–8). For both graphs, the curves were fitted by least squares non-linear analysis of regression (see text for details).

effect on sumatriptan-evoked contractions compared to 5-HT-elicited responses with concentration ratios of 6.6 ± 2.8 ($n = 4$) for sumatriptan and 3.3 ± 0.2 ($n = 3$) for 5-HT, in the presence of GR127935, 1 nM and 10 nM, respectively. Estimates of the apparent pA_2 values for GR127935 versus sumatriptan and 5-HT (with EC_{50} values calculated from the depressed maximums) were 9.4 ± 0.3 and 8.4 ± 0.03, respectively, which were calculated from a single concentration of GR127935 (1 and 10 nM, respectively). No change in the sensitivity to sumatriptan or 5-HT was observed in the vehicle treated tissues (concentration ratio < 2).

Effects of nifedipine on sumatriptan-induced contractions

Nifedipine (0.3 and 3 nM) and vehicle had no effect on sumatriptan evoked contractions (Fig. 4).

3.2. Guinea-pig jugular vein

Effects of ketanserin on sumatriptan-induced relaxation

Sumatriptan caused concentration-dependent relaxations of guinea-pig jugular vein pre-contracted with the thromboxane mimetic, U-46619, giving a pEC_{50} value of 6.3 ± 0.16 ($n = 16$). Ketanserin (1 μM) had no effect on sumatriptan-induced relaxation (concentration ratio < 2; $n = 4$). Although, a higher concentration of ketanserin (10 μM) inhibited sumatriptan-evoked relaxations in three preparations out of five, causing a reduction in the maximum response (Fig. 5) and in the remaining two tissues, ketanserin (10 μM) was without effect (concentration ratio < 2). Thus, giving an estimate of an apparent pA_2 of 5.9 ± 0.06 for three tissues (calculated from the EC_{50} values from the depressed maximum). The overall mean concentration ratio for ketanserin (10 μM) obtained for all five tissues was 5.4 ± 2.2. Also, there was no inhibition of U-46619 (30 nM)-elicited contraction in the presence of ketanserin (1 and 10 μM) compared to the initial reference response to U-46619 in the same tissue (n = 9).

Effect of GR127935 on sumatriptan-induced relaxation

Sumatriptan evoked relaxations of guinea-pig jugular vein were antagonised by GR127935 (1 nM) giving an apparent pA_2 of 10 ± 0.2 (concentration ratio = 16.3 ± 9.7; $n = 4$). GR127935 (1 nM) reduced the maximum responses evoked by sumatriptan and a higher concentration of GR127935 (10 nM; $n = 4$) completely abolished these responses (Fig. 6). The second addition of U-46619 (30 nM) in the same tissue was not inhibited by GR127935 (1 and 10 nM; $n = 8$).

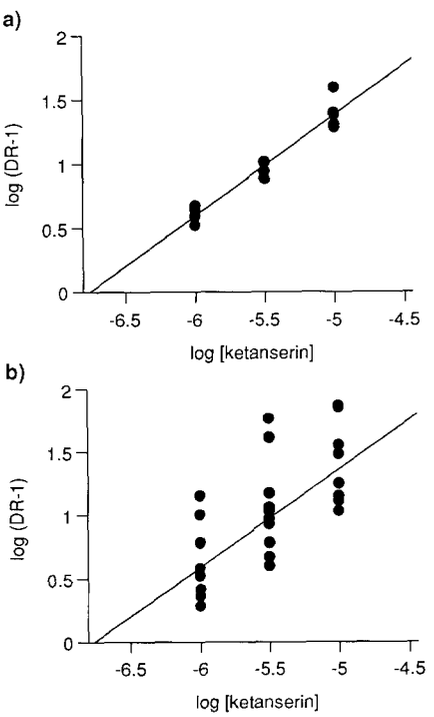

Fig. 2. Schild plots depicting antagonism of (a) 5-HT and (b) sumatriptan-induced contractions in rabbit saphenous vein by ketanserin. Each point represents data obtained from a separate preparation. The gradient of the best-fit straight line was determined by linear regression.

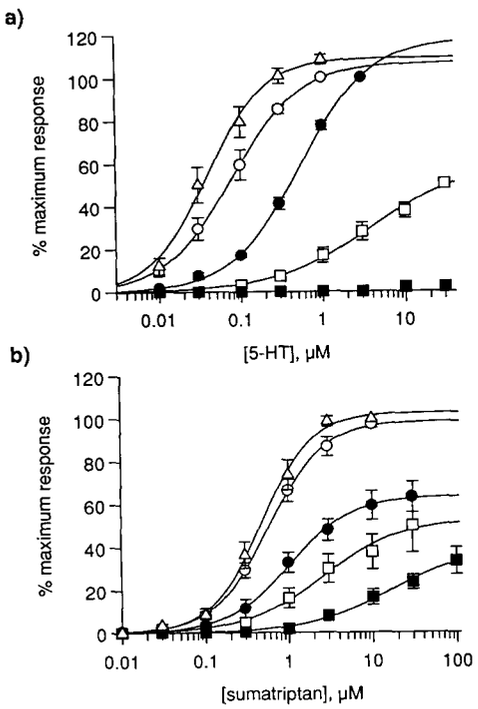

Fig. 3. Concentration-effect curves to (a) 5-HT in the absence (open circle) and presence of GR1273935 (10 nM, filled circle, 30 nM, open square and 100 nM, filled square) and vehicle (open triangle) and (b) sumatriptan in the absence (open circle) and presence of GR127935 (1 nM, filled circle, 3 nM, open square and 10 nM, filled square) and vehicle (open triangle) in rabbit saphenous vein. Each point, expressed as a percentage of the maximum contractile response obtained in the initial curve, is the mean value ± S.E.M. ($n = 3$–5). Curves were fitted by least squares non-linear analysis of regression (see text for details).

Effect of nifedipine on U-46619-induced contraction

Nifedipine in concentrations up to 1 μM had no effect on U-46619-elicited contractions of guinea-pig jugular vein. Higher concentrations of the antagonist (up to 10 μM) caused approximately 80% inhibition (Fig. 7).

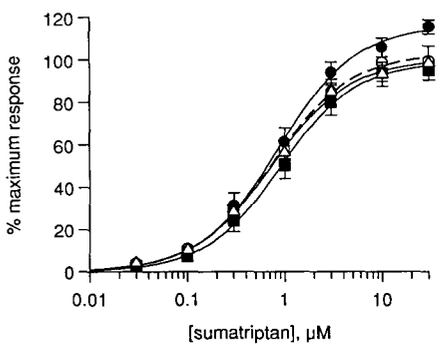

Fig. 4. Concentration-effect curves to sumatriptan in the absence (open circle and broken line) and presence of nifedipine (0.3 nM, filled square and 3 nM, open triangle) and vehicle (filled circle), in rabbit saphenous vein. Each point, expressed as a percentage of the maximum contractile response obtained in the initial curve, is the mean value ± S.E.M. ($n = 5$). Curves were fitted by least squares non-linear analysis of regression (see text for details).

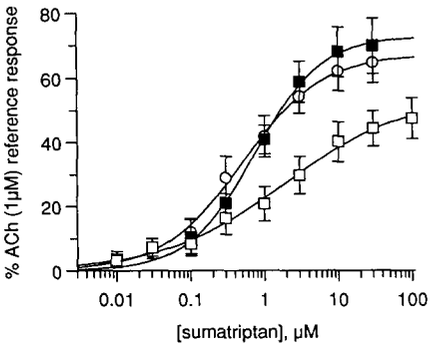

Fig. 5. Concentration-effect curves to sumatriptan in the absence (open circle) and presence of ketanserin (1 μM, filled square and 10 μM, open square) in guinea-pig jugular vein. Each point, expressed as a percentage of the relaxation evoked by acetylcholine (1 μM), is the mean value ± S.E.M. ($n = 3$–4). Curves were fitted by least squares non-linear analysis of regression (see text for details).

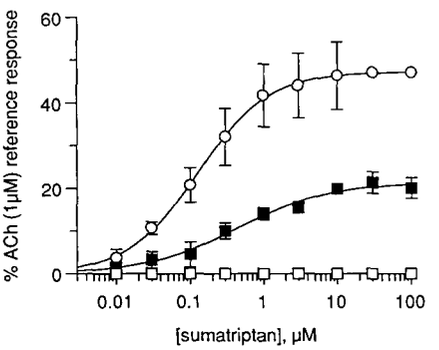

Fig. 6. Concentration-effect curves to sumatriptan in the absence (open circle) and presence of GR127935 (1 nM, filled square and 10 nM, open square) in guinea-pig jugular vein. Each point, expressed as a percentage of the relaxation evoked by acetylcholine (1 μM), is the mean value ± S.E.M. ($n = 4$). Curves were fitted by least squares non-linear analysis of regression (see text for details).

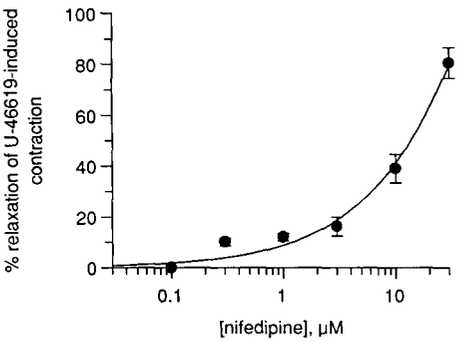

Fig. 7. Concentration-effect curve to nifedipine in guinea-pig jugular vein pre-contracted with U-46619 (30 nM). Each point, expressed as percentage relaxation of U-46619-induced contraction, is the mean value ± S.E.M. ($n = 6$). Curves were fitted by least squares non-linear analysis of regression (see text for details).

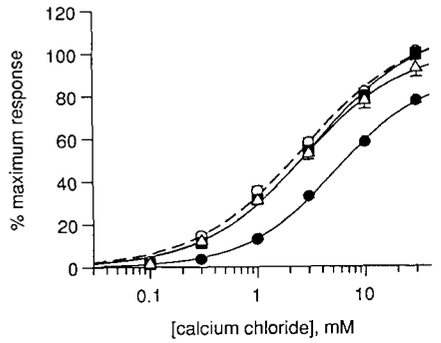

Fig. 8. Concentration-effect curves to $CaCl_2$ in the absence (open circle) and presence of ketanserin (300 nM, filled circle), GR127935 (1 μM, filled square) or vehicle (open triangle) in guinea-pig ileum longitudinal muscle/myenteric plexus. Each point, expressed as a percentage of the maximum response obtained in the initial curve, is the mean value ± S.E.M. ($n = 4$). Curves were fitted by least squares non-linear analysis of regression (see text for details).

3.3. Guinea-pig ileum longitudinal muscle / myenteric plexus

Effect of ketanserin and GR127935 on $CaCl_2$-induced contractions of guinea-pig ileum longitudinal muscle / myenteric plexus

Fig. 8 shows that ketanserin (300 nM) caused a rightward shift in the position of the concentration-effect curves to $CaCl_2$, without depressing the maximum, giving an apparent pA_2 of 6.5 ± 0.05 ($n = 4$), whereas, GR127935 (1 μM) had no effect on $CaCl_2$-induced contractions ($n = 4$). The apparent pA_2 value for nifedipine, in this preparation, was 10.5 (data not shown). No vehicle effects were evident with respect to $CaCl_2$-induced contractions observed in guinea-pig ileum longitudinal muscle/myenteric plexus ($n = 4$; see Fig. 8).

4. Discussion

4.1. Antagonistic effects of ketanserin and GR127935 on sumatriptan and 5-HT-induced contractions in rabbit saphenous vein

In rabbit saphenous vein, 5-HT was found to be more potent than sumatriptan in causing contractions (pEC_{50} values = 7.6 and 6.2, respectively) and this is consistent with previous reports, where Martin and Maclennan (1990) obtained $p[A_{50}]$ values of 7.75 and 6.60 for 5-HT and sumatriptan, respectively, in the same preparation. The novel and selective 5-HT_{1D} receptor antagonist GR127935, antagonised the responses to 5-HT and sumatriptan in rabbit saphenous vein and this observation is consistent with previously reported effects of GR127935 on sumatriptan-induced contractions of dog saphenous vein (Clitherow et al., 1994). Thus, the inhibitory effect of GR127935 confirms the presence of 5-HT_{1D} receptors in rabbit saphenous vein.

In the present study GR127935 reduced the maximum responses to sumatriptan in rabbit saphenous vein and a similar observation was made for this antagonist in dog saphenous vein (Clitherow et al., 1994). It is unlikely that the inhibitory effects elicited by GR127935 are due to the blockade of Ca^{2+} influx, via voltage-operated Ca^{2+} channels, since GR127935 was devoid of Ca^{2+} channel blocking properties in concentrations up to 1 μM, in depolarised strips of guinea-pig ileum longitudinal muscle/myenteric plexus. The non-surmountable inhibitory effect of GR127935 seems likely to reflect its high lipophilicity and hence slow dissociation kinetics rather than a non-competitive interaction with the agonist-receptor complex, since the inhibitory effects are slowly reversible and other chemically related antagonists show a competitive action (Clitherow et al., 1994). Interestingly, in contrast to ketanserin, which did not differentiate between 5-HT and sumatriptan (see below), GR127935, was more effective in inhibiting sumatriptan-induced contractions than 5-HT-mediated responses with apparent pA_2 values (with EC_{50} values calculated from curves with a reduced maximum response) of 9.4 for sumatriptan and 8.4 for 5-HT. The explanation for this difference in the effect of GR127935 is not clear, however, it may reflect differences in the kinetics of the agonist-antagonist receptor interaction.

In rabbit saphenous vein, ketanserin (1 μM) was found to inhibit contractions elicited by sumatriptan (concentration ratio, of 6.5) and 5-HT (concentration ratio of 5.1) and this effect was found to be concentration-dependent and competitive in nature, giving virtually identical pA_2 values (6.76 and 6.75, respectively) for both the agonists. Thus, the contractile response to both sumatriptan and 5-HT appeared to be mediated via an interaction at the same receptor.

It is possible that the inhibitory effect of ketanserin on 5-HT_{1D} receptor-mediated responses in rabbit saphenous vein could be attributed to non-specific actions of this antagonist, since ketanserin has been reported to have weak Ca^{2+} channel blocking properties (Van Neuten and Vanhoutte, 1981). However, this seems unlikely to be the case, although ketanserin did inhibit $CaCl_2$-induced contraction of guinea-pig ileum longitudinal muscle/myenteric plexus (giving an apparent pA_2 of 6.6 which indeed was a similar value to the pA_2 obtained for ketanserin versus sumatriptan in rabbit saphenous vein). In rabbit saphenous vein, relatively high concentrations of the L-type Ca^{2+}channel

blocker nifedipine (up to 3 nM, which is a concentration 100-fold higher than that required to inhibit contractions elicited by $CaCl_2$, in guinea-pig ileum longitudinal muscle/myenteric plexus, giving an apparent pA_2 value of 10.5 for the latter) had no inhibitory effect on contractions induced by sumatriptan (apparent pA_2 value of < 8.5). This eliminates the possibility that the antagonistic effects of ketanserin on sumatriptan-induced responses in rabbit saphenous vein were due to Ca^{2+} channel blockade. The antagonism is more likely to reflect the direct interaction of ketanserin at 5-HT_{1D} receptors

4.2. Antagonistic effects of ketanserin and GR127935 on sumatriptan-induced relaxation in guinea-pig jugular vein

Sumatriptan evoked endothelium- and concentration-dependent relaxation of pre-contracted guinea-pig jugular vein, giving a pEC_{50} value of 6.3 similar to that obtained for rabbit saphenous vein, 6.2 (see above) and to the value of 6.58 reported by Gupta (1992) for guinea-pig jugular vein. GR127935 antagonised the relaxation responses induced by sumatriptan, which confirms that 5-HT_{1D} receptors have been activated in guinea-pig jugular vein. In guinea-pig jugular vein GR127935 also acted as a non-surmountable antagonist, which can be attributed to its physiochemical properties (see above). When comparing the effects of GR127935 in rabbit saphenous vein and guinea-pig jugular vein, the inhibition appears to be more pronounced in guinea-pig jugular vein than in rabbit saphenous vein with apparent pA_2 values of 10 and 9.4, for the two tissues, respectively.

Ketanserin (1 μM) had no effect on sumatriptan-induced relaxation (concentration ratio of < 2), which is in contrast to the effect of ketanserin (1 μM) observed in rabbit saphenous vein where a concentration ratio of 6.5 was observed. However, in guinea-pig jugular vein, at a higher concentration, ketanserin (10 μM) did result in a non-competitive antagonism of sumatriptan-induced relaxations, giving a concentration ratio of 5.4 (with EC_{50} values calculated from curves with depressed maximum responses), although this value was derived from three tissues out of five, whereas, in the remaining two tissues sumatriptan evoked relaxations were insensitive to ketanserin (10 μM). Thus, ketanserin displayed a differential effect between rabbit saphenous vein and guinea-pig jugular vein, being at least 10-fold more active in rabbit saphenous vein. Gupta (1992) reported that ketanserin (3 μM) caused a 10-fold rightward displacement of sumatriptan elicited relaxation, however, no data were shown and it is not clear whether there was a depression of the maximum of the concentration-effect curve to sumatriptan.

4.3. Interpretation of the differences in the inhibitory effects of the 5-HT_{1D} receptor antagonists in rabbit saphenous vein and guinea-pig jugular vein

Both ketanserin and GR127935, antagonised 5-HT- and sumatriptan-evoked responses in rabbit saphenous vein and guinea-pig jugular vein with ketanserin having a greater effect in rabbit saphenous vein compared to guinea-pig jugular vein and GR127935 had a greater effect in guinea-pig jugular vein compared to rabbit saphenous vein. The differential effects of these antagonists may reflect species differences in 5-HT_{1D} receptor pharmacology. Indeed, in guinea-pig tissues such as iliac artery (Sahin-Erdemli et al., 1991) and jugular vein (this study) responses to sumatriptan are insensitive to ketanserin (1 μM) and in contrast, rabbit preparations i.e. saphenous vein (Martin et al., 1990), renal artery (Choppin and O'Connor, 1993) and basilar artery (Tilford and Baxter, 1994) are sensitive to ketanserin (1 μM). However, the cloning of the two 5-HT_{1D} receptor subtypes 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors (Hartig et al., 1992) demonstrates that intraspecies differences do exist and for cloned human 5-HT_{1D} receptors it has been shown that ketanserin has high affinity (20–70-fold) for the 5-$HT_{1D\alpha}$ receptor subtype over the 5-$HT_{1D\beta}$ receptor subtype (Weinshank et al., 1991; Kaumann et al., 1993). Furthermore, this would set a criterion where the degree of sensitivity, if any, to ketanserin, would determine which subtype was present in a tissue containing 5-HT_{1D} receptors. Indeed, on this basis Kaumann et al. (1993) has established the presence of 5-$HT_{1D\beta}$ receptors in human arteries as no effect was observed with ketanserin (1 μM) on sumatriptan-induced contraction.

The present study shows that ketanserin (1 μM) exerts a differential effect on sumatriptan-evoked responses in rabbit saphenous vein and guinea-pig jugular vein, in that ketanserin-sensitive 5-HT_{1D} receptors are activated in rabbit saphenous vein and ketanserin-insensitive 5-HT_{1D} receptors are present in guinea-pig jugular vein. This pharmacological profile would indicate that 5-$HT_{1D\alpha}$ receptors predominate in rabbit saphenous vein and 5-$HT_{1D\beta}$ receptors are more prevalent in guinea-pig jugular vein. To further support this finding, GR127935 also displayed a differential effect (pK_i values of 9.9 and 8.9 for human 5-$HT_{1D\beta}$ and 5-$HT_{1D\alpha}$, respectively; Clitherow et al., 1994), although small, where this antagonist was 6-fold more effective in inhibiting sumatriptan-induced responses in guinea-pig jugular vein than in rabbit saphenous vein, which would infer the predominance of 5-$HT_{1D\beta}$ receptors in guinea-pig jugular vein.

It is also important to note that in both rabbit saphenous vein and guinea-pig jugular vein, ketanserin produced varying degrees of inhibition of sumatriptan-induced responses which is evident in the Schild plot

constructed for ketanserin versus sumatriptan in rabbit saphenous vein (Fig. 2) and the varying degrees of inhibition of sumatriptan-induced relaxations by ketanserin (10 μM) in guinea-pig jugular vein where two tissues out of five were insensitive to ketanserin (concentration ratio < 2). This variability of the effect of ketanserin on sumatriptan-mediated responses may indicate the presence of mixed populations of 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors.

Since ketanserin has now been shown to differentiate between 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors (Kaumann et al., 1994) it may be of interest to reassess the results of previous papers where on the basis of ketanserin sensitivity, responses have been characterised as being 5-HT_2 receptor-mediated (e.g. human saphenous vein, Bax et al., 1992; dog coronary artery, Frenken and Kaumann, 1985), however, it may be reasonable to consider the possible activation of 5-$HT_{1D\alpha}$ receptor subtype. Until better tools with greater selectivity are designed, to date, ketanserin and GR127935 are the most appropriate antagonists to investigate 5-HT_{1D} receptor subtypes.

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