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Short communication

Selective antagonism of human 5-HT_{1D} and 5-HT_{1B} receptor-mediated responses in stably transfected C6-glial cells by ketanserin and GR 127,935

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Abstract

The antagonist effects of ketanserin and 2'-methyl-4'-(5-methyl-1,2,4)oxadiazol-3-yl)-biphenyl-[4-carboxylic acid 4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide (GR 127,935) were compared on naratriptan-induced inhibition of cAMP formation in C6-glial cell lines stably expressing human 5-HT_{1D} or 5-HT_{1B} receptor sites. Ketanserin demonstrated potent (pA_2: 7.76), competitive antagonism of naratriptan-induced inhibition of forskolin (100 μM)-stimulated cAMP formation in C6-glial/5-HT_{1D} cells. Whereas GR 127,935 was ineffective as an antagonist in these cells, it produced an intrinsic activity (pEC_{50}: 6.98) that was sensitive to ketanserin (10 μM) blockade. Unlike ketanserin, GR 127,935 potently antagonised the naratriptan response in C6-glial/5-HT_{1B} cells while also depressing the maximum response. The differential antagonist effects of ketanserin and GR 127,935 on naratriptan responses elicited in C6-glial/5-HT_{1D} and C6-glial/5-HT_{1B} cells demonstrate these compounds do selectively block human 5-HT_{1D} and 5-HT_{1B} receptors, respectively.

Keywords: 5-HT_{1D} receptor antagonist; Ketanserin; 5-HT_{1B} receptor antagonist; GR 127,935; 5-HT_{1D} receptor, human, cloned; 5-HT_{1B} receptor, human, cloned; cAMP formation; (Transfected rat C6-glial cell line)

1. Introduction

The human serotonin 5-HT_{1D} receptor is complex since it is encoded by a subfamily of two distinct genes, 5-HT_{1D} (also designated 5-$HT_{1D\alpha}$) and 5-HT_{1B} (also designated 5-$HT_{1D\beta}$), that are located on different human chromosomes. These receptor subtypes show a relatively low (63%) overall amino acid identity with 77% amino acid identity within the transmembrane regions (Weinshank et al., 1992). They also demonstrate a selective tissue distribution; 5-HT_{1D} and 5-HT_{1B} receptors are highly expressed in brain raphe nuclei and striatum, respectively (Peroutka, 1994). The contractile response of human cerebral arteries is probably mediated by 5-HT_{1B} receptors since they contain mRNA transcripts for 5-HT_{1B}, but not 5-HT_{1D}, receptors (Hamel et al., 1993). Rebeck et al. (1994) have demonstrated the presence of selective 5-HT_{1D}, but not 5-HT_{1B}, receptor mRNA in human trigeminal ganglia. Both receptor subtypes may possibly be involved in cardiovascular function, vasospasm, migraine, depression, anxiety and movement disorders. Nevertheless, the precise function of each receptor subtype in man remains to be defined.

Human 5-HT_{1D} and 5-HT_{1B} receptor subtypes were first found to display binding properties that seemed indistinguishable (Weinshank et al., 1992). More recent studies illustrate these receptor subtypes can be pharmacologically differentiated using the 5-HT_2 receptor antagonists ketanserin and ritanserin (Kaumann et al., 1994; Peroutka, 1994; Pauwels and Colpaert, 1995). Both compounds show potent binding affinity for and are apparently silent antagonists at the human 5-HT_{1D} receptor subtype. A comparative pharmacological study with rat C6-glial cell lines expressing a similar number of cloned human 5-HT_{1D} or 5-HT_{1B} receptors led us to conclude that 1-naphthylpiperazine, metergoline and the putative 5-HT_{1D} receptor antagonist 2'-methyl-4'-(5-methyl-1,2,4)oxadiazol-3-yl)-biphenyl-[4-carboxylic acid 4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide (GR 127,935) selectively activate

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human 5-HT_{1D} receptors (Pauwels et al., 1995). These findings further differentiate the pharmacology of human 5-HT_{1D} and 5-HT_{1B} receptors.

In this study, we investigated the relative antagonist selectivity of ketanserin and GR 127,935 at human 5-HT_{1B} and 5-HT_{1D} receptors in terms of their effects on agonist dose-response curves. Naratriptan was chosen as a 5-HT_{1D} receptor agonist as it shows similar binding affinity for human 5-HT_{1D} and 5-HT_{1B} receptor sites. The antagonist effects were measured against naratriptan-induced inhibition of cAMP formation in C6-glial cell lines containing cloned human 5-HT_{1D} or 5-HT_{1B} receptor sites.

2. Materials and methods

2.1. Cell culture

C6-glial cells, permanently transfected with a pRcRSV plasmid containing a cloned human 5-HT_{1D} receptor gene (C6-glial/5-HT_{1D}) or $pcDNA_3$ plasmid containing a cloned human 5-HT_{1B} receptor gene (C6-glial/5-HT_{1B}), were grown in 24-well tissue culture plates with 1.0 ml Dulbecco's modified Eagle medium supplemented with 10% heat-inactivated foetal calf serum as described previously (Pauwels et al., 1995).

2.2. 5-HT_{1D} receptor binding to membrane preparations of C6-glial / 5-HT_{1D} and C6-glial / 5-HT_{1B} cells

Membrane preparations were prepared from transfected C6-glial cells in 50 mM Tris-HCl pH 7.7 containing 4 mM $CaCl_2$, 10 μM pargyline and 0.1% ascorbic acid as described previously (Pauwels et al., 1995). Binding assays were performed with 0.5 nM [^{3}H]5-carboxamidotryptamine (5-CT) in both the absence and the presence of naratriptan or 1 μM 5-HT to determine non-specific binding. K_i values were calculated according to the equation $K_i = IC_{50}/(1 + C/K_d)$ with C the concentration and K_d the equilibrium dissociation constant of [^{3}H]5-CT (0.12 and 0.22 nM for C6-glial/5-HT_{1D} and C6-glial/5-HT_{1B}, respectively; Pauwels et al., 1995).

2.3. 5-HT_{1D} and 5-HT_{1B} receptor-mediated inhibition of forskolin-stimulated cAMP formation

Inhibition of forskolin-stimulated cAMP formation by 5-HT was measured as previously described (Pauwels et al., 1995). Cultures were washed with 1.0 ml controlled salt solution and incubated for 5 min at 37°C with 1.0 ml controlled salt solution containing 1 mM isobutylmethylxanthine in the presence of 100 μM forskolin in both the absence and the presence of naratriptan, GR 127,935 or 1 μM 5-HT to determine maximal cAMP inhibition. Basal accumulation of cAMP was measured in the absence of forskolin and compound. Antagonist activity of ketanserin or GR 127,935 was tested after pre-incubation of the compound for 15 min. The reaction was stopped by the addition of 0.1 ml ice-cold $HClO_4$ to a final concentration of 0.04 M and neutralized afterwards. The cellular cAMP content was assayed using a radioimmunoassay kit. Inhibition of forskolin-induced cAMP formation was calculated as the percentage of that obtained with 1 μM 5-HT. EC_{50} values (concentration of test agent yielding 50% of the inhibition induced by 1 μM 5-HT) were derived. In experiments using antagonists, concentration ratios were calculated and used to obtain estimates of apparent pA_2 values using the following equation: apparent pA_2 = log (concentration ratio − 1) − log (antagonist concentration).

2.4. Materials

C6-glial cells (CCL 107, rat) were obtained from ATCC (Rockville, USA). Culture media and tissue culture plates were from Gibco Biocult. Laboratories (Paisley, UK). Plasmids were obtained from Invitrogen (San Diego, USA). [^{3}H]5-Carboxamidotryptamine (5-CT) (15–30 Ci/mmol) was from New England Nuclear (Les Ulis, France). The radioimmunoassay kit for cAMP was from Immunotech (Marseille, France). 2'-Methyl-4'-(5-methyl-(1,2,4)oxadiazol-3-yl)-biphenyl-[4-carboxylic acid (4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide (GR 127,935) has been kindly prepared according to a patent procedure (European Patent Application 0533268 A1) by Drs. S. Halazy and C. Jorand. Naratriptan was synthesized by Mr. J.L. Maurel. Ketanserin was obtained from Sigma (St. Louis, USA).

3. Results

3.1. Effects of ketanserin on naratriptan- and GR 127,935-induced inhibition of forskolin-stimulated cAMP formation in C6-glial / 5-HT_{1D} cells

Forskolin (100 μM)-induced cAMP formation in the C6-glial/5-HT_{1D} cell line was inhibited in the presence of 1 μM 5-HT by 78%. Naratriptan inhibited forskolin-stimulated cAMP formation to the same extent as 5-HT with an EC_{50} of 0.92 ± 0.19 nM (n = 8), fully in agreement with its binding affinity (K_i: 0.73 ± 0.14 nM, n = 4). Ketanserin caused a concentration-dependent antagonism of naratriptan-induced inhibition of cAMP formation causing a parallel displacement of the concentration-effect curves, without depressing the maximum responses (Fig. 1A). The concentration ratio obtained for naratriptan in the presence of ketanserin (1 μM) was 54. The Schild plot for ketanserin (0.1, 0.3, 1 and 10 μM) versus naratriptan yielded an apparent pA_2 value of 7.76 ± 0.10 (Fig. 1B). The slope of the Schild plot was 0.96 ± 0.05, which is not

significantly different from unity, consistent with competitive antagonism. GR 127,935 was ineffective as an antagonist (0.1 nM to 100 nM) against naratriptan (not shown); however, it induced 87% inhibition of forskolin-stimulated cAMP formation at submicromolar concentrations in C6-glial/5-HT_{1D} cells, revealing an EC_{50} value of 104 ± 20 nM ($n = 8$). Ketanserin (10 μM) caused an almost parallel displacement of the GR 127,935 concentration-effect curve;

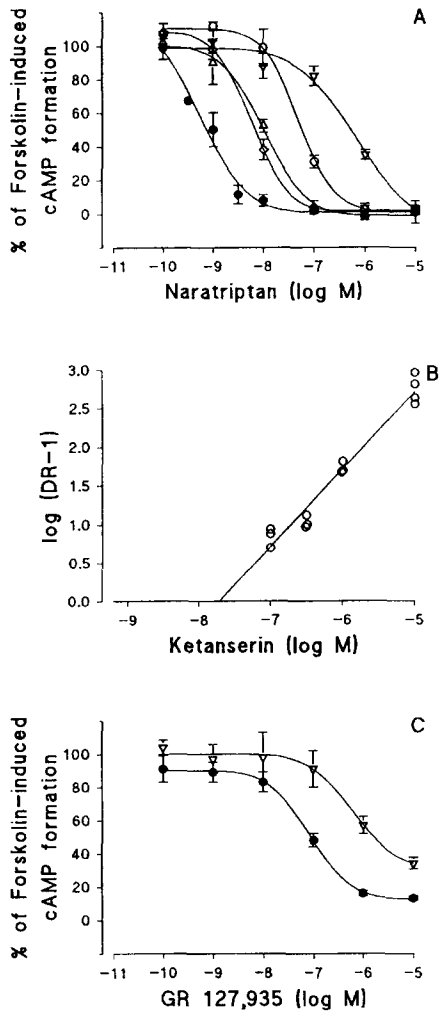

Fig. 1. Effects of ketanserin on naratriptan- and GR 127,935-induced inhibition of forskolin-stimulated cAMP formation in C6-glial/5-HT_{1D} cells. A: Concentration-effect curves to naratriptan in the absence (●, EC_{50}: 0.92 ± 0.19 nM) and presence of ketanserin (0.1 μM, ◇, EC'_{50}: 7.5 ± 1 nM; 0.3 μM, △, EC'_{50}: 10.8 ± 1.1 nM; 1 μM, ○, EC'_{50}: 50 ± 5 nM; and 10 μM, ▽, EC'_{50}: 543 ± 118 nM). Curves were constructed using mean values ± S.E.M. of 3 (with ketanserin) to 8 (without ketanserin) independent experiments, each performed in triplicate. B: Schild plot showing antagonism of naratriptan-induced inhibition of forskolin-stimulated cAMP formation by ketanserin. Each point represents a value of an independent experiment. The gradient of the best-fit straight line was determined by linear regression. C: Concentration-effect curve to GR 127,935 in the absence (●) and presence of 10 μM ketanserin (▽). Curves were constructed using mean values ± S.E.M. in the absence (EC_{50}: 104 ± 20 nM, $n = 8$) or presence of ketanserin (EC'_{50}: 2067 ± 546 nM, $n = 3$), respectively.

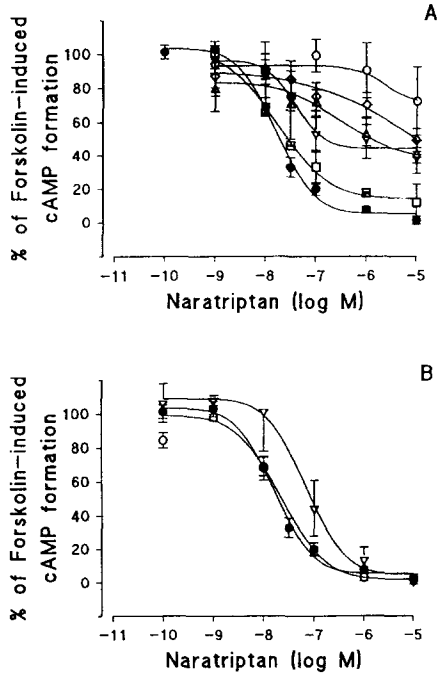

Fig. 2. Effects of GR 127,935 and ketanserin on naratriptan-induced inhibition of forskolin-stimulated cAMP formation in C6-glial/5-HT_{1B} cells. A: Concentration-effect curves to naratriptan in the absence (●, EC_{50}: 16.6 ± 2.2 nM) and presence of GR 127,935 (3 nM, □, EC'_{50}: 22 ± 1 nM; 10 nM, ▽, EC'_{50}: partial inhibition, 39 nM; 30 nM, △, EC'_{50}: partial inhibition, 316 nM; 100 nM, ◇, EC'_{50}: 10000 nM; and 1000 nM, ○, EC'_{50}: > 10000 nM). Curves were constructed using mean values ± S.E.M. of 3–6 independent experiments, each performed in triplicate. Partial inhibition indicates reduction of the maximum response, this value was taken into account to determine the corresponding EC'_{50} value. B: Concentration-effect curves to naratriptan in the absence (●, EC_{50}: 16.6 ± 2.2 nM) and presence of ketanserin (1 μM, ○, EC'_{50}: 20.5 ± 3.5 nM; 10 μM, ▽, EC'_{50}: 108 ± 72 nM). Curves were constructed using mean values ± S.E.M. of 2–6 independent experiments, each performed in triplicate. Ketanserin (10 μM) did not inhibit forskolin-stimulated cAMP formation.

the concentration ratio obtained for GR 127,935 in the presence of 10 μM ketanserin was 20 (Fig. 1C).

3.2. Effects of GR 127,935 and ketanserin on naratriptan-induced inhibition of forskolin-stimulated cAMP formation in C6-glial/5-HT_{1B} cells

The C6-glial/5-HT_{1B} cell line displayed marked (> 90%) inhibition of 100 μM forskolin-stimulated cAMP formation in the presence of 1 μM 5-HT. Naratriptan showed an agonist efficacy similar to that of 5-HT; half-maximal inhibition of forskolin-stimulated cAMP formation by naratriptan was attained at a 30 times higher concentration (16.6 ± 2.2 nM, $n = 6$) than its respective binding affinity (0.54 ± 0.07 nM, $n = 4$). GR 127,935 antagonised the naratriptan-elicited response in a concentration-dependent manner; potency as well as maximal effects were reduced at 10 nM GR 127,935 and higher

concentrations (Fig. 2A). Ketanserin (10 μM) did not show intrinsic activity. It was neither effective as an antagonist against the naratriptan-induced response; the concentration ratio obtained for naratriptan in the presence of ketanserin was maximally 6.5 at 10 μM (Fig. 2B) consistent with its lack of binding affinity (IC_{50}: > 10000 nM) for 5-HT_{1B} sites.

4. Discussion

The relative antagonist selectivity of ketanserin and GR 127,935 was investigated against naratriptan-mediated cAMP responses in rat C6-glial cell lines expressing human 5-HT_{1B} and 5-HT_{1D} receptors. Naratriptan was found to be 3 times more potent than sumatriptan (Pauwels et al., 1995) in causing inhibition of stimulated cAMP formation at cloned human 5-HT_{1B} as well as 5-HT_{1D} receptor sites. Ketanserin antagonised the naratriptan response in C6-glial/5-HT_{1D} cells and this effect was concentration-dependent and competitive, yielding a pA_2 value of 7.76. Antagonist activity at these receptor sites with GR 127,935 could not be detected; however, GR 127,935 showed intrinsic activity at submicromolar concentrations. The maximal effect of GR 127,935 was almost similar to that of naratriptan; its potency was much less, however, and about 100-fold different from its corresponding binding affinity (Clitherow et al., 1994). The intrinsic activity of GR 127,935 was antagonised by ketanserin although less effectively than against naratriptan (concentration ratio 20 versus 590 at 10 μM ketanserin). In contrast to ketanserin, GR 127,935 potently antagonised the naratriptan response in C6-glial/5-HT_{1B} cells and reduced the maximum response. Similar findings were obtained for the antagonism of 5-CT by GR 127,735 (pK_B value: 8.89) in transfected Chinese hamster ovary K_1/5-HT_{1B} cells (Pauwels and Palmier, 1995). The insurmountable inhibitory effect of GR 127,935 seems likely to reflect its high lipophilicity and slow dissociation kinetics rather than a non-competitive interaction with the agonist-receptor complex, since the inhibitory effect is slowly reversible (Clitherow et al., 1994). Insurmountable antagonism has also been observed for GR 127,935 in contraction studies of dog saphenous vein (Clitherow et al., 1994) and rabbit saphenous vein (Razzaque et al., 1995), and relaxation studies of pre-contracted guinea-pig jugular vein (Razzaque et al., 1995). Antagonism of sumatriptan-mediated responses by ketanserin has been observed in rabbit tissues including saphenous vein (Martin and Maclennan, 1990; Razzaque et al., 1995), renal artery (Choppin and O'Connor, 1993) and basilar artery (Tilford and Baxter, 1994). In contrast, responses to sumatriptan are apparently insensitive to ketanserin (1 μM) in guinea-pig tissues such as jugular vein (Razzaque et al., 1995) and ileac artery (Sahin-Erdemli et al., 1991). Kaumann et al. (1993) putatively established the presence of 5-HT_{1B} receptors in human arteries as no effect was observed with ketanserin (1 μM) on sumatriptan-induced contractions. Different proportions of functional 5-HT_{1D} and 5-HT_{1B} receptors may underlie these pharmacological differences between guinea-pig, human and rabbit preparations. Finally, it may be of interest to reconsider results where on the basis of ketanserin sensitivity, responses have been characterized as being 5-HT_2 receptor-mediated (e.g. human saphenous vein, dog coronary artery; see: Razzaque et al., 1995). It may be reasonable to consider in these preparations the possible activation of 5-HT_{1D} besides 5-HT_2 receptors. In summary, ketanserin and GR 127,935 were found in the present study to selectively antagonise naratriptan responses in C6-glial/5-HT_{1D} and C6-glial/5-HT_{1B} cells, respectively.

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