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Short communication

Stereoselectivity of 8-OH-DPAT enantiomers at cloned human 5-HT_{1D} receptor sites

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Abstract

The cAMP responses of (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and its enantiomers were measured at cloned human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors in transfected C6-glial cells. *R*(+)-8-OH-DPAT demonstrated potent intrinsic activity (EC_{50} value: 30 nM) at 5-$HT_{1D\alpha}$ receptor sites, its maximal effect being comparable to that of sumatriptan. Racemic 8-OH-DPAT and *S*(−)-8-OH-DPAT showed similar agonist efficacy but were respectively 2 and 75 times less potent than *R*(+)-8-OH-DPAT. This differs from the lack of stereoselectivity of the 8-OH-DPAT enantiomers for 5-HT_{1A} receptors.

Keywords: 8-OH-DPAT ((±)-8-hydroxy-2-(di-*n*-propylamino)tetralin); Stereoselectivity; 5-HT_{1D} receptor

1. Introduction

The discovery that the anxiolytic agent buspirone, which also has antidepressant properties, binds with high affinity to 5-HT_{1A} receptors (Traber and Glaser, 1987) has stimulated research on these receptors. Key findings have been the identification of the 5-HT_{1A} receptor binding site (Pedigo et al., 1981), the demonstration of its high affinity for (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (Gozlan et al., 1983) and the finding that the G-21 clone encodes the human 5-HT_{1A} receptor (Fargin et al., 1988).

The great majority of pharmacological investigations of 8-OH-DPAT have been performed with the racemate rather than its enantiomers. It was unexpected that the enantiomers differed only slightly in potency (Arvidsson et al., 1981) and therefore showed a lack of stereoselectivity. Whereas the affinities of the *R*(+)- and *S*(−)-enantiomers of 8-OH-DPAT for 5-HT_{1A} receptors are similar (K_i = 4.1 and 6.1 nM, respectively, Hacksell et al., 1993), their efficacies are different. In the forskolin-stimulated adenylyl cyclase assay, *R*(+)-8-OH-DPAT behaves as an apparently full agonist, that is, it decreases the cAMP production to the same extent as 5-HT itself, whereas the *S*(−)-enantiomer is a partial agonist as it decreases cAMP levels to about 50% of the maximal reduction induced by 5-HT (Cornfield et al., 1991). This emphasizes the importance of using a stereoselective compound instead of the racemate for pharmacological studies.

Recently, we observed besides the reported receptor binding affinities for 8-OH-DPAT, submicromolar affinity for cloned human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor sites (Pauwels et al., 1995). In this study, the potencies and efficacies of the 8-OH-DPAT enantiomers on inhibition of cAMP formation in C6-glial cell lines, stably expressing 5-$HT_{1D\alpha}$ or 5-$HT_{1D\beta}$ receptor sites, were measured.

2. Materials and methods

2.1. Cell culture

C6-glial cells, permanently transfected with a pRcRSV plasmid containing a cloned human 5-$HT_{1D\alpha}$ receptor gene (C6-glial/5-$HT_{1D\alpha}$) or $pcDNA_3$ plasmid containing a cloned human 5-$HT_{1D\beta}$ receptor gene (C6-glial/5-$HT_{1D\beta}$), 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).

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2.2. 5-HT_{1D} receptor binding to membrane preparations of 6-glial / 5-$HT_{1D\alpha}$ and C6-glial / 5-$HT_{1D\beta}$ 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) both in the absence and presence of 8-OH-DPAT and its enantiomers, or 1 μM 5-HT to de-termine 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-$HT_{1D\alpha}$ and C6-glial/5-$HT_{1D\beta}$, respectively; Pauwels et al., 1995).

2.3. 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ 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 isobutylmethyl-xanthine in the presence of 100 μM forskolin both in the absence and the presence of sumatriptan, 8-OH-DPAT and its enantiomers or 1 μM 5-HT to determine maximal cAMP inhibition. Basal accumulation of cAMP was measured in the absence of forskolin and compound. 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.

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).

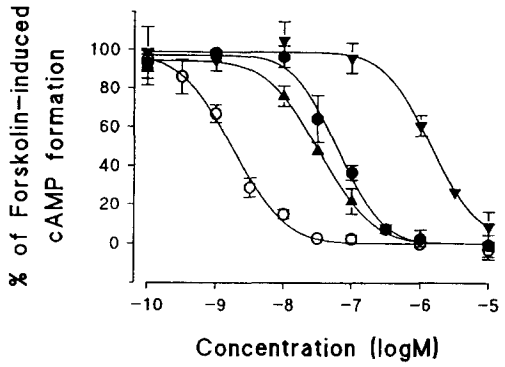

Fig. 1. Forskolin-induced cAMP formation in cultures of C6-glial/5-$HT_{1D\alpha}$ cells in the presence of sumatriptan, racemic 8-OH-DPAT and its enantiomers. C6-glial cells, transfected with a pRcRSV plasmid containing a cloned human 5-$HT_{1D\alpha}$ receptor gene, were exposed for 5 min to 100 μM forskolin in the presence of 1 mM isobutylmethylxanthine with increasing concentrations of sumatriptan (○), 8-OH-DPAT (●), $R(+)$-8-OH-DPAT (▲) or $S(-)$-8-OH-DPAT (▼). Cellular cAMP content was assayed using a radioimmunoassay kit. Inhibition of 100 μM forskolin-induced cAMP formation (187 ± 6 pmol/well, $n = 12$) was calculated as the percentage of that obtained with 1 μM 5-HT (78 ± 2.3%). EC_{50} values are summarized in Table 1. Curves were constructed using mean values ± S.E.M. of 3 to 11 independent experiments, each performed in triplicate.

[^{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). The 8-OH-DPAT enantiomers were obtained from RBI (Natick, USA).

3. Results

8-OH-DPAT showed a 3-fold higher binding affinity for 5-$HT_{1D\alpha}$ (K_i: 44.3 nM) than 5-$HT_{1D\beta}$ (K_i: 125.1 nM) receptor sites in stably transfected C6-glial cells using [^{3}H]5-CT as a radioligand. The activity resided in the $R(+)$-enantiomer, the $S(-)$-enantiomer showed 15 and 23 times less 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ binding affinity, respectively (Table 1). Fig. 1 compares the 5-$HT_{1D\alpha}$ receptor-mediated effects of 8-OH-DPAT and its enantiomers with that of sumatriptan on inhibition of forskolin (100 μM)-stimulated cAMP formation. Whereas sumatriptan was most potent (EC_{50}: 2 ± 0.29 nM, $n = 11$), $R(+)$-8-OH-

Table 1
K_i values of sumatriptan, 8-OH-DPAT and its enantiomers for inhibition of [^{3}H]5-CT binding to cloned human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor sites in stably transfected C6-glial cell lines, and their EC_{50} values for inhibition of 100 μM forskolin-induced cAMP formation

Receptor site		Sumatriptan	8-OH-DPAT	$R(+)$-8-OH-DPAT	$S(-)$-8-OH-DPAT
5-$HT_{1D\alpha}$	K_i (nM)	2.3	44.3 ± 2.4 (3)	28.8 ± 3.5 (2)	434 ± 17.8 (2)
	EC_{50} (nM)	2.0 ± 0.29 (11)	60.4 ± 10.5 (9)	30 ± 3.1 (3)	2267 ± 706 (3)
5-$HT_{1D\beta}$	K_i (nM)	2.9	125.1 ± 30.6 (3)	75.5 ± 11.2 (2)	1727 ± 150 (2)
	EC_{50} (nM)	78 ± 10 (12)	723 ± 85 (7)	415 ± 186 (2)	> 10000 (2)

Values are given as the means ± S.E.M. Number of independent experiments, each performed in triplicate, is given in parentheses. Binding data for sumatriptan were taken from Pauwels et al. (1995).

DPAT showed its half-maximal agonist effect at 30 nM, fully in agreement with its binding affinity (Table 1). Racemic 8-OH-DPAT and its *S*(−)-enantiomer were 2 and 75 times less potent as agonists, respectively. Similar agonist efficacies were observed with sumatriptan, racemic 8-OH-DPAT as well as its enantiomers; their E_{max} values were similar to that of 5-HT. The agonist activity of these compounds at 5-$HT_{1D\beta}$ receptor sites was less marked; the half-maximal agonist effects were measured at 415 nM and 723 nM for *R*(+)-8-OH-DPAT and 8-OH-DPAT, respectively (Table 1). Moreover, *R*(+)-8-OH-DPAT and 8-OH-DPAT did not exert full agonist activity; the maximal inhibition of cAMP formation observed was between 83 and 87% relative to that of sumatriptan or 5-HT.

4. Discussion

Besides high affinity for 5-HT_{1A} receptor sites, *R*(+)-8-OH-DPAT binds to cloned human 5-$HT_{1D\alpha}$ receptor sites with a K_i value of 29 nM, i.e., about 7-fold higher than its K_i value for the 5-HT_{1A} receptor site (Hacksell et al., 1993). Whereas almost similar binding affinities were observed with the racemic mixture and the enantiomers of 8-OH-DPAT at 5-HT_{1A} receptor sites (Cornfield et al., 1991), stereoselectivity was found for human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor sites by measuring the binding affinity as well as agonist potency. *R*(+)-8-OH-DPAT showed a 5-$HT_{1D\alpha}$ receptor selectivity in as much as it showed 14 times less intrinsic activity at 5-$HT_{1D\beta}$ receptor sites. The inactive enantiomer had little effect on the observed intrinsic activity at both 5-HT_{1D} receptor subtypes. The presence of this compound can only reduce the concentration of the active enantiomer by half. Racemic 8-OH-DPAT was indeed 2 times less active than *R*(+)-8-OH-DPAT. Similar agonist efficacies were observed with 8-OH-DPAT and its enantiomers for 5-$HT_{1D\alpha}$ receptor sites in contrast to the different efficacies observed for 5-HT_{1A} receptor sites (Cornfield et al., 1991). Although these ligand-receptor interactions are not well understood, the 5-$HT_{1D\alpha}$ receptor-mediated agonist activity of *R*(+)-8-OH-DPAT should be taken into account when analysing its activity in vivo. Moreover, 5-HT_{1D} receptors may have a functional role in the raphe cell body region (Starkey and Skingle, 1994), traditionally thought to be under 5-HT_{1A} receptor control.

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