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Actions of the prototypical 5-HT_{1A} receptor agonist 8-OH-DPAT at human α_2-adrenoceptors: (+)8-OH-DPAT, but not (−)8-OH-DPAT is an α_{2B} subtype preferential agonist

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ABSTRACT

8-OH-DPAT [8-hydroxy-2-(di-n-propylamino)tetralin] is the prototypical agonist at serotonin 5-HT_{1A} receptors; however, activity at other targets contributes to the functional effects of the compound as well. We examined the properties of 8-OH-DPAT and its enantiomers at recombinant human (h)α_2-adrenoceptor subtypes, using a panel of radioligand binding and functional tests. In competition binding experiments using [^{3}H]-RX821002, about 10-fold selectivity of (+)8-OH-DPAT for the hα_{2B} subtype (pK_i about 7) over hα_{2A}- and hα_{2C}-adrenoceptors was observed. In contrast, the S(−) enantiomer of 8-OH-DPAT showed similar weak affinities for the three receptor subtypes (pK_is<6). The binding affinity of (+)8-OH-DPAT at the hα_{2B}- and the hα_{2A}-adrenoceptor was found sensitive to GTPγS, a receptor/G protein-uncoupling agent, indicating agonist properties of the drug. Furthermore, using [^{35}S]GTPγS binding determination at CHO-hα_{2B} or CHO-hα_{2A} cell membranes and G protein coupled inwardly rectifying potassium (GIRK) current recordings in *Xenopus* oocytes expressing hα_{2B}, partial agonist activity of (+)8-OH-DPAT at the respective receptors was confirmed in these two different functional assays. Potency of (+)8-OH-DPAT for stimulation of [^{35}S]GTPγS incorporation was lower at the hα_{2A}- than at the hα_{2B}-adrenoceptor, consistent with binding affinities. Thus, (+)8-OH-DPAT and, as a consequence, racemic (±)8-OH-DPAT are partial agonists at hα_2-adrenoceptors with selectivity for the hα_{2B} subtype, a property that might contribute to the effects of the compound described in native systems.

1. Introduction

8-OH-DPAT [8-hydroxy-2-(di-n-propylamino)tetralin] is considered as the prototypical agonist at serotonin 5-HT_{1A} receptors (Caliendo et al., 2005) and has been largely used for in vitro and in vivo characterization of 5-HT_{1A} receptor-mediated effects. Accordingly, the tritium-labelled compound represents the reference agonist radioligand to specifically label 5-HT_{1A} receptors (Gozlan et al., 1983). 8-OH-DPAT is a racemic mixture of the R(+) and the S(−) isomer, which are characterized by different functional activities: both exhibit similar high potency, but while the R(+) enantiomer is a potent full or near full 5-HT_{1A} receptor agonist, the S(−) enantiomer exhibits only partial agonist activity (Cornfield et al., 1991; Fowler et al., 1992; Nelson, 1991). As a consequence, racemic (±)8-OH-DPAT includes both of these properties and thus acts as agonist with differential relative efficacies depending on the particular functional readout coupled to 5-HT_{1A} receptor activation (Dumuis et al., 1988; Pauwels et al., 1997).

Apart from its actions at 5-HT_{1A} receptors, 8-OH-DAT has been shown to possess affinities at a number of other targets. The most prominent of these is the 5-HT_7 receptor, where 8-OH-DPAT acts as partial agonist with submicromolar affinity (Rauly-Lestienne et al., 2007). This property of 8-OH-DPAT has in fact led to the characterization of the compound as a mixed 5-HT_{1A}/5-HT_7 agonist (Bonaventure et al., 2002; Ruat et al., 1993).

With respect to non-serotonergic targets, interactions of 8-OH-DPAT with D_2-like dopaminergic and α-adrenoceptors are the best characterized. Among dopaminergic receptors, 8-OH-DPAT shows some preference for the D_3 subtype, but binds with moderate affinity to D_2 receptors as well (K_i around 1 μM according to Lejeune et al., 1997; Yu et al., 1996). As concerns its functional effects, 8-OH-DPAT has been found to exhibit partial agonist activity at D_2 receptors both in vivo and in vitro (Matuszewich et al., 1999; Rinken et al., 1999; Smith and Cutts, 1990).

Albeit 8-OH-DPAT has some affinity at α_1-adrenoceptors (Yoshio et al., 2001), more powerful interactions with receptors of the α_2 adrenergic type have been observed. In binding studies, 8-OH-DPAT exhibits moderate affinity for α_2 sites (Brown et al., 1990; Gobert et al., 1995; Renouard et al., 1994) and the radioligand [^{3}H]-8-OH-DPAT has been found to label α_2-adrenoceptor sites in mouse brain (Bonaventure

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et al., 2004). In line with this, a number of early studies with 8-OH-DPAT indicated that some of its central effects were mediated by α_2-adrenoceptors, albeit an indirect mode of action could not be excluded in most cases (Chaouloff and Jeanrenaud, 1987; Doods et al., 1988; Fozard et al., 1987; Marsden and Martin, 1986; Millan and Colpaert, 1991). Other results moreover support a functionally relevant interaction of 8-OH-DPAT with peripheral α_2-adrenoceptors in different tissues (Crist and Surprenant, 1987; Tiniakov and Scrogin, 2006).

α_2-adrenoceptors can be subdivided into the α_{2A}, α_{2B} and α_{2C} subtypes, the α_{2A}-adrenoceptor being the most widely expressed in the CNS. These subtypes exhibit a high degree of functional and pharmacological similarity (Fairbanks et al., 2009), but nevertheless a particular pattern of tissue distribution and functional effects can be attributed to each of them (Gyires et al., 2009; Knaus et al., 2007; Philipp et al., 2002). Interestingly, some indications for subtype-specificity of 8-OH-DPAT at α_2-adrenoceptors have been presented (Borton et al., 1991; Brown et al., 1990; Renouard et al., 1994). However, subtype-selective actions of 8-OH-DPAT at adrenoceptors have not been studied in detail. Here, we test the actions of the drug and its enantiomers at recombinant human (h)α_2-adrenoceptors using radioligand binding and several approaches on receptor functional activity.

2. Materials and methods

2.1. Membrane preparation

Membranes were prepared from CHO or C6 glial cells stably expressing human (h)α_2-adrenoceptor subtypes. At confluence, cells were washed twice with phosphate buffered saline (PBS), pH 7.4, and stored at −80 °C. Cells were harvested from dishes in a buffer containing 10 mM Tris–HCl (pH 7.5) supplemented with 0.1 mM EDTA and centrifuged for 10 min at 45000 g. The pellet was homogenised in the same buffer using a polytron and recentrifuged. The final membrane pellet was dissolved in Tris/EDTA buffer and stored at −80 °C until used.

2.2. Radioligand binding

Competition binding assays at C6 glial cells stably expressing hα_{2A}-, hα_{2B}- or hα_{2C}-adrenoceptors were performed as previously described (Wurch et al., 1999). For competition binding experiments, C6-hα_2 membranes (10–20 µg of protein) were resuspended in 50 mM Tris buffer, pH 7.6, and incubated for 120 min at 25 °C with the radioligand, [^{3}H]-RX821002 [(1,4-[6,7(n)-^{3}H]benzodioxan-2-methoxy-2-yl)-2-imidazoline hydrochloride]; GE Healthcare Europe GmbH, Orsay, France, and test compounds in a final volume of 0.5 ml. Concentrations of [^{3}H]-RX821002 were about 2 nM, 10 nM and 4 nM, respectively, for hα_{2A}-, hα_{2B}- or hα_{2C}-adrenoceptors (K_ds 1.2 nM, 9.2 nM and 2.2 nM, respectively). Non-specific binding was defined with phentolamine (10 µM). In a subset of experiments, GTPγS was added at a final concentration of 100 µM. Incubations were terminated by rapid filtration through Whatman GF/B filters (presoaked for 1 h in 0.1% polyethylenimine) using a 96 well filtermate harvester (PerkinElmer Life Science, Boston, MA). The filters were washed three times, and radioactivity retained on the filters was determined by liquid scintillation counting using a TopCount microplate scintillation counter (PerkinElmer Life Science).

2.3. [^{35}S]GTPγS binding experiments

Receptor-linked G protein activation at hα_2-adrenoceptors was determined by measuring the incorporation of [^{35}S]GTPγS (>1000 Ci/mmole; GE Healthcare Europe GmbH, Orsay, France) into membranes of CHO cells stably expressing hα_{2A}- or hα_{2B}-adrenoceptors. Briefly, membranes were preincubated 30 min at 30 °C with receptor ligands in a buffer containing 20 mM HEPES, 0.3 µM GDP, 3 mM $MgCl_2$, 100 mM NaCl, pH 7.4. The reaction was started by addition of 0.5 nM [^{35}S]GTPγS in a final volume of 0.5 ml and incubation was performed for an additional 30 min. Experiments were terminated by rapid filtration through Unifilter-96 GF/B filter (PerkinElmer Life Sciences) using a Filtermate harvester (PerkinElmer Life Sciences, Boston, MA). Radioactivity retained on the filters was determined by liquid scintillation counting using a TopCount microplate scintillation counter (PerkinElmer Life Sciences). Basal binding is defined as 0% whereas adrenaline (10 µM)-stimulated [^{35}S]GTPγS binding performed in each experiment is defined as 100%.

2.4. Xenopus laevis oocyte expression and GIRK current recordings

The plasmids containing the coding sequence for hα_{2A}- or hα_{2B}-adrenoceptors were subcloned into the *Xenopus* high expression vector pGEMHE. Plasmids pSP/GIRK1 and pBScMXT/GIRK2 encoding the GIRK1 and GIRK2 potassium channel subunits were prepared as described (Heusler et al., 2005). Plasmids were linearised with *Nhe*I (pGEMHE/hα_2-adrenoceptors), *EcoR*I (pSP/GIRK1) or *Sal*I (pBScMXT/GIRK2) and in vitro transcription of RNA was performed using the T7 (pGEMHE/hα_2-adrenoceptors), SP6 (pSP/GIRK1) or T3 (pBScMXT/GIRK2) mMessage mMachine transcription kit (Ambion, Austin, TX). RNA was purified using the RNeasy RNA cleanup kit (Qiagen, Courtaboeuf, France), quantified spectrometrically, diluted to the appropriate concentration in RNAse-free water and stored at −80 °C prior to use.

Isolation and separation of oocytes were performed as previously described (Heusler et al., 2005). Defolliculated oocytes were injected with a roughly 50 nl volume of a solution containing the cRNAs coding for the GIRK 1 and GIRK 2 channel subunits at a concentration of 20 pg cRNA/oocyte each with addition of hα_{2B}-adrenoceptor cRNA at 1 ng/oocyte (no measurable ligand-induced currents were observed in oocytes injected with receptor or GIRK channel RNA alone, not shown). After injection, oocytes were kept at 17 °C in ND96 solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM HEPES, pH 7.5 with NaOH) supplemented with 50 mg/l gentamicin. Donor animals were handled and cared for in an AALAAC-approved institution and the protocols were carried out in compliance with French regulations and with local Ethical Committee guidelines for animal research (protocol approved by the ethics committee under number 242).

Whole-cell oocyte currents were recorded using a Geneclamp 500 amplifier (Axon Instruments, Union City, CA) and the two-electrode voltage clamp technique, as described previously (Heusler et al., 2005). Briefly, oocytes were placed in a recording chamber where they were continuously superfused with ND96 solution (2.5–3.5 ml/min). GIRK currents were recorded in high-potassium solution (hK^+, similar to ND96, but containing 96 mM KCl, 2 mM NaCl) at a holding potential of −70 mV. Receptor ligands dissolved in hK^+ were applied by superfusion. All ligands were applied at a concentration of 10 µM, except where stated otherwise. At the end of each experiment, $BaCl_2$ (1 mM, dissolved in hK^+) was applied to quantify the GIRK-independent currents in hK^+. Evaluation of receptor-independent effects of ligands on GIRK currents was performed as described (Heusler et al., 2005). For drugs exhibiting receptor-independent GIRK current inhibitions in this assay, ligand efficacy values in the assay on receptor activation were corrected for the respective value (see Results). The pClamp 8 software (Axon Instruments) was used for data acquisition.

2.5. Drugs

GTPγS, (−)adrenaline, clonidine, (±)8-OH-DPAT and (+)8-OH-DPAT were purchased from Sigma RBI, (−)8-OH-DPAT was synthesized in-house.

2.6. Data analysis

Isotherms were analysed by non-linear regression using the program Prism (GraphPad Software, San Diego, CA) to yield EC_{50} or