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[^{3}H]7-OH-DPAT is capable of labeling dopamine D_2 as well as D_3 receptors

Antonio M. Gonzalez, David R. Sibley *

Molecular Neuropharmacology Section, Experimental Therapeutics Branch, National Institute of Neurological Disorders & Stroke, National Institutes of Health, Bethesda, MD 20892, USA

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Abstract

The binding of [^{3}H](+)-7-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin ([^{3}H]7-OH-DPAT) to dopamine D_2 and D_3 receptors expressed in Chinese hamster ovary (CHO) cells was investigated and compared with [^{3}H]methylspiperone. [^{3}H]7-OH-DPAT labeled the D_3 receptor in the CHO cells in a guanine nucleotide-insensitive fashion and exhibited a K_d of about 0.5 nM. In the presence of $MgCl_2$. [^{3}H]7-OH-DPAT was also found to label the D_2 receptor in CHO cells with high affinity (3.6 nM). The binding of [^{3}H]7-OH-DPAT to the D_2 receptor was sensitive to guanine nucleotides suggesting occupancy of a high affinity G protein-coupled state of the receptor. These results suggest that caution should be exercised when using [^{3}H]7-OH-DPAT to label the dopamine D_3 receptor in brain tissues.

Keywords: 7-OH-DPAT (7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin); Dopamine D_2 receptor; Dopamine D_3 receptor

Molecular biological studies have recently identified a family of dopamine D_2-like receptors which include the D_2, D_3 and D_4 receptor subtypes. The D_3 receptor is of significant interest as it demonstrates high affinity for many neuroleptic drugs and is expressed predominantly in limbic brain areas including the olfactory tubercle, nucleus accumbens and islands of Calleja. These characteristics have suggested that the D_3 receptor may play an important role in the therapeutic action of antipsychotic drugs.

A major problem in understanding the physiological role of the D_3 receptor is a lack of suitably selective pharmacologic agents. Recently (±)-7-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin (7-OH-DPAT) has been reported to be a selective ligand for the dopamine D_3 receptor and [^{3}H]7-OH-DPAT has been used to label D_3-like binding sites in rat brain membranes (Levesque et al., 1992). In the initial study of Levesque et al. (1992), (±)-7-OH-DPAT was reported to exhibit a K_i of 61 nM for the rat D_2 receptor whereas the rat D_3 receptor showed a K_i of 0.78 nM. Subsequently, the enantiomers of 7-OH-DPAT have been investigated and the *R*-(+)-isomer has been reported to be 220-fold (Damsma et al., 1993) and 64-fold (Baldessarini et al., 1993) selective for the D_3 receptor compared to the D_2 receptor. Based on these initial studies, a number of groups have begun to use [^{3}H]7-OH-DPAT to selectively label D_3 receptors in brain tissues (Herroelen et al., 1994; Hillefors-Berglund and Von Euler, 1994).

Recently, however, the selectivity of 7-OH-DPAT has come into question as [^{3}H]7-OH-DPAT has been shown to label sigma binding sites in bovine caudate nucleus (Schoemaker, 1993) and a related ligand, [^{125}I]-(*R*)-*trans*-7-hydroxy-2-[*N*-propyl-*N*-3′-iodo-2′-propenyl)-amino]tetralin, has been suggested to label D_2 receptors in cell lines (Burris et al., 1994). We now report that under commonly employed experimental conditions, [^{3}H]7-OH-DPAT is capable of directly labeling the D_2 receptor with high affinity. These results suggest that caution should be exercised when using [^{3}H]-7-OH-DPAT as a selective ligand for the D_3 receptor.

The methods for cell culture and radioligand binding assays were generally as described in Zhang et al. (1994). Briefly, Chinese hamster ovary (CHO) cells

* Corresponding author. National Institute of Neurological Disorders & Stroke, National Institutes of Health, Building 10, Room 5C-108, 9000 Rockville Pike, Bethesda, MD 20892, USA.

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stably expressing the rat dopamine D_{2L} and D_3 receptors were cultured in F-12 media supplemented with 10% fetal bovine serum and 1 mM sodium pyruvate at 37°C in a humidified atmosphere containing 5% CO_2. Radioligand binding assays were performed by preparing membranes from the cells and incubating them with varying concentrations of either [^{3}H]methylspiperone (86 Ci/mmol, Dupont/NEN) or [^{3}H]-(+)-7-OH-DPAT (110 Ci/mmol, Amersham) in Tris-HCl, pH 7.4 at 25°C, 10 mM $MgCl_2$, and 1 mM EDTA for 1 h at 25°C. Non-specific binding was defined using 1 μM (+)-butaclamol for D_2 receptor binding and 10 μM (+)-butaclamol for D_3 receptor binding. The assays were terminated by filtration through GF/B glass fiber filters and the trapped radioactivity was quantitated via scintillation spectroscopy.

In our initial series of experiments, we compared the D_3 receptor binding of [^{3}H]7-OH-DPAT to that of [^{3}H]methylspiperone, a radiolabeled D_2-like antagonist ligand. Fig. 1A shows representative saturation isotherms for [^{3}H]methylspiperone and [^{3}H]7-OH-DPAT binding to membranes prepared from CHO cells transfected with the rat D_3 receptor. [^{3}H]-Methylspiperone bound with high affinity ($K_d = 0.50 \pm 0.02$ nM, $n = 7$) and exhibited a maximum binding capacity (B_{max}) of 2260 ± 303 fmol/mg protein, $n = 7$. [^{3}H]7-OH-DPAT also labeled the D_3 receptor with high affinity ($K_d = 0.49 \pm 0.08$ nM, $n = 4$) and exhibited a binding capacity of 2130 ± 241 fmol/mg protein, $n = 4$. Adding the guanine nucleotide guanylyl-5'-imidodiphosphate (GppNHp) to the assay buffer did not significantly affect the binding of [^{3}H]7-OH-DPAT to the D_3 receptor: $K_d = 0.71 \pm 0.16$ nM, $n = 3$; $B_{max} =$ 2240 ± 70 fmol/mg protein, $n = 3$. These results are similar to those initially reported for the D_3 receptor binding of [^{3}H]7-OH-DPAT by Levesque et al. (1992).

We next investigated the interaction of [^{3}H]7-OH-DPAT with the D_2 receptor using CHO cells transfected with the D_{2L} receptor isoform (Zhang et al., 1994). In preliminary experiments, we determined that [^{3}H]methylspiperone exhibits a maximum binding capacity of 1580 ± 150 fmol/mg protein, $n = 5$, to the D_2 receptors on these cells (data not shown). Surprisingly, we found that [^{3}H]7-OH-DPAT also labeled the D_2 receptors with relatively high affinity (Fig. 1B) exhibiting a K_d of 3.6 ± 0.14 nM, $n = 3$ and a B_{max} of 240 ± 25 fmol/mg protein, $n = 3$. The addition of GppNHp to the assay buffer completely abolished the specific binding of [^{3}H]7-OH-DPAT to the dopamine D_2 receptors (Fig. 1B).

These results clearly indicate that [^{3}H]7-OH-DPAT is capable of labeling the dopamine D_2 receptor in addition to the D_3 receptor subtype. The data further suggest that it is the G protein-coupled, high affinity agonist binding state of the D_2 receptor which is recognized by [^{3}H]7-OH-DPAT. This is indicated by the observation that [^{3}H]7-OH-DPAT labels fewer D_2 receptor sites than the radiolabeled antagonist, [^{3}H]-methylspiperone, and that the binding can be completely inhibited by guanine nucleotides which convert the high affinity state to low affinity. The affinity of [^{3}H]7-OH-DPAT for the uncoupled state of the D_2 receptor is thus too low to be identified using filtration binding assays. It should be noted that the D_2 receptors on the CHO cells are coupled to decreasing intracellular cAMP levels through a pertussis toxin-sensitive

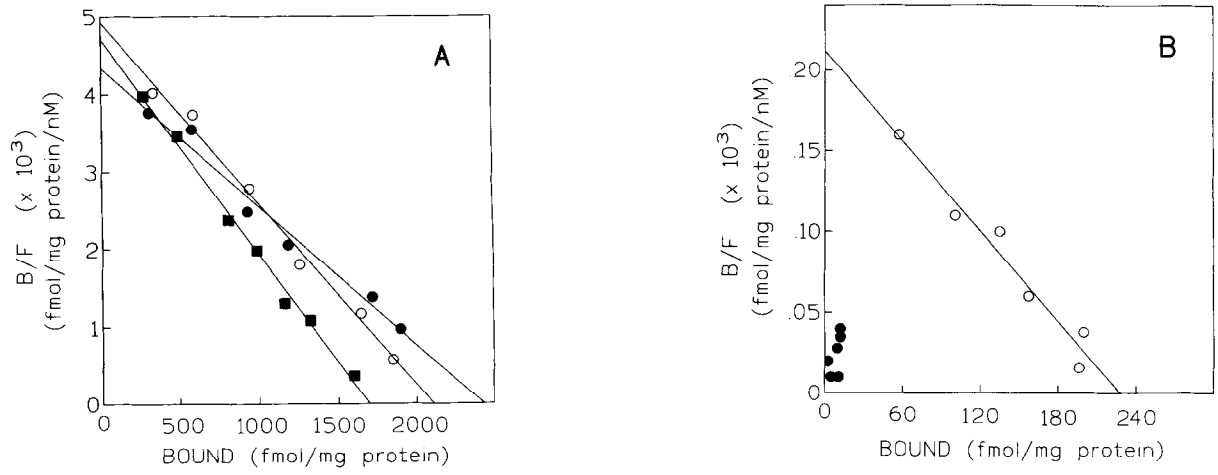

Fig. 1. Scatchard analysis of specific [^{3}H]methylspiperone and [^{3}H]7-OH-DPAT binding to dopamine D_2 or D_3 receptors. Membranes from cells expressing either D_2 or D_3 receptors were prepared and saturation binding assays performed as described in the text. A: D_3 receptor binding. [^{3}H]Methylspiperone (■) binding revealed a K_d of 0.51 nM and a B_{max} of 1720 fmol/mg protein; [^{3}H]7-OH-DPAT (○) binding revealed a K_d of 0.53 nM and a B_{max} of 2140 fmol/mg protein whereas in the presence of 0.2 mM GppNHp (●) the [^{3}H]7-OH-DPAT binding parameters were: $K_d = 0.78$ nM and $B_{max} = 2290$ fmol/mg protein. B: D_2 receptor binding. [^{3}H]7-OH-DPAT (○) binding revealed a K_d of 2.1 nM and a B_{max} of 214 fmol/mg protein. In the presence of 0.2 mM GppNHp (●), there was no specific binding of [^{3}H]7-OH-DPAT. Representative experiments are shown while average binding parameter values are given in the text.

G protein (Zhang et al., 1994). In contrast, we have found no evidence for functional G protein coupling of the D_3 receptor in transfected CHO cells as assessed using a number of second messenger assays (unpublished observations). Not surprisingly, [^{3}H]7-OH-DPAT binding to the D_3 receptor is insensitive to guanine nucleotides.

The ability of [^{3}H]7-OH-DPAT to label the D_2 receptor is most likely dependent on the conditions of the radioligand binding assay. In our experiments, we have employed a low ionic strength buffer with a high concentration of Mg^{2+} which is known to favor the formation of the G protein-coupled state of the D_2 receptor. In contrast, in the study of Levesque et al. (1992), the binding assays were conducted in the complete absence of divalent cations. Although Na^+ has also been suggested to decrease the affinity of agonists for the D_2 receptor, we found that its inclusion (120 mM) in the assay buffer did not significantly affect the D_2 receptor binding of [^{3}H]7-OH-DPAT (unpublished observations).

In summary, caution should be exercised when attempting to utilize [^{3}H]7-OH-DPAT as a D_3-selective radioligand in brain tissues. The assay conditions should be carefully chosen to eliminate D_2 receptor interactions and the pharmacology of the labeled receptor sites should be characterized. Our results also raise a more general caveat concerning 7-OH-DPAT as a D_3-selective ligand. Since the G protein-coupled form of the D_2 receptor is believed to represent its functional state, then for agonist ligands it might be more appropriate to use their affinities for this coupled state to estimate D_2/D_3 selectivity ratios. Thus, under the conditions of this study, 7-OH-DPAT would demonstrate only about 7-fold selectivity for the D_3 receptor which is significantly less than that initially suggested.

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