

Lack of Discrimination by Agonists for D₂ and D₃ Dopamine Receptors

Kevin D. Burris, Ph.D., Mary A. Pacheco, Ph.D., Theresa M. Filtz, Ph.D., Mei-Ping Kung, Ph.D., Hank F. Kung, Ph.D., and Perry B. Molinoff, M.D.

The affinities of D₃ dopamine receptors for antagonists are similar to those of D₂ receptors. D₃ receptors have been reported, however, to have affinities nearly 100-fold higher than those of D₂ receptors for some agonists, including (±)-7-hydroxy-*n,n*-dipropyl-aminotetralin (7-OH-DPAT) and quinpirole. This has led to the use of these agonists to try to identify functional responses mediated by D₃ receptors *in vivo*. However, D₂ receptors exist in multiple states having high and low affinities for agonists. The G protein-coupled state of D₂ receptors is believed to be the functional state of these receptors. When receptors were labeled with the D₂ receptor antagonist [¹²⁵I]-(S)-3-iodo-N-[(1-ethyl-2-pyrrolidinyl)methyl]-5,6-dimethoxysalicylamide ([¹²⁵I]-NCQ-298) under conditions that promote uncoupling of receptors from G proteins, the affinities of D₃ receptors were approximately 130-fold higher than those of D₂ receptors for 7-OH-DPAT and

quinpirole. When receptors were labeled with the D₂ receptor agonist [¹²⁵I]-(R)trans-7-hydroxy-2-[*N*-propyl-N-(3'-iodo-2'-propenyl)-amino]tetralin ([¹²⁵I]-7-OH-PIPAT) under conditions that favor interactions of receptors with G proteins, the affinities of D₃ receptors were less than sevenfold higher than the affinities of D₂ receptors for the same drugs. Similarly, small differences in the affinities of D₂ and D₃ receptors for other agonists were seen when receptors were labeled with [¹²⁵I]-7-OH-PIPAT. These data demonstrate that putative D₃ receptor-selective agonists also interact with a high-affinity, G protein-coupled state of D₂ receptors. The similarities in affinities of the agonist-preferring state of D₂ and D₃ receptors means that currently available agonists cannot be used to discriminate between behavioral effects mediated by D₂ and D₃ receptors. [*Neuropsychopharmacology* 12:335-345, 1995]

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The D₂ subtype of dopamine receptor is thought to be an important site of action for antipsychotic drugs (Creese et al. 1976; Seeman et al. 1976). Recently, two new D₂-like receptor subtypes, termed D₃ and D₄

receptors, have been identified (Sokoloff et al. 1990; Van Tol et al. 1991). Based on their reported high affinity for many antipsychotic drugs and a relatively restricted distribution in limbic areas of the brain, D₃ receptors have been proposed as a possible target site for antipsychotic drug development (Schwartz et al. 1992). Currently, little is known about the physiological role of D₃ receptors. Studies of the properties and function of D₃ receptors in the brain have been hindered by a lack of selective drugs.

D₃ receptors are both structurally and pharmacologically similar to D₂ receptors. Both have seven putative membrane-spanning regions characteristic of neurotransmitter receptors that couple to guanine nucleotide-binding regulatory proteins (G proteins). D₃ receptors share approximately 75% amino acid homology with D₂ receptors within the membrane-spanning regions and 50% amino acid homology overall (Sokoloff

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"Agonist" and "antagonist" refer to the reported functions of these drugs at D₂ receptors.

From the departments of Pharmacology (KDB, MAP, TMF, HFK, and PBM) and Radiology (MPK, HFK), University of Pennsylvania School of Medicine, Philadelphia, PA.

Address correspondence to: Kevin D. Burris, Ph.D., CNS Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492.

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et al. 1990). The affinities of D₃ receptors for many antagonists, including those used therapeutically in the treatment of psychoses, are comparable to the affinities of D₂ receptors for the same drugs (Sokoloff et al. 1990). However, in studies using transfected cells, D₃ receptors have been reported to have affinities nearly 100-fold higher than those of D₂ receptors for some agonists, including quinpirole and the putative dopamine autoreceptor agonist (\pm)-7-hydroxy-n,n-dipropylaminotetralin (7-OH-DPAT) (Lévesque et al. 1992).

The identification of 7-OH-DPAT as a D₃ receptor-selective drug has stimulated the use of this agent in laboratory animals to identify functional responses mediated by D₃ receptors (Caine and Koob 1993; Daly and Waddington 1993; Damsma et al. 1993). However, the validity of using 7-OH-DPAT—or other agonists—to discriminate between effects mediated by D₂ and D₃ receptors is problematic. Multiple signaling pathways including inhibition of adenylyl cyclase activity, inhibition of K⁺ currents, Ca²⁺ mobilization, an increased rate of extracellular acidification, and potentiation of arachidonic acid release have been linked to activation of D₂ receptors (Neve et al. 1989; Vallar and Meldolesi 1989; Kanterman et al. 1991; Lledo et al. 1992). Although reports of functional effects of activation of D₃ receptors have appeared (Chio et al. 1994; Pilon et al. 1994; Potenza et al. 1994; Seabrook et al. 1994; Tang et al. 1994a), no functional responses have been consistently linked to the activation of D₃ receptors. Therefore, receptor selectivity has been defined using affinity values determined by competition of radiolabeled antagonist binding to receptors expressed in transfected cells.

D₂ receptors exist in multiple states having high and low affinities for agonists (Zahniser and Molinoff 1978; DeLean et al. 1982; Sibley et al. 1982; McDonald et al. 1984; George et al. 1985; Grigoriadis and Seeman 1985; Lahti et al. 1992). The agonist-preferring high-affinity state is believed to involve the formation of a ternary complex composed of agonist, the D₂ receptor, and a G protein (Wregget and DeLean 1984). To fully characterize the selectivity of a receptor for an agonist it is necessary to determine the affinities of multiple states of D₂ receptors for the agonist. Both G protein-coupled and uncoupled states of D₂ receptors are believed to have high affinity for radiolabeled antagonists. Computer-assisted analysis of data from the competition for radiolabeled antagonist binding has been used to determine the affinities of drugs for multiple populations of noninteracting receptor subtypes coexisting within the same tissue (Molinoff et al. 1981). Although these analytical methods are not directly transferrable to the study of interconverting affinity states of a single receptor subtype, similar techniques have been used to investigate the properties of multiple-affinity states of D₂ receptors (Sibley et al. 1982; McDonald et al. 1984; George et al. 1985; Chio et al. 1994). The inhibi-

tion of radiolabeled antagonist binding in the presence of guanine nucleotides provides a good estimate of the affinity of the uncoupled state of receptors for the agonist. An accurate determination of the affinity of an agonist for the G protein-coupled state of receptors cannot be obtained using an antagonist radioligand. An accurate estimate can be obtained in assays with radiolabeled agonists.

Based on the reported selectivity of D₃ receptors for 7-OH-DPAT, an iodinated derivative, [¹²⁵I](R)*trans*-7-hydroxy-2-[N-propyl-N-(3'-iodo-2'-propenyl)-amino]-tetralin ([¹²⁵I]-7-OH-PIPAT), was recently developed (Chumpradit et al. 1993; Foulon et al. 1993). In addition to D₃ receptors, a guanine nucleotide-sensitive state of D₂ receptors was found to bind [¹²⁵I]-7-OH-PIPAT with high affinity under conditions that promote the interaction of receptors with G proteins (Burris et al. 1994). The availability of [¹²⁵I]-7-OH-PIPAT, therefore, provides a radioligand with which the G protein-coupled state of D₂ receptors can be directly labeled. In the present study the affinities of the G protein-coupled state of D₂ receptors for drugs were determined by competition for [¹²⁵I]-7-OH-PIPAT binding under conditions (MgCl₂ present) that favor receptor interactions with G proteins. In addition, the affinities of the uncoupled state of D₂ receptors for drugs were determined by competition for the binding of [¹²⁵I]-NCQ-298, a D₂ receptor antagonist that binds with high affinity to D₂ and D₃ receptors (Hall et al. 1990; Filtz et al. 1993; Boundy et al. 1993a, 1993b) under conditions (guanine nucleotide present) that promote the uncoupling of receptors from G proteins. The affinity of D₂ receptors for [¹²⁵I]-NCQ-298 is increased when assays are carried out in the presence of sodium (Hall et al. 1990); therefore, NaCl (50 mM) was also included in assays with [¹²⁵I]-NCQ-298. For purposes of comparison, the affinities of D₃ receptors for the drugs were also determined under both conditions.

The affinities of D₂ receptors for agonists were much lower than those of D₃ receptors for the same drugs when assays were carried out under conditions that promote uncoupling of receptors from G proteins. However, the affinities of D₂ receptors for agonists were similar to the affinities of D₃ receptors for the same drugs under conditions that favor coupling of receptors to G proteins. Given the range of selectivity obtained in vitro with agonists, it is not possible to use differences in the in vivo potency of agonists, such as 7-OH-DPAT, to ascribe particular behavioral responses to activation of D₃ receptors.

MATERIALS AND METHODS

Materials and Drugs

Tissue culture dishes (100 × 20 mm) were purchased from Corning (Corning, NY). Eagle's minimum essen-

tial medium (EMEM), L-glutamine, and G418 sulfate were purchased from Gibco Laboratories (Grand Island, NY). Fetal bovine serum was purchased from Hyclone Laboratories, Inc. (Logan, UT). 3-Isobutyl-1-methylxanthine (IBMX) was purchased from Aldrich Chemical Company Inc. (Milwaukee, WI). Tris[hydroxymethyl]aminomethane base (Tris), ethylenediamine tetraacetic acid disodium (Na_2EDTA), L-ascorbic acid, forskolin, and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO). Guanylylimidodiphosphate tetralithium [Gpp(NH)p] and adenylylimidodiphosphate tetralithium [App(NH)p] were purchased from Boehringer Mannheim Corporation (Indianapolis, IN). (+)Butaclamol hydrochloride, (–)quinpirole hydrochloride, dopamine hydrochloride, (–)apomorphine hydrochloride, (\pm)-7-hydroxy-n,n-dipropyl-aminotetralin hydrogen bromide, and S(–)sulpiride were purchased from Research Biochemicals Int. (Natick, MA). [^3H]-Adenine (26.9 Ci/mmol) was purchased from DuPont NEN (Wilmington, DE). [^{125}I]-NCQ-298 (2,200 Ci/mmol), [^{125}I]-7-OH-PIPAT (2,200 Ci/mmol), and (R,S)-7-OH-PIPAT were synthesized as previously described (Chumpradit et al. 1993; Foulon et al. 1993). In later experiments [^{125}I]-NCQ-298 (2,200 Ci/mmol) was provided by DuPont NEN.

Tissue Culture

HEK-293 cells, a human embryonic kidney cell line (Graham et al. 1977), were transfected with cDNA encoding the human D_{2L} (D_2 long isoform) and the rat D_3 receptor as previously described (Boundy et al., 1993b; Burris et al. 1994). Cells were grown in monolayer culture at 37°C in 5% CO_2 and fed with EMEM supplemented with 10% fetal bovine serum and 6418 (0.5 mg/ml). Cells were fed every fourth day and subcultured or harvested on day 7.

Preparation of Membranes

Cells were washed with ice-cold phosphate-buffered saline (138 mM NaCl, 4.1 mM KCl, 5.1 mM Na_2HPO_4 , 1.5 mM KH_2PO_4 , and 11.1 mM glucose, pH 7.4) and then incubated for 5 to 10 minutes with ice-cold 10 mM Tris buffer containing 5 mM Na_2EDTA . Cells were removed from plates, homogenized with a Brinkmann polytron, and centrifuged at 32,000/g for 20 minutes. The resulting supernatant was discarded, and the pellet resuspended by homogenization in 50 mM Tris buffer (pH 7.7 at 25°C) containing 1 mM Na_2EDTA and frozen at –70°C. On the day of an experiment membranes were thawed, incubated for 20 minutes at 37°C, and centrifuged for 20 minutes at 32,000/g. Supernatants were discarded and pellets resuspended by homogenization in 50 mM Tris buffer (pH 7.7 at 25°C).

Radioligand Binding

Binding assays were carried out as previously described (Burris et al. 1994). Crude membranes (0.5–5 μg of protein per assay tube) were incubated for 60 minutes at 37°C with [^{125}I]-7-OH-PIPAT or [^{125}I]-NCQ-298 and drugs as indicated in a total volume of 100 μl . When using [^{125}I]-7-OH-PIPAT the assay buffer consisted of 50 mM Tris (pH 7.7 at 25°C) containing 2 mM MgCl_2 and 0.1% BSA. For [^{125}I]-NCQ-298 the assay buffer consisted of 50 mM Tris (pH 7.7 at 25°C) containing 50 mM NaCl, 100 μM Gpp(NH)p, and 0.1% BSA. Assays were terminated by the addition of 5 ml of ice-cold wash buffer (20 mM Tris for experiments involving [^{125}I]-7-OH-PIPAT or 10 mM Tris containing 154 mM NaCl for experiments involving [^{125}I]-NCQ-298). Filtration was carried out using a Brandel cell harvester with glass-fiber filters (Schleicher and Schuell #32, previously soaked in 0.3% polyethylenimine) followed by washing with 15 ml of ice-cold wash buffer. Protein concentrations were determined by the method of Bradford (1976) using BSA as a standard.

Maximum binding and K_d values were determined by Scatchard (1949) transformation of saturation binding data using unweighted linear regression analysis. Competition curves were analyzed by nonlinear regression for a one-site fit using an iterative curve-fitting program.

Accumulation of cAMP

Accumulation of cAMP was determined by a modification of the method of Jones et al. (1987) as described by Filtz et al. (1993). Cells were grown to confluency in 100-mm culture plates and incubated for 18 hours in EMEM supplemented with [^3H]-adenine (3 $\mu\text{Ci}/\text{ml}$). Cells were rinsed twice with PBS at 37°C, then scraped from the plate and centrifuged for 5 minutes at 500/g. The resulting supernatant was discarded and the pellet resuspended in EMEM at 37°C. Cells were incubated in glass tubes for 10 minutes at 37°C in EMEM containing 1.4 mM IBMX and 13 mM HEPES, pH 7.4 (assay buffer). Drugs were added in assay buffer containing forskolin (final concentration of forskolin = 10 μM). After 7 minutes, reactions were stopped by the addition of 1 ml of 7.5% trichloroacetic acid at 4°C. [^3H]-cAMP was separated from [^3H]-ATP and [^3H]-ADP by sequential chromatography over Dowex and alumina columns.

RESULTS

Comparison of the Binding of [^{125}I]-7-OH-PIPAT to D_2 and D_3 Receptors

We have previously reported that [^{125}I]-7-OH-PIPAT binds with high affinity to both D_2 and D_3 receptors

(Burris et al. 1994). The binding of [125 I]-7-OH-PIPAT to D_2 , but not to D_3 , receptors is reduced in the presence of Gpp(NH)p. In the presence of $MgCl_2$ (2 mM), [125 I]-7-OH-PIPAT bound with a K_D value of 0.4 nM ($B_{max} = 660$ fmol/mg of protein) to receptors on membranes prepared from HEK- D_2 cells (Figure 1A). Gpp(NH)p decreased specific binding of [125 I]-7-OH-PIPAT in a concentration-dependent manner (Figure 1A, inset). The effect was selective for guanine nucleotides since Gpp(NH)p was at least two orders of magnitude more potent than App(NH)p. The maximum concentration of Gpp(NH)p tested (100 μ M) decreased specific binding by 80% compared to control.

In membranes prepared from HEK-293 cells expressing transfected D_3 receptors (HEK- D_3 cells) [125 I]-7-OH-PIPAT bound with a K_D value of 0.1 nM and a density of approximately 180 fmol/mg of protein (Figure 1B). In contrast to the effect seen with cells expressing D_2 receptors, 100 μ M Gpp(NH)p had no effect on

the binding of [125 I]-7-OH-PIPAT to D_3 receptors (Figure 1B, inset).

Stimulation of D_2 Receptors by 7-OH-PIPAT and 7-OH-DPAT

Guanine nucleotide-sensitive binding of [125 I]-7-OH-PIPAT to D_2 receptors suggests that [125 I]-7-OH-PIPAT is an agonist at D_2 receptors. The ability of racemic 7-OH-PIPAT to stimulate D_2 receptors was examined directly in HEK- D_2 cells. Forskolin (10 μ M) increased cAMP accumulation two- to fourfold over basal levels. Increasing concentrations of 7-OH-PIPAT potently ($IC_{50} = 2.6$ nM) inhibited the increase in cAMP accumulation stimulated by forskolin (Figure 2). Butaclamol (1 μ M), a D_2 receptor antagonist, completely blocked the inhibition of cAMP accumulation by 100 nM 7-OH-PIPAT (Figure 2, inset).

7-OH-DPAT has been reported to inhibit release of

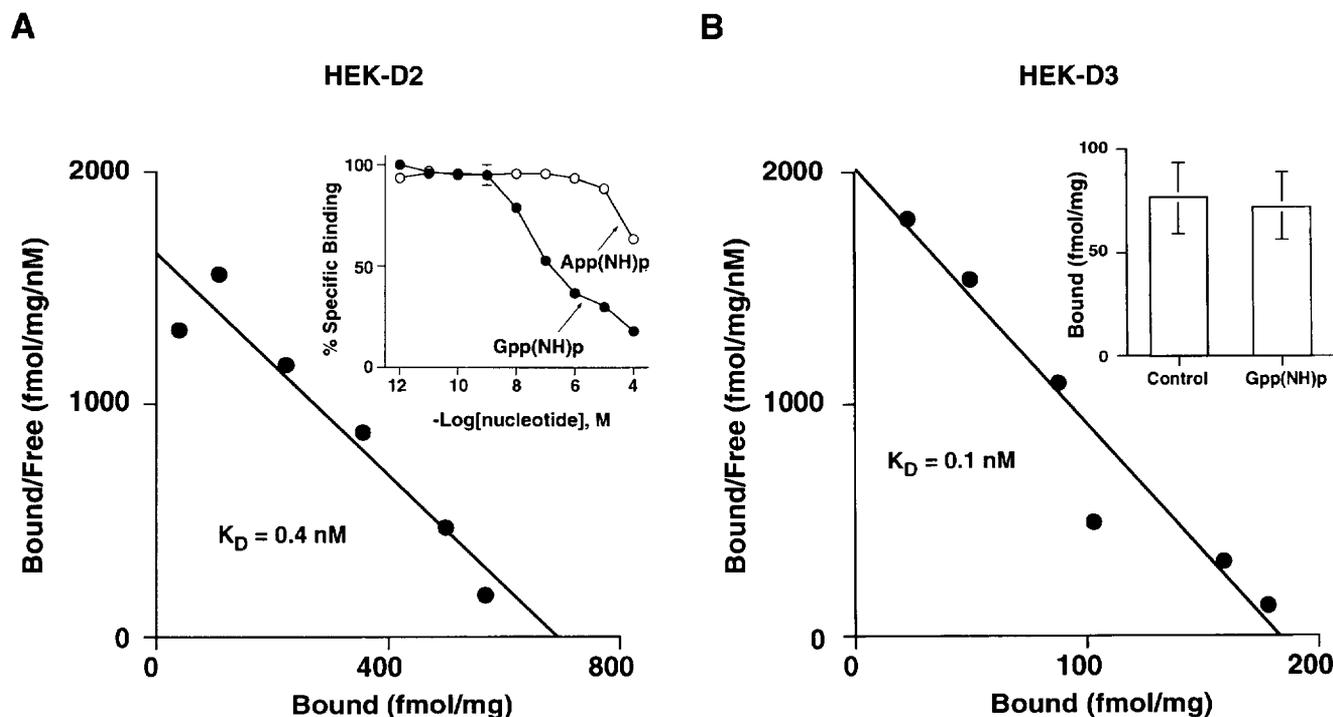


Figure 1. Binding of [125 I]-7-OH-PIPAT to D_2 and D_3 receptors. (A) Membranes prepared from HEK- D_2 cells were incubated with increasing concentrations of [125 I]-7-OH-PIPAT (0.03–3.17 nM) in the presence of 2 mM $MgCl_2$. Data shown are the means of specific binding for two separate tissue preparations assayed in triplicate. The inset shows the effect of Gpp(NH)p and App(NH)p on the binding of [125 I]-7-OH-PIPAT. Membranes were incubated with 0.54 nM [125 I]-7-OH-PIPAT and increasing concentrations of Gpp(NH)p or App(NH)p in the presence of 2 mM $MgCl_2$. Data shown are the means of specific binding for three separate tissue preparations assayed in triplicate. Nonspecific binding was determined with 2 μ M butaclamol. (B) Membranes prepared from HEK- D_3 cells were incubated with increasing concentrations of [125 I]-7-OH-PIPAT (0.01–1.33 nM) in the presence of 2 mM $MgCl_2$. Data shown are the means of specific binding for three separate tissue preparations assayed in triplicate. The inset shows the effect of Gpp(NH)p on the binding of [125 I]-7-OH-PIPAT. Membranes were incubated with 0.15 nM [125 I]-7-OH-PIPAT in the absence or presence of 100 μ M Gpp(NH)p. Data shown are the means of specific binding for three separate tissue preparations assayed in duplicate. Nonspecific binding was determined with 2 μ M butaclamol.

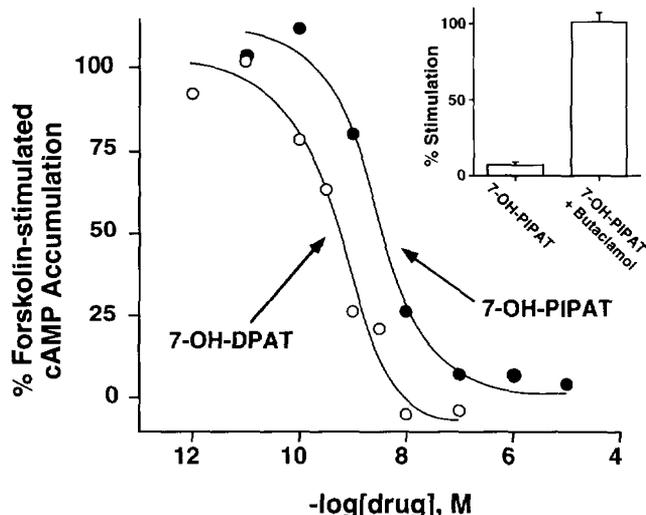


Figure 2. Inhibition of forskolin-stimulated accumulation of [³H]-cAMP in HEK-D₂ cells. Cells were incubated with 10 μM forskolin and 1.4 mM IBMX in the absence or presence of increasing concentrations of 7-OH-DPAT or 7-OH-PIPAT. Data are expressed as a percentage of the stimulation of cAMP accumulation elicited by 10 μM forskolin. Accumulation of cAMP was measured as a percentage of [³H]-cAMP to [³H]-ATP and [³H]-ADP. Data shown are the means of two experiments (7-OH-PIPAT) or three experiments (7-OH-DPAT). The inset shows the effect of 100 nM 7-OH-PIPAT alone and in the presence of 1 μM (+)butaclamol on [³H]cAMP accumulation elicited by 10 μM forskolin.

dopamine in rat striatum through stimulation of D₂-like receptors (Mulder et al. 1987). Therefore, it was of interest to examine the ability of 7-OH-DPAT to inhibit forskolin-stimulated cAMP accumulation in HEK-D₂ cells. Increasing concentrations of 7-OH-DPAT potently (IC₅₀ = 0.6 nM) inhibited forskolin-stimulated cAMP accumulation in HEK-D₂ cells (Figure 2). In contrast to their actions at D₂ receptors, neither 7-OH-PIPAT nor

7-OH-DPAT (at concentrations up to 100 nM) inhibited forskolin-stimulated cAMP accumulation in HEK-D₃ cells (data not shown).

Binding of Agonists and Antagonists to D₂ and D₃ Receptors

D₂ receptors exist in multiple states having low and high affinities for agonists (DeLean et al. 1982; Sibley et al. 1982; McDonald et al. 1984; George et al. 1985; Grigoriadis and Seeman 1985; Lahti et al. 1992). The affinities of D₂ receptors for agonists and antagonists were compared for receptors labeled with [¹²⁵I]-7-OH-PIPAT and with [¹²⁵I]-NCQ-298 under conditions that promote, respectively, coupling or uncoupling of receptors to G proteins. To facilitate comparison, identical assay conditions were used in studies of the properties of D₃ receptors.

When receptors were labeled with [¹²⁵I]-NCQ-298, D₂ and D₃ receptors displayed similar potencies for sulpiride, a D₂ receptor antagonist (Figure 3A, Table 1). When receptors were labeled with [¹²⁵I]-7-OH-PIPAT, the affinity of D₃ receptors for sulpiride was slightly less than the affinity of D₂ receptors for sulpiride (Figure 3B, Table 1). D₂ receptors displayed a 16-fold higher affinity for butaclamol than did D₃ receptors when receptors were labeled with [¹²⁵I]-NCQ-298 (Table 1). The limited selectivity of D₂ receptors for butaclamol was also seen when receptors were labeled with [¹²⁵I]-7-OH-PIPAT (Table 1).

D₃ receptors have been reported to have higher affinities for agonists than do D₂ receptors (Lévesque et al. 1992). When receptors were labeled with [¹²⁵I]-NCQ-298 in the presence of Gpp(NH)p and NaCl, D₃ receptors displayed, as expected, much higher affinities for most agonists than did D₂ receptors (Table 1). However, in the absence of Gpp(NH)p and NaCl, when

Table 1. Affinities of Agonists and Antagonists for D₂ and D₃ Receptors Labeled with [¹²⁵I]-NCQ-298 and [¹²⁵I]-7-OH-PIPAT

	[¹²⁵ I]-NCQ-298			[¹²⁵ I]-7-OH-PIPAT		
	K _i (nM)		D ₂ /D ₃ Selectivity Index	K _i (nM)		D ₂ /D ₃ Selectivity Index
	HEK-D ₂	HEK-D ₃		HEK-D ₂	HEK-D ₃	
Agonist						
7-OH-DPAT	165 ± 10 (3)	1.2 ± 0.1 (3)	138	2.6 ± 0.4 (6)	0.4 ± 0.1 (3)	7
Quinpirole	1065 ± 95 (3)	8.0 ± 0.4 (3)	133	6.4 ± 1.3 (5)	1.7 ± 0.2 (2)	4
Dopamine	1908 ± 652 (3)	44 ± 5 (3)	43	5.8 ± 0.4 (3)	5.6 ± 0.5 (3)	1
Apomorphine	168 ± 14 (3)	31 ± 3 (6)	5	4.9 ± 0.2 (4)	14 ± 2 (6)	0.4
Antagonist						
Sulpiride	12 ± 4 (3)	14 ± 1 (6)	0.9	60 ± 6 (2)	142 ± 17 (2)	0.4
Butaclamol	0.20 ± 0.03 (3)	3.2 ± 0.2 (3)	0.06	0.69 ± 0.05 (3)	5.0 ± 0.4 (3)	0.1

Competition experiments were carried out as described in Figure 3. K_i values were determined by the method of Cheng and Prusoff (1973). Data are the mean ± SEM, n ≥ 3 experiments, or mean ± range, n = 2 experiments. The selectivity index is defined as the ratio of the K_i for D₂ compared to D₃ receptors. A high-selectivity index implies higher affinity for D₃ than for D₂ receptors.

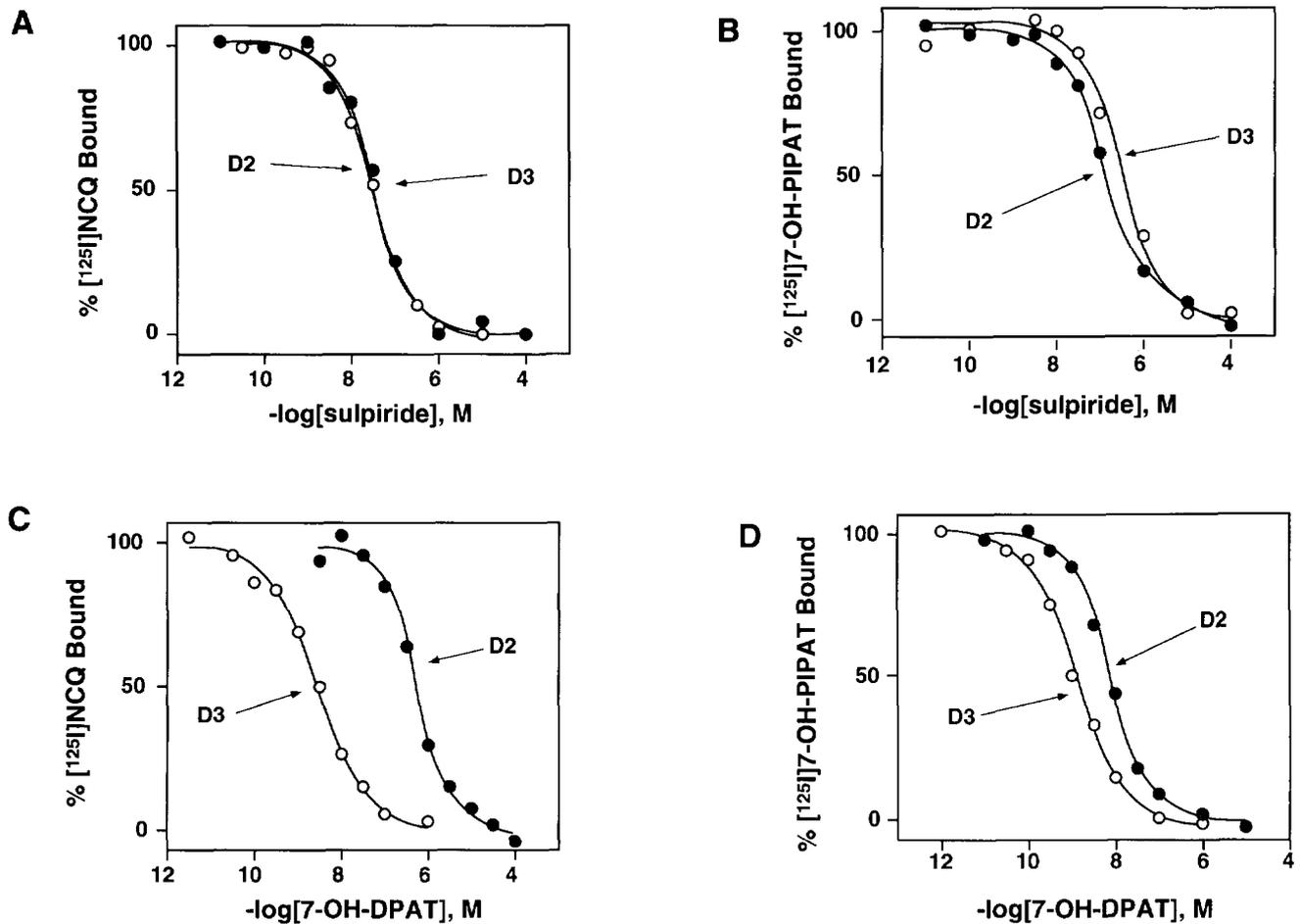


Figure 3. Inhibition of the binding of [125 I]-7-OH-PIPAT and [125 I]-NCQ-298 to D₂ and D₃ receptors expressed in HEK-293 cells. (A) Membranes were added to 0.15 nM [125 I]-NCQ-298 in the presence of 50 mM NaCl, 100 μ M Gpp(NH)p, and increasing concentrations of sulpiride. Data shown are the means of specific binding for three separate tissue preparations assayed in triplicate. (B) Membranes were added to 0.62 nM (D₂) or 0.14 nM (D₃) [125 I]-7-OH-PIPAT in the presence of 2 mM MgCl₂ and increasing concentrations of sulpiride. Data shown are the means of specific binding for two separate tissue preparations assayed in triplicate. (C) Membranes were added to 0.15 nM [125 I]-NCQ-298 in the presence of 50 mM NaCl, 100 μ M Gpp(NH)p, and increasing concentrations of 7-OH-DPAT. Data shown are the means of specific binding for three separate tissue preparations assayed in triplicate. (D) Membranes were added to 0.72 nM (D₂) or 0.16 nM (D₃) [125 I]-7-OH-PIPAT in the presence of 2 mM MgCl₂ and increasing concentrations of 7-OH-DPAT. Data shown are the means of specific binding for three separate tissue preparations assayed in triplicate. Nonspecific binding was determined with 2 μ M butaclamol.

[125 I]-7-OH-PIPAT was used to label receptors, the affinities of D₃ receptors for agonists were similar to the affinities of D₂ receptors for the same agonists (Table 1). In particular, under conditions that promote uncoupling of G proteins from receptors, D₃ receptors displayed an affinity for 7-OH-DPAT nearly 140-fold higher than that of D₂ receptors (Figure 3C, Table 1). However, the affinity of D₃ receptors for 7-OH-DPAT was only slightly higher than that of D₂ receptors when the receptors were examined under conditions that favor receptor coupling to G proteins (Figure 3D, Table 1). Similarly, the affinities of D₃ receptors for

quinpirole and dopamine were much higher than the affinities of D₂ receptors for the agonists in the presence of Gpp(NH)p and NaCl when [125 I]-NCQ-298 was used to label receptors; however, when Gpp(NH)p and NaCl were not present, and when [125 I]-7-OH-PIPAT was used, receptors bound quinpirole and dopamine with nearly equal affinities (Table 1).

Despite the different assay conditions, D₃ receptors labeled with [125 I]-7-OH-PIPAT and [125 I]-NCQ-298 displayed comparable affinities for agonists. In contrast, the G protein-coupled state of D₂ receptors (i.e., receptors labeled with [125 I]-7-OH-PIPAT) had 30- to

330-fold higher affinity for agonists than did the uncoupled state of D₂ receptors [i.e., [¹²⁵I]-NCQ-298-labeled receptors assayed in the presence of Gpp(NH)p]. 7-OH-DPAT, quinpirole, and dopamine displayed marked selectivity for D₃ receptors compared with D₂ receptors when Gpp(NH)p and NaCl were present in the assay and receptors were labeled with [¹²⁵I]-NCQ-298 (Table 1). However, this selectivity was reduced or absent when assays were carried out in the absence of Gpp(NH)p and NaCl and when receptors were labeled with [¹²⁵I]-7-OH-PIPAT (Table 1). Unlike the other agonists, apomorphine was only slightly D₃ receptor-selective when receptors were labeled with [¹²⁵I]-NCQ-298 (Table 1). However, when receptors were labeled with [¹²⁵I]-7-OH-PIPAT apomorphine displayed an approximately threefold selectivity for D₂ receptors (Table 1).

D₃ receptors labeled with [¹²⁵I]-7-OH-PIPAT bound agonists with two- to eightfold higher affinities than did receptors labeled with [¹²⁵I]-NCQ-298 (Table 1). Guanine nucleotides and NaCl have been shown to decrease the affinity of D₂ receptors for agonists (Grigoriadis and Seeman 1985; Neve et al. 1991). The presence of Gpp(NH)p and/or NaCl in the assay with [¹²⁵I]-NCQ-298 might also play a role in the decrease in the affinity of D₃ receptors for agonists. Therefore, the effects of Gpp(NH)p and NaCl on the affinity of D₃ receptors for 7-OH-DPAT were investigated. D₃ receptors bound 7-OH-DPAT with equally high affinity in the absence and presence of 100 μM Gpp(NH)p (Table 2). However, when 50 mM NaCl was added, the affinity of [¹²⁵I]-7-OH-PIPAT-labeled D₃ receptors for 7-OH-DPAT was reduced to a value approaching that obtained for [¹²⁵I]-NCQ-298-labeled receptors (Table 2). The affinity of D₃ receptors for the antagonist sulpiride also appeared to be a function of assay conditions. D₃ receptors labeled with [¹²⁵I]-NCQ-298 had a 10-fold higher affinity for sulpiride than did D₃ receptors labeled with [¹²⁵I]-7-OH-PIPAT (Table 1). D₃ receptors labeled with [¹²⁵I]-7-OH-PIPAT bound sulpiride with nearly equal affinity in the absence or presence of 100

μM Gpp(NH)p (Table 2). However, in the presence of 50 mM NaCl, the affinity of [¹²⁵I]-7-OH-PIPAT-labeled D₃ receptors for sulpiride was increased to a value similar to that seen for receptors labeled with [¹²⁵I]-NCQ-298 (Table 2).

DISCUSSION

Putative D₃ dopamine receptor-selective agonists have been used recently in studies of the biochemical properties and physiological roles of D₃ receptors (Caine and Koob 1993; Daly and Waddington 1993; Damsma et al. 1993; Meller et al. 1993). D₃ receptors and D₂ receptors have similar structural and pharmacological properties (Sokoloff et al. 1990). High densities of D₂ receptors exist in many of the same brain regions (e.g., nucleus accumbens and olfactory tubercle) in which D₃ receptors are found (Lévesque et al. 1992; Levant et al. 1993). Furthermore, the density of D₂ receptors is at least an order of magnitude higher than that of D₃ receptors (Boundy et al. 1993b). Therefore, agonists must have a high degree of selectivity if they are to effectively discriminate between actions at D₃ and D₂ receptors. D₃ receptors have been reported to have 100-fold higher affinity than D₂ receptors for 7-OH-DPAT based on affinity values determined by competition for binding of radiolabeled antagonists to receptors expressed in transfected cells (Lévesque et al. 1992). However, D₂ receptors exist in multiple states with varying affinities for agonists. High-affinity binding of agonists to D₂ receptors is dependent, in part, on coupling of the receptors to G proteins. In the original report in which the properties of D₂ and D₃ receptors were compared, assays were carried out under conditions (no MgCl₂ and 50 mM NaCl) that minimize high-affinity binding of agonists to D₂ receptors. In the present study, affinities of D₂ and D₃ receptors for 7-OH-DPAT and other agonists were determined directly by competition for the binding of a radiolabeled agonist under conditions that promote coupling of receptors to G proteins.

Table 2. Effect of Gpp(NH)p and NaCl on Binding of 7-OH-DPAT and Sulpiride to D₃ Receptors Labeled with [¹²⁵I]-7-OH-PIPAT or [¹²⁵I]-NCQ-298

	[¹²⁵ I]-7-OH-PIPAT			[¹²⁵ I]-NCQ-298
	Control	Gpp(NH)p	Gpp(NH)p + NaCl	Gpp(NH)p + NaCl
7-OH-DPAT	0.18 ± 0.01	0.20 ± 0.02	0.56 ± 0.11 ^a	0.73 ± 0.13 ^b
Sulpiride	165 ± 13	151 ± 38	19 ± 5 ^c	14 ± 1 ^c

K_i values were determined by the method of Cheng and Prusoff (1973) using IC₅₀ values obtained in competition experiments as described in Figure 3 except that MgCl₂ was not included in the [¹²⁵I]-7-OH-PIPAT binding assay. The data are the mean ± SEM for three separate tissue preparations assayed in duplicate or triplicate.

^a *p* < .05.

^b *p* < .025.

^c *p* < .001 for assay containing Gpp(NH)p and NaCl compared with control.

[¹²⁵I]-7-OH-PIPAT has been shown to label a guanine nucleotide-sensitive state of D₂ receptors (Burris et al. 1994 and present study). Furthermore, racemic 7-OH-PIPAT potently activated D₂ receptors coupled to the inhibition of cAMP accumulation. These data suggest that [¹²⁵I]-7-OH-PIPAT labeled a functional G protein-coupled state of D₂ receptors. When receptors expressed in HEK-293 cells were labeled with the antagonist [¹²⁵I]-NCQ-298 under conditions that inhibit interactions of receptors with G proteins, D₃ receptors displayed greater than a 100-fold higher affinity for 7-OH-PIPAT than did D₂ receptors. This is consistent with the affinity of D₃ receptors for 7-OH-PIPAT determined by inhibition of the binding of the antagonist [¹²⁵I]-iodosulpiride to receptors expressed in CHO cells (Lévesque et al. 1992). However, under conditions that favor coupling of receptors with G proteins, the affinity of D₃ receptors was only sevenfold higher than the affinity of D₂ receptors for 7-OH-PIPAT. Similarly, D₃ receptors had much higher affinities than did D₂ receptors for the agonists dopamine and quinpirole under conditions that promote the uncoupled state of receptors, whereas the selectivity of D₃ receptors over D₂ receptors was diminished when the G protein-coupled state of the receptors was examined. The reported selectivity of agonists for D₃ receptors is, therefore, dependent on assay conditions.

The existence of multiple states of D₂ receptors having high and low affinities for agonists is well established. In membranes prepared from rat and bovine striatum and pituitary, which express high densities of D₂ receptors, high- and low-affinity components have been identified in studies of the inhibition of radiolabeled antagonist binding (George et al. 1985; McDonald et al. 1984; Sibley et al. 1982). Labeled agonists such as [³H]-apomorphine, [³H]-*N-n*-propyl-norapomorphine, and [³H]-quinpirole have been used to directly label a guanine nucleotide-sensitive state of D₂ receptors having high affinity for agonists (Creese et al. 1979; Levant et al. 1992; Seeman and Schaus 1991; Sibley et al. 1982; Titeler and Seeman 1979). However, ³H-labeled agonists often display a high degree of nonspecific binding. Furthermore, interpretation of results of studies with brain tissue are complicated by the existence of multiple D₂-like receptor subtypes. Clonal cell lines expressing transfected receptor cDNA allow the study of single subtypes of dopamine receptor. The D₂ agonist [³H]-U-86170 binds to a guanine nucleotide-sensitive state of D₂ receptors transfected in CHO cells (Lahti et al. 1992). The affinities of [³H]-U-86170-labeled D₂ receptors for agonists were similar to affinities determined for D₂ receptors labeled with [¹²⁵I]-7-OH-PIPAT, which is consistent with the finding that both radioligands label a G protein-coupled agonist-preferring state of the receptors.

The agonist-preferring high-affinity state is thought

to be the functional state of D₂ receptors. In cells from the anterior pituitary, activation of D₂ receptors by agonists results in inhibition of prolactin release (George et al. 1985). This effect appears to be mediated through inhibition of adenylyl cyclase activity. The potency of agonists, as measured in studies of the inhibition of adenylyl cyclase activity and prolactin release, correlates with the affinity of the same agonists for the high-affinity state of D₂ receptors (McDonald et al. 1984; George et al. 1985). Whereas high-affinity binding of agonists to D₂ receptors is seen in membrane preparations, studies using radioligand binding assays with intact cells have not revealed the existence of D₂ receptors with a high affinity for agonists (Sibley et al. 1983). High-affinity binding of agonists to β receptors on intact cells has been seen under preequilibrium conditions (Toews et al. 1983), which is consistent with the hypothesis that agonists rapidly convert receptors from a state having high affinity for agonists to a state having low affinity for agonists (Pittman and Molinoff 1980). Nevertheless, functional studies using intact cells have demonstrated that agonists potently activate D₂ receptors (George et al. 1985; Neve et al. 1989; Lahti et al. 1992; Chio et al. 1994) suggesting that in vivo a high-affinity interaction between receptors and agonists occurs. However, the high-affinity state cannot be readily measured using radioligand binding assays in intact cells due to rapid conversion of receptors to a low-affinity state. In the present study, the potency of 7-OH-PIPAT for inhibiting forskolin-stimulated accumulation of cAMP measured in intact cells was similar to the affinity of 7-OH-PIPAT for D₂ receptors labeled with [¹²⁵I]-7-OH-PIPAT. Furthermore, we have previously determined that quinpirole and dopamine potently (IC₅₀ values of 1 and 16 nM, respectively) inhibit cAMP accumulation in HEK-D₂ cells (Filtz et al. 1993). These values are similar to affinities determined in membrane preparations for the G protein-coupled state of D₂ receptors labeled with [¹²⁵I]-7-OH-PIPAT, but not for the state of D₂ receptors labeled with [¹²⁵I]-NCQ-298. This suggests that the affinities of the G protein-coupled state of D₂ receptors for agonists determined in membrane preparations reflect the affinities of receptors for the agonists in vivo.

In contrast to the wide range of affinities for agonists at G protein-coupled and uncoupled states of D₂ receptors, D₃ receptors displayed less than a 10-fold higher affinity for agonists at receptors labeled with [¹²⁵I]-7-OH-PIPAT than receptors labeled with [¹²⁵I]-NCQ-298. This small difference in affinity appears to be a consequence of the presence of NaCl in the assay with [¹²⁵I]-NCQ-298. Binding of [¹²⁵I]-7-OH-PIPAT to D₃ receptors was not decreased by adding Gpp(NH)p, suggesting that high-affinity binding of agonists to a G protein-coupled state of D₃ receptors in HEK-293 cells is insensitive to Gpp(NH)p or that D₃ receptors

bind agonists with high affinity in the absence of an interaction with G proteins. In addition, Gpp(NH)p did not decrease the affinity of D₃ receptors for 7-OH-DPAT. Similarly, D₃ receptors expressed in a variety of cell types have been reported to exist in a high-affinity state for agonists that is resistant to the effects of guanine nucleotides (Sokoloff et al. 1990, 1992; McAllister et al. 1993; Freedman et al. 1994; Tang et al. 1994b). Studies with CHO cells have in some instances revealed slight decreases in the affinities of D₃ receptors for agonists in the presence of guanine nucleotides (Sokoloff et al. 1992; Castro and Strange 1993; Chio et al. 1994; MacKenzie et al. 1994). The lack of large shifts in receptor affinity may be due to the absence of appropriate G proteins with which D₃ receptors can couple. Alternatively, it has not been determined with which signal transduction pathway D₃ receptors are coupled. Therefore, agonists at D₃ receptors have not been unambiguously defined. Chio et al. (1994) have recently reported functional effects of agonists on D₃ receptors transfected into CHO cells. Similar to D₂ receptors, D₃ receptors inhibit forskolin-stimulated cAMP accumulation, increase extracellular acidification, and stimulate mitogenesis, although the responses are considerably weaker than those seen at D₂ receptors. Furthermore, coupling of D₃ receptors to the inhibition of dopamine release and the regulation of Ca²⁺ conductance has been reported (Seabrook et al. 1994; Tang et al. 1994a).

In HEK-D₃ cells neither sensitivity to guanine nucleotides nor activation of D₃ receptors coupled to inhibition of cAMP accumulation was detected. However, binding of agonists to D₃ receptors, like that of D₂ receptors (Grigoriadis and Seeman 1985; Neve et al. 1991), was sensitive to NaCl. A decrease in the affinity of D₃ receptors for agonists upon addition of NaCl has been reported in studies with CHO cells expressing transfected D₃ receptors (MacKenzie et al. 1994). The ability of sodium to reduce the affinity of D₂ receptors for agonists is believed to involve an aspartate (Asp. 80) within the second membrane-spanning region of the receptor (Neve et al. 1991). A corresponding aspartate residue is present in the D₃ receptor, which may confer sodium sensitivity (Sokoloff et al. 1990; Schwartz et al. 1992).

With regard to antagonists, D₂ receptors displayed limited selectivity for butaclamol compared to D₃ receptors when receptors were labeled with [¹²⁵I]-NCQ-298. The affinities of D₃ receptors for butaclamol were similar whether receptors were labeled with [¹²⁵I]-NCQ-298 or [¹²⁵I]-7-OH-PIPAT. In contrast, the D₂ receptor antagonist sulpiride bound with 10-fold higher affinity to D₃ receptors labeled with [¹²⁵I]-NCQ-298 than to receptors labeled with [¹²⁵I]-7-OH-PIPAT. The presence of NaCl in the [¹²⁵I]-NCQ-298 assay, but not in the [¹²⁵I]-7-OH-PIPAT assay, appeared to mediate this difference in affinity. Sulpiride is a benzamide

that requires sodium for high-affinity binding to D₂ receptors (Neve et al. 1991). Furthermore, the binding of [¹²⁵I]-iodosulpiride to D₃ receptors is increased in the presence of NaCl (Sokoloff et al. 1992). Similarly, the affinity of [¹²⁵I]-7-OH-PIPAT-labeled D₃ receptors for sulpiride was increased in the presence of NaCl, which is consistent with the presence of a sodium-sensitive site on the D₃ receptor.

The present results add to the emerging evidence that D₂ and D₃ receptors have similar pharmacological and biochemical properties. Nevertheless, the reports that 7-OH-DPAT and other D₂ agonists are selective for D₃ receptors have stimulated the use of these compounds in laboratory animals to identify functional responses mediated by D₃ receptors (Caine and Koob 1993; Daly and Waddington 1993; Damsma et al. 1993). For example, it was recently reported that 7-OH-DPAT, quinpirole, and apomorphine were effective in reducing cocaine self-administration in rats (Caine and Koob 1993). 7-OH-DPAT and quinpirole were more potent than apomorphine, a pattern suggesting the involvement of D₃ receptors rather than D₂ receptors in the reinforcing effects of cocaine. Central to the conclusion that these agonists elicit their effects on cocaine self-administration through interaction at D₃ receptors were reports that D₃ receptors have a high affinity for all three drugs, whereas D₂ receptors have a high affinity for apomorphine, but a low affinity for 7-OH-DPAT and quinpirole. However, as seen in the present study, D₂ receptors have either high or low affinities for 7-OH-DPAT, quinpirole, and apomorphine, depending on assay conditions. The G protein-coupled state of D₂ receptors had a high affinity for all three drugs. Furthermore, all three drugs are potent agonists at D₂ receptors (Chio et al. 1994; Lahti et al. 1992; Neve et al. 1989; and present results). In HEK-D₂ cells the potency of 7-OH-DPAT for activating D₂ receptors and the affinity of the G protein-coupled state of the D₂ receptor for 7-OH-DPAT were similar to the affinity of D₃ receptors for 7-OH-DPAT. Similar potencies of 7-OH-DPAT for activating D₂ and D₃ receptors have recently been reported (Chio et al. 1994; Liu et al. 1993; Potenza et al. 1994). These results suggest that 7-OH-DPAT and other agonists do not have sufficient selectivity to distinguish the subtype of receptor through which behavioral effects of agonists such as 7-OH-DPAT are mediated. Therefore, the elucidation of functional responses mediated by activation of D₃ receptors may require the development of subtype-selective dopamine receptor antagonists.

In summary, [¹²⁵I]-7-OH-PIPAT has been shown to interact with a functional G protein-coupled state of D₂ receptors that has a high affinity for agonists. Comparison of the affinities of D₂ and D₃ receptors for agonists defines the range of selectivity obtained with currently available agonists. When the agonist-labeled

G protein-coupled state of D₂ receptors is considered, D₃ receptor selectivity ascribed to agonists is diminished. The use of drugs to determine behavioral effects mediated by activation of D₃ receptors will require identifying compounds that exhibit a high degree of selectivity under a broad range of conditions.

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REFERENCES

- Boundy VA, Luedtke RR, Artymyshyn RP, Filtz TM, Molinoff PB (1993a): Development of polyclonal anti-D₂ dopamine receptor antibodies using sequence-specific peptides. *Mol Pharmacol* 43:666–676
- Boundy VA, Luedtke RR, Gallitano AL, Smith JE, Filtz TM, Kallen RG, Molinoff PB (1993b): Expression and characterization of the rat D₃ dopamine receptor: Pharmacologic properties and development of antibodies. *J Pharmacol Exp Ther* 264:1002–1011
- Bradford M (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254
- Burris KD, Filtz TM, Chumpradit S, Kung M-P, Foulon C, Hensler JG, Kung HF, Molinoff PB (1994): Characterization of [¹²⁵I](R)-trans-7-hydroxy-2-[N-propyl-N-(3'-iodo-2'-propenyl)amino]tetralin binding to dopamine D₃ receptors in rat olfactory tubercle. *J Pharmacol Exp Ther* 268:935–942
- Caine SB, Koob GF (1993): Modulation of cocaine self-administration in the rat through D-3 dopamine receptors. *Science* 260:1814–1816
- Castro SW, Strange PG (1993): Coupling of D₂ and D₃ dopamine receptors to G-proteins. *Fed Eur Biochem Soc* 315:223–226
- Cheng Y-C, Prusoff WH (1973): Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I₅₀) of an enzymatic reaction. *Biochem Pharmacol* 22:3099–3108
- Chio CL, Lajiness ME, Huff RM (1994): Activation of heterologously expressed D₃ dopamine receptors: Comparison with D₂ dopamine receptors. *Mol Pharmacol* 45: 51–60
- Chumpradit S, Kung M-P, Kung HF (1993): Synthesis and optical resolution of (R)- and (S)-trans-7-hydroxy-2-[N-propyl-N-(3'-iodo-2'-propenyl)amino]tetralin: A new D₃ dopamine receptor ligand. *J Med Chem* 36:4308–4312
- Creese I, Burt DR, Snyder SH (1976): Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* 192:481–483
- Creese I, Padgett L, Fazzani E, Lopez F (1979): ³H-N-n-propylnorapomorphine: A novel agonist ligand for central dopamine receptors. *Eur J Pharmacol* 56:411–412
- Daly SA, Waddington JL (1993): Behavioural effects of the putative D-3 dopamine receptor agonist 7-OH-DPAT in relation to other "D-2-like" agonists. *Neuropharmacology* 32:509–510
- Damsma G, Bottema T, Westerink BHC, Tepper PG, Dijkstra D, Pugsley TA, Mackenzie RG, Heffner TG, Wikström H (1993): Pharmacological aspects of R-(+)-7-OH-DPAT, a putative dopamine D₃ receptor ligand. *Eur J Pharmacol* 249:R9–R10
- DeLean A, Kilpatrick BF, Caron MG (1982): Dopamine receptor of the porcine anterior pituitary gland. Evidence for two affinity states discriminated by both agonists and antagonists. *Mol Pharmacol* 22:290–297
- Filtz TM, Artymyshyn RA, Guan W, Molinoff PB (1993): Paradoxical regulation of dopamine receptors in transfected 293 cells. *Mol Pharmacol* 44:371–379
- Foulon C, Kung M-P, Kung HF (1993): Synthesis of (R,S)-2'-trans-7-hydroxy-2-[N-n-propyl-N-(3'-iodo-2'-propenyl)amino]tetralin (trans-7-OH-PIPAT): A new D₃ dopamine receptor ligand. *J Med Chem* 36:1499–1500
- Freedman SB, Patel S, Marwood R, Emms F, Seabrook GR, Knowles MR, McAllister G (1994): Expression and pharmacological characterization of the human D₃ dopamine receptor. *J Pharmacol Exp Ther* 268:417–426
- George SR, Watanabe M, DiPaolo T, Falardeau P, Labrie F, Seeman P (1985): The functional state of the dopamine receptor in the anterior pituitary is in the high-affinity form. *Endocrinology* 117:690–697
- Graham FL, Smiley J, Russell WC, Nairn R (1977): Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J Gen Virol* 36:59–72
- Grigoriadis D, Seeman P (1985): Complete conversion of brain D₂ dopamine receptors from the high- to the low-affinity state for dopamine agonists, using sodium ions and guanine nucleotide. *J Neurochem* 44:1925–1935
- Hall H, Hogberg T, Halldin C, Köhler C, Ström P, Ross SB, Larsson SA, Farde L (1990): NCQ 298, a new selective iodinated salicylamide ligand for the labelling of dopamine D₂ receptors. *Psychopharmacology* 103:6–18
- Jones SB, Toews ML, Turner JT, Bylund DB (1987): α -Adrenergic receptor-mediated sensitization of forskolin-stimulated cyclic AMP production. *Proc Natl Acad Sci USA* 84:1294–1298
- Kanterman RY, Mahan LC, Briley EM, Monsma FJ Jr, Sibley DR, Axelrod J, Felder CC (1991): Transfected D₂ dopamine receptors mediate the potentiation of arachidonic acid release in Chinese hamster ovary cells. *Mol Pharmacol* 39:364–369
- Lahti RA, Figur LM, Piercey MF, Ruppel PL, Evans DL (1992): Intrinsic activity determinations at the dopamine D₂ guanine nucleotide-binding protein-coupled receptor: Utilization of receptor state binding affinities. *Mol Pharmacol* 42:432–438
- Levant B, Grigoriadis DE, DeSouza EB (1992): Characterization of [³H]quinpirole binding to D₂-like dopamine receptors in rat brain. *J Pharmacol Exp Ther* 262:929–935
- Levant B, Grigoriadis DE, DeSouza EB (1993): [³H]Quinpirole binding to putative D₂ and D₃ dopamine receptors in rat brain and pituitary gland: A quantitative autoradiographic study. *J Pharmacol Exp Ther* 264:991–1001
- Lévesque D, Diaz J, Pilon C, Martres M-P, Giros B, Souil E, Schott D, Morgat J-L, Schwartz J-C, Sokoloff P (1992):

- Identification, characterization, and localization of the dopamine D₃ receptor in rat brain using 7-[³H]hydroxy-N,N-di-n-propyl-2-aminotetralin. *Proc Natl Acad Sci USA* 89:8155–8159
- Liu J-C, Cox RF, Greif GJ, Freedman JE, Waszczak BL (1993): Electrophysiological effects of 7-OH-DPAT on nigrostriatal vs mesolimbic dopamine systems. *Soc Neurosci Abstr* 19:1374
- Lledo PM, Homburger V, Bockaert J, Vincent J-D (1992): Differential G protein-mediated coupling of D₂ dopamine receptors to K⁺ and Ca²⁺ currents in rat anterior pituitary cells. *Neuron* 8:455–463
- MacKenzie RG, VanLeeuwen D, Pugsley TA, Shih Y-H, Demattos S, Tang L, Todd RD, O'Malley KL (1994): Characterization of the human dopamine D₃ receptor expressed in transfected cell lines. *Eur J Pharmacol* 266:79–85
- McAllister G, Knowles MR, Patel S, Marwood R, Emms F, Seabrook GR, Graziano M, Borkowski D, Hey PJ, Freedman SB (1993): Characterization of a chimeric hD₃/D₂ dopamine receptor expressed in CHO cells. *FEBS Lett* 324:81–86
- McDonald WM, Sibley DR, Kilpatrick BF, Caron MG (1984): Dopaminergic inhibition of adenylate cyclase correlates with high affinity agonist binding to anterior pituitary D₂ dopamine receptors. *Mol Cell Endocrinol* 36:201–209
- Meller E, Bohmarker K, Goldstein M, Basham DA (1993): Evidence that striatal synthesis-inhibiting autoreceptors are dopamine D₃ receptors. *Eur J Pharmacol* 249:R5–R6
- Molinoff PB, Wolfe BB, Weiland GA (1981): Quantitative analysis of drug-receptor interactions. II. Determination of the properties of receptor subtypes. *Life Sci* 29:427–443
- Mulder TBA, de Vries JB, Dijkstra D, Wiechers JW, Grol CJ, Horn AS (1987): Further in vitro and in vivo studies with the putative presynaptic dopamine agonist N,N-dipropyl-7-hydroxy-2-aminotetralin. *Naunyn-Schmiedeberg's Arch Pharmacol* 336:494–501
- Neve KA, Henningsen RA, Bunzow JR, Civelli O (1989): Functional characterization of a rat dopamine D-2 receptor cDNA expressed in a mammalian cell line. *Mol Pharmacol* 36:446–451
- Neve KA, Cox BA, Henningsen RA, Spanoyannis A, Neve RL (1991): Pivotal role for aspartate-80 in the regulation of dopamine D₂ receptor affinity for drugs and inhibition of adenylate cyclase. *Mol Pharmacol* 39:733–739
- Pilon C, Lévesque D, Dimitriadou V, Griffon N, Martres M-P, Schwartz J-C, Sokoloff P (1994): Functional coupling of the human D₃ dopamine receptor in a transfected NG 108-15 neuroblastoma-glioma hybrid cell line. *Eur J Pharmacol* 268:129–139
- Pittman RN, Molinoff PB (1980): Interactions of agonists and antagonists with B-adrenergic receptors on intact L6 muscle cells. *J Cyclic Nucl Res* 6:421–435
- Potenza MN, Graminski GF, Schmauss C, Lerner MR (1994): Functional expression and characterization of human D₂ and D₃ dopamine receptors. *J Neurosci* 14:1463–1476
- Scatchard G (1949): The attractions of proteins for small molecules and ions. *Ann N Y Acad Sci* 51:660–672
- Schwartz J-C, Giros B, Martres M-P, Sokoloff P (1992): The dopamine receptor family: Molecular biology and pharmacology. *Semin Neurosci* 4:99–108
- Seabrook GR, Kemp JA, Freedman SB, Patel S, Sinclair HA, McAllister G (1994): Functional expression of human D₃ dopamine receptors in differentiated neuroblastoma × glioma NG 108-15 cells. *Br J Pharmacol* 111:391–393
- Seeman P, Schaus JM (1991): Dopamine receptors labelled by [³H]quinpirole. *Eur J Pharmacol* 203:105–109
- Seeman P, Lee T, Chau-wong M, Wong K (1976): Antipsychotic drug doses and neuroleptic/dopamine receptors. *Nature* 261:717–718
- Sibley DR, DeLean A, Creese I (1982): Anterior pituitary dopamine receptors. Demonstration of interconvertible high and low affinity states of the D-2 dopamine receptor. *J Biol Chem* 257:6351–6361
- Sibley DR, Mahan LC, Creese I (1983): Dopamine receptor binding on intact cells. Absence of a high-affinity agonist-receptor binding state. *Mol Pharmacol* 23:295–302
- Sokoloff P, Giros B, Martres M-P, Bouthenet M-L, Schwartz J-C (1990): Molecular cloning and characterization of a novel dopamine receptor (D₃) as a target for neuroleptics. *Nature* 347:146–151
- Sokoloff P, Andrieux M, Besancon R, Pilon C, Martres M-P, Giros B, Schwartz J-C (1992): Pharmacology of human dopamine D₃ receptor expressed in a mammalian cell line: Comparison with D₂ receptor. *Eur J Pharmacol* 225:331–337
- Tang L, Todd RD, O'Malley KL (1994a): Dopamine D₂ and D₃ receptors inhibit dopamine release. *J Pharmacol Exp Ther* 270:475–479
- Tang L, Todd RD, Heller A, O'Malley KL (1994b): Pharmacological and functional characterization of D₂, D₃ and D₄ dopamine receptors in fibroblast and dopaminergic cell lines. *J Pharmacol Exp Ther* 268:495–502
- Titeler M, Seeman P (1979): Selective labelling of different dopamine receptors by a new [³H]-ligand: [³H]-N-n-propylnorapomorphine. *Eur J Pharmacol* 56:291–292
- Toews ML, Harden TK, Perkins JP (1983): High-affinity binding of agonists to β-adrenergic receptors on intact cells. *Proc Natl Acad Sci USA* 80:3553–3557
- Vallar L, Meldolesi J (1989): Mechanisms of signal transduction at the dopamine D₂ receptor. *Trends Pharmacol Sci* 10:72–77
- Van Tol HHM, Bunzow JR, Guan H-C, Sunahara RK, Seeman P, Niznik HB, Civelli O (1991): Cloning of the gene for a human dopamine D₄ receptor with high affinity for the antipsychotic clozapine. *Nature* 350:610–614
- Wregget KA, DeLean A (1984): The ternary complex model. Its properties and application to ligand interactions with the D₂-dopamine receptor of the anterior pituitary gland. *Mol Pharmacol* 26:214–227
- Zahniser NR, Molinoff PB (1978): Effect of guanine nucleotides on striatal dopamine receptors. *Nature* 275:453–455