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In vitro affinity of piribedil for dopamine D_3 receptor subtypes, an autoradiographic study

Alfredo Cagnotto, Luca Parotti, Tiziana Mennini *

Istituto di Ricerche Farmacologiche 'Mario Negri', via Eritrea 62, 20157 Milan, Italy

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Abstract

Receptor binding autoradiography, using the selective ligand [^{3}H]7-OH-(*R*)DPAT (*R*(+)-2-dipropylamino-7-hydroxy 1,2,3,4-tetrahydronaphthalene), showed that piribedil is a potent inhibitor at dopamine D_3 receptors in limbic regions (island of Calleja), with affinity (IC_{50}) between 30 and 60 nM. The in vitro IC_{50} of piribedil for inhibition of [^{3}H]spiperone binding to receptors of the dopamine D_2-like family (D_2, D_3 and D_4), ranged between 10^{-7} and 10^{-6} M in different brain regions (medial and lateral caudate putamen, olfactory tubercles, and nucleus accumbens). At the highest concentration tested (10^{-5} M) piribedil inhibited dopamine D_1 receptor binding by < 50%. It is concluded that piribedil has 20 times higher affinity for dopamine D_3 than for dopamine D_2-like receptors, and very low affinity for the dopamine D_1 receptor subtype in rat brain. How this pattern of receptor affinity is related to the pharmacological profile of piribedil deserves further investigation.

Keywords: Piribedil; Dopamine receptor; Autoradiography

1. Introduction

Piribedil is a direct dopamine receptor agonist used for the treatment of Parkinson's disease (Jenner, 1992; Rondot and Ziegler, 1992) and of other clinical disorders involving dysfunction of the dopaminergic system (Dourish, 1983). It binds to dopamine D_2 receptors in vitro, as assessed with striatal homogenates (Hall et al., 1983). However, since its affinity is only moderate (10^{-7}–10^{-6} M), several studies have been carried out to determine whether active metabolites contribute to piribedil's activity.

Whatever the proposed mechanisms of action of the metabolites, pharmacokinetic studies have clearly demonstrated that, after piribedil doses active on the dopaminergic system, mainly unchanged piribedil is found in rat brain, with only traces of its hydroxylated and N-oxide metabolites (Sarati et al., 1991). These data suggest that, in the rat brain, metabolites are probably irrelevant to the drug's dopaminergic agonist activity.

Another possibility for explaining the dopaminergic activity of piribedil is its interaction with different dopamine receptor subtypes. In addition to the dopamine D_1 and dopamine D_2 receptors, dopamine D_3 (Sokoloff et al., 1990) and dopamine D_4 (Van Tol et al., 1991) receptors have recently been described in rat and human brain.

The affinity of drugs for the dopamine receptor subtypes has usually been determined using cloned receptors transfected and expressed in cell cultures (Damsa et al., 1995; Sokoloff et al., 1990; Lévesque et al., 1992). However, the pharmacological profile of receptors expressed in transfected cells and in native tissue do not always agree in detail, and may differ by about one order of magnitude (Landwehrmeyer et al., 1993).

Unfortunately, selective ligands, like [^{3}H]7-OH-(*R*)DPAT (*R*(+)-2-dipropylamino-7-hydroxy-1,2,3,4-tetrahydronaphthalene), are available only for the dopamine D_3 receptor subtype, that is, however, mainly expressed in brain regions, such as the islands of Calleja (Lévesque et al., 1992; Landwehrmeyer et al., 1993; Diaz et al., 1995), that cannot be utilized for homogenate receptor binding.

We therefore use quantitative autoradiography in rat brain sections to determine the affinity of piribedil for the dopamine D_3 receptor subtype in the islands of Calleja. For comparison, its affinity for dopamine D_1- and D_2-like receptors has also been determined, using [^{3}H]SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-te-

* Corresponding author. Tel.: +39 (0)2 390141; fax: +39 (0)2 3546277; telex: 331268 NEGRI I.

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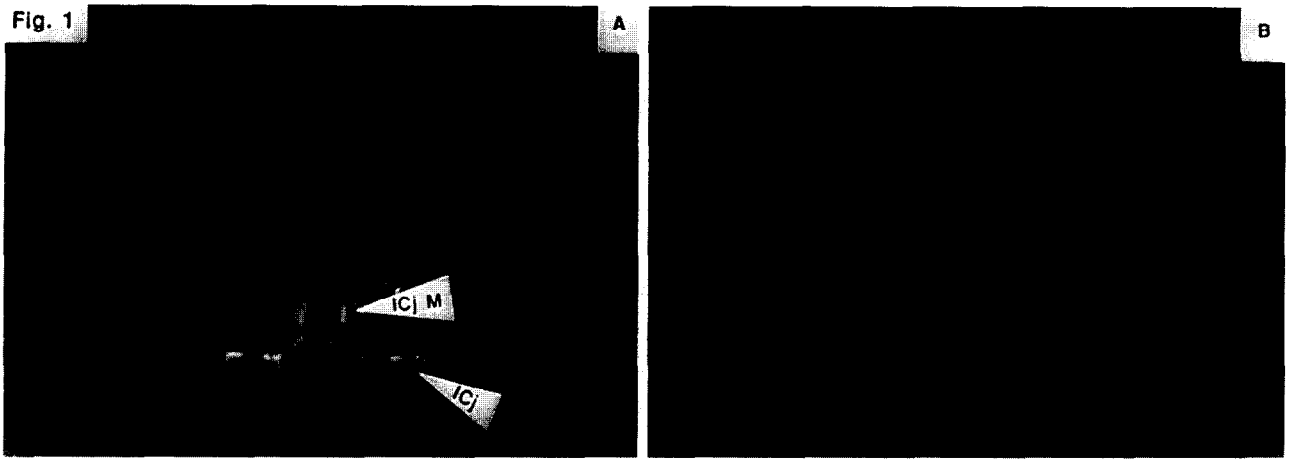

Fig. 1. Representative autoradiograms of dopamine D_3 receptor binding in rat brain coronal sections. The slices were incubated with 0.5 nM [^{3}H]7-OH-(*R*)DPAT, in the absence (A) or presence (B) of 10^{-5} M piribedil. The light regions correspond to sites with high binding. ICjM, island of Calleja, major island; ICj, island of Calleja.

trahydro-1*H*-3-benzazepine) to selectively label dopamine D_1 receptors, and [^{3}H]spiperone binding to heterogeneous dopamine D_2, D_3 and D_4 receptors in two brain regions (caudate-putamen and limbic areas) with a different relative expression of dopamine D_2 receptors.

2. Material and methods

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. No. 116, G.U., Suppl. 40, Feb. 18, 1992) and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, Dec. 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985).

Male Sprague-Dawley rats (CRL: CD(SD)BR, Charles River, Italy) were killed by decapitation and the brains were rapidly removed, frozen in liquid nitrogen and stored at −80°C until use. Consecutive coronal sections (14 μm) were cut at −16°C at the level of the anterior caudate-putamen, including nucleus accumbens, olfactory tubercles and islands of Calleja (plates 12–13 of the Paxinos and Watson (1982) rat brain atlas) using a cryostat (Microm HM 500 OM). The sections were thaw-mounted on gelatin-coated glass slides and stored at −20°C until the binding experiments.

[^{3}H]SCH 23390 binding to dopamine D_1 receptors was measured as follows (Morelli et al., 1990): after 5 min pre-incubation in 50 mM Tris-HCl, pH 7.4, containing 120 mM NaCl, the slices were incubated for 30 min at room temperature in 50 mM Tris-HCl, pH 7.4, with the addition of 120 mM NaCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM KCl and 1 nM [^{3}H]SCH 23390 (specific activity 71.1 Ci/mmol, NEN). Non-specific binding was determined in the presence of 1 μM of SCH 23390. Mianserin, 50 nM, was added to the incubation buffer to avoid binding to 5-HT receptors.

The binding of [^{3}H]spiperone to mixed dopamine D_2, D_3 and D_4 receptors was measured with the same incubation protocol and the same buffer as used for [^{3}H]SCH 23390 binding; 0.4 nM [^{3}H]spiperone (specific activity 25.0 Ci/mmol NEN) was added to the incubation buffer and non-specific binding was determined in the presence of 100 μM (−)-sulpiride. Incubations were stopped with two 5-min rinses in ice-cold 50 mM Tris-HCl, pH 7.4, and one rapid dip in ice-cold distilled water (Morelli et al., 1990).

[^{3}H]7-OH-(*R*)DPAT binding to dopamine D_3 receptors was done as described by Lévesque et al. (1992). Tissue

Table 1
Specific [^{3}H]ligands binding to different dopamine receptor subtypes (fmol/mg tissue ± S.D.)

	[^{3}H]SCH 23390	[^{3}H]Spiperone	[^{3}H]7-OH-(*R*)DPAT
Lateral caudate-putamen	245.17 ± 11.19	133.49 ± 9.38	N.D.
Medial caudate-putamen	230.34 ± 9.14	103.75 ± 5.86	N.D.
Nucleus accumbens	181.80 ± 18.39	63.08 ± 9.94	N.D.
Olfactory tubercle	180.73 ± 16.05	51.59 ± 19.20	N.D.
Major island of Calleja	N.D.	N.D.	19.41 ± 5.14
Island of Calleja	N.D.	N.D.	22.96 ± 3.68

The values are means ± S.D. for six animals per group.
N.D., not detectable.

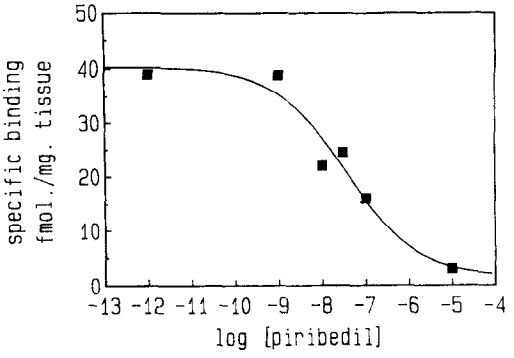

Fig. 2. Competition curve for piribedil on [^{3}H]7-OH-(*R*)DPAT binding in major island of Calleja. Piribedil was tested with consecutive coronal sections and at different concentrations, from 10^{-5} M to 10^{-9} M. Log [M] piribedil was plotted against specific ligand binding (fmol/mg tissue). Each point is the mean value of triplicate samples, varying < 10%. Curve fitting was done with the 'Allfit' program.

slices were preincubated in 50 mM Hepes-Na, pH 7.5, containing 1 mM EDTA and 0.1% bovine serum albumin 3 times for 5 min at 22°C, then incubated in the same fresh buffer containing 0.5 nM [^{3}H]7-OH-(*R*)DPAT (specific activity 139.0 Ci/mmol Amersham) for 1 h at 22°C. Eliprodil, 200 nM, was added to the incubation buffer to avoid binding to sigma receptors (Schoemaker, 1993; Wallace and Booze, 1995). Non-specific binding was determined in the presence of 1 μM dopamine. Incubations were stopped with four rinses in ice-cold 50 mM Hepes-Na, pH 7.5, containing 100 mM NaCl and one rapid dip in ice-cold distilled water.

After washing, the slides were dried overnight under a stream of cold air, apposed to Hyperfilm (Amersham) and placed in an X-ray box. After appropriate exposure the films were developed in Kodak D19 at 18°C for 10 min and optical densities of autoradiograms were determined with an RAS 3000 image analyser (Loats Associate). The radioactivity per mg of tissue was determined using tissue-equivalent plastic standards (Amersham).

Piribedil was tested in vitro at 5 different concentrations, from 10^{-5} to 10^{-9} M, in consecutive coronal sections, in triplicate. IC_{50} were calculated using the 'Allfit' program running on an IBM XT personal computer (De Lean et al., 1978).

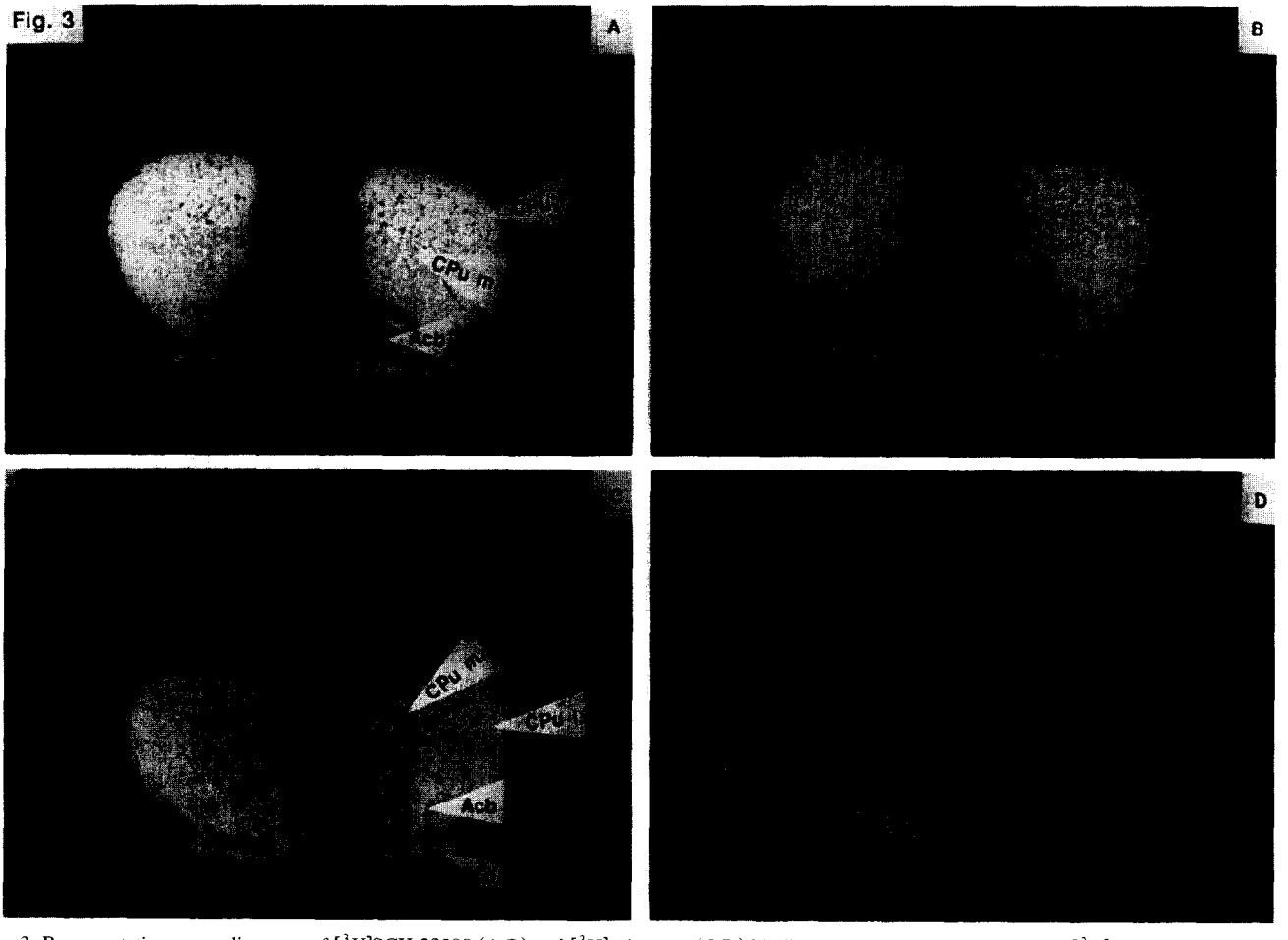

Fig. 3. Representative autoradiograms of [^{3}H]SCH 23390 (A,B) and [^{3}H]spiperone (C,D) binding in rat brain coronal sections. [^{3}H]SCH 23390 was used at 1 nM in the absence (A) or presence (B) of 10^{-5} M piribedil. [^{3}H]Spiperone was used at 0.4 nM in the absence (C) or presence (D) of 10^{-5} M piribedil. The light regions correspond to sites with high binding. CPu, caudate-putamen; Acb, nucleus accumbens; Tu, olfactory tubercle.

3. Results

Fig. 1 shows representative autoradiographic images of the in vitro labelling of the dopamine D_3 receptor subtype in anterior coronal sections of rat brain, and the effect of a maximal concentration of piribedil (10^{-5} M). [^{3}H]7-OH-(*R*)DPAT significantly bound to dopamine D_3 receptors in island of Calleja (Fig. 1A, Table 1). Non-specific binding was almost negligible under our experimental conditions. 10^{-5} M piribedil (Fig. 1B) completely inhibited dopamine D_3 receptor binding.

The complete dose-response curve for piribedil for inhibition of [^{3}H]7-OH-(*R*)DPAT in the major island of Calleja is shown in Fig. 2. The calculated IC_{50} is 30 ± 14 nM. Similar results were obtained in the island of Calleja (IC_{50} 62 ± 28 nM).

Dopamine D_1 receptors (Fig. 3A) were intensively labelled by [^{3}H]SCH 23390 in the lateral and medial caudate-putamen and, to a similar extent, in olfactory tubercle and nucleus accumbens (Table 1). Specific binding was almost identical to total binding. At 10^{-5} M, piribedil (Fig. 3B) only slightly inhibited dopamine D_1 receptor binding in caudate-putamen (30–35%), olfactory tubercle (25%) and nucleus accumbens (41%).

[^{3}H]Spiperone (Fig. 3C) labels a mixed population of dopamine D_2, D_3 and D_4 receptors with different regional distribution. The cortical binding in Fig. 3C was not inhibited by (−)-sulpiride (not shown) or by 10^{-5} piribedil (Fig. 3D), confirming that it represents binding to 5-HT_2 receptors. In dopaminergic regions, [^{3}H]spiperone binding was higher in caudate-putamen, lower in nucleus accumbens and olfactory tubercle (Table 1). Non-specific binding was only 7% of total binding. Piribedil 10^{-5} M inhibited [^{3}H]spiperone binding in caudate-putamen (82 and 89% in medial and lateral, respectively), olfactory tubercle and nucleus accumbens (69 and 62%) (Fig. 3D). When complete dose-response curves were plotted, the corresponding IC_{50} were: 288 ± 63 and 737 ± 96 nM in medial and lateral caudate-putamen, 559 ± 156 nM in olfactory tubercle and 1189 ± 194 nM in nucleus accumbens.

4. Discussion

The present study used receptor binding autoradiography to determine the affinity of piribedil for dopamine receptor subtypes in the rat brain. This approach associates the use of partially selective labelled ligands with the selective localization of a single receptor subtype in a defined brain region, allowing the evaluation of drug affinity for receptor subtypes in native tissue.

[^{3}H]Spiperone is a non-selective ligand that binds to mixed dopamine D_2, D_3 and D_4 receptors. The regional distribution of these receptor subtypes is different, with the dopamine D_2 receptors mainly localized in striatum (where dopamine D_3 and D_4 receptors are not significantly expressed), and dopamine D_3 receptors mainly present in the islands of Calleja where dopamine D_2 receptor expression is very low or undetectable (Landwehrmeyer et al., 1993). The mRNA for the dopamine D_4 receptor is found in limbic and cortical regions with relatively lower levels in the basal ganglia (Van Tol et al., 1991). Therefore, the binding of [^{3}H]spiperone in the caudate can be considered as selective for dopamine D_2 receptors (Landwehrmeyer et al., 1993). We confirm previous findings with rat brain homogenates (Hall et al., 1983), indicating that piribedil has moderate affinity for the striatal dopamine D_2 receptor subtype in vitro. In other brain regions, such as the nucleus accumbens and olfactory tubercles, the affinity of piribedil for dopamine D_2-like receptors is in the range of that found in the striatum.

We also found that piribedil has no significant effect on the dopamine D_1 receptor subtype in rat brain, consistent with its reported lack of stimulation of adenylate cyclase activity in rat striatum (Miller and Iversen, 1974).

The most important finding of our study was that piribedil is a potent inhibitor at dopamine D_3 receptors with affinity between 30 and 60 nM, i.e. one order of magnitude higher than for striatal dopamine D_2 receptors.

Our results are in contrast with the reported affinity ratio (0.5) of piribedil for cloned human dopamine $D_2 : D_3$ receptors (Millan et al., 1995). However, it has already been remarked that the pharmacological profile of receptors expressed in transfected cells and in native tissue may differ by about one order of magnitude (Landwehrmeyer et al., 1993).

The validity of labelling dopamine D_3 receptors in the rat brain with [^{3}H]7-OH-(*R*)DPAT has been demonstrated both in membrane homogenates (Lévesque et al., 1992) and in autoradiographic studies (Landwehrmeyer et al., 1993; Levant et al., 1995; Lévesque et al., 1992), particularly in the presence of inhibitors of σ receptors, for which it has some affinity (Schoemaker, 1993; Wallace and Booze, 1995). In addition the islands of Calleja are considered the brain region where dopamine D_3 receptors are predominant (Lévesque et al., 1992), matching very closely the distribution of dopamine D_3 receptor mRNA (Landwehrmeyer et al., 1993). The functional role of dopamine D_3 receptors in the island of Calleja is not yet clear, although they may be involved in reproductive/endocrine functions. However, dopamine D_3 receptors are present in other brain regions also, for example the nucleus accumbens, the ventral caudate-putamen, septal areas, hypothalamus and cerebellar cortex (Lévesque et al., 1992; Landwehrmeyer et al., 1993; Levant et al., 1995), suggesting that these receptors are involved in several physiological functions.

In summary, we found that piribedil in vitro has 10 times higher affinity for dopamine D_3 than D_2 receptors, and no significant affinity for the dopamine D_1 receptor in rat brain. How this pattern of receptor affinity is related to its pharmacological profile deserves further investigation.

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