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[^{3}H]-YM-09151-2 binding sites in human brain postmortem

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ABSTRACT

The controversial and limited data on the distribution of dopamine (DA) receptors of type 4 (D_4) in the human brain prompted us to explore their density and pharmacological characteristics in the prefrontal cortex, striatum and hippocampus, through a series of binding assays. Brain samples were taken during autopsy from seven subjects. Tissue homogenates were incubated with increasing concentration of [^{3}H]-YM-09151-2, a D_2-like receptor antagonist, and L-745,870 and/or sulpiride to define the non-specific binding, while PPAP was used to block sigma receptors. The results showed a low density of D_4 receptors in the hippocampus only, with a preponderance of D_2/D_3 and sigma receptors in the prefrontal cortex and striatum. In conclusion, these findings underline that it is possible to label D_4 receptors by means of [^{3}H]-YM-09151-2, provided that D_2, D_3 and sigma receptors are blocked.

1. Introduction

Dopamine (DA) receptors are classified into two major classes called D_1- and D_2-like, on the basis, respectively, of the high and low affinity for the neurotransmitter. The D_1-like class includes D_1 and D_5 receptors, while the D_2, D_3, D_4 subtypes belong to the D_2-like class (Civelli et al., 1991). Much interest has been directed towards the D_4 subtype after the demonstration that clozapine, the prototype of atypical antipsychotics, shows a high affinity for it (Meltzer, 1994). Both the clinical effectiveness and the low risk of extrapyramidal side effects of clozapine have been related to its preferential activity on D_4 receptors which, have been hypothesized to be involved not only in the pathophysiology of different psychotic disorders, but also of Parkinson's disease, mood disorders and hypercynetic syndrome (Tarazi and Baldessarini, 1999; Tarazi et al., 2004). However, data on D_4 receptors in these conditions are few and indirect (Davis et al., 1991; MacQueen et al., 2003; Kempf et al., 2005; Maletic et al., 2007). In fact, D_2 and D_4 receptor had been evaluated by different authors in the prefrontal cortex of schizophrenic patients through the messenger ribonucleic acid (mRNA) levels, and the ensuing findings were controversial, as both increased or similar levels of expression were reported, in comparison with those of healthy subjects (Mulcrone and Kerwin, 1996; Roberts et al., 1996; Stefanis et al., 1998). Others employed an indirect binding assay, while subtracting the binding of [^{3}H]-raclopride, which labels only the D_2 and D_3 receptors, from that of [^{3}H]-YM-09151-2, which labels all D_2-like receptors, while reporting a sixfold increase of D_4 receptors density in the striatum of schizophrenic patients (Seeman et al., 1993a). However, the density of D_2-like receptors, evaluated by means of [^{11}C]-methylspiperone, resulted higher in schizophrenic than in healthy subjects, but similar in the two groups when [^{11}C]-raclopride was employed (Hagberg et al., 1998). Given that methylspiperone labels all D_2-like receptors, these results seemed to support the presence of D_4 receptors in the striatum. The interpretation of these data is, however, complicated by the evidence that [^{11}C]-methylspiperone binds only to the D_2-like receptors in monomeric configuration, at variance with raclopride that binds to both monomeric and oligomeric configurations, and [^{3}H]-YM-09151-2 labels also the sigma and serotonin receptors of type 1a and 2a ($5HT_{1A}$ and $5HT_{2A}$) (Helmeste et al., 1996, 1997; Ujike et al., 1996; Tang et al., 1997).

These controversies may also be related to the fact that little is known on the distribution of D_2-like receptors, and particularly of D_4 ones, in the human brain in normal conditions.

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D_4 receptors have been described at high density in the prefrontal cortex by means of a binding method employing [^{3}H]NGD 94–1 (Primus et al., 1997). However, in a recent study exploring D_4 receptors in human prefrontal cortex by means of the binding of [^{3}H]-YM-09151-2, their density resulted inconsistent (Marazziti et al., 2007). Therefore, given the paucity of available information, the aim of the present study, which can be considered an extension of the previous, was to explore and characterize the distribution and density of D_4 receptors in the prefrontal cortex, striatum and hippocampus of human brain postmortem, by comparing and/or subtracting the values of binding parameters obtained with [^{3}H]-YM-09151-2 and different compounds to define the non-specific binding. As a consequence, the presence of sigma sites in human prefrontal cortex and striatum was evaluated as well.

2. Experimental procedure

2.1. Subjects

Prefrontal cortex, striatum and hippocampus samples were taken postmortem, during autoptic sessions, from seven subjects (four men and three women; age, mean ± SD: 61 ± 5 years). They were immediately frozen in dry ice and rapidly stored to −80°. Autolysis time (i.e., the time between death and freezing the samples) ranged between 7 h and 25 h, an interval which does not interfere with binding assays, as demonstrated in preliminary experiments (data not shown). All subjects had died for causes not involving primarily the brain (four from myocardial infarction, two from pulmonary embolism and one from respiratory failure), had not suffered from any psychiatric or neurological disorders and were not administered psychotropic drugs, as recorded by their medical charts. The study was approved by the Ethics Committee at Pisa University.

2.2. Preparation of brain tissue homogenates

Brain tissue samples were defrosted and separated by white substance. Tissues were re-suspended in D_4 buffer (120 mM NaCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, EDTA 1 mM, pH 7.4) to yield 4 mg original wet weight per ml, according to the method of Seeman et al. (1993a,b), and then homogenized with an Ultraturrax in D_4 buffer. The homogenates were not washed and centrifuged, because a consistent loss of receptors ranging between 15% and 60% may occur during these steps.

2.3. Binding of [^{3}H]-YM-09151-2

Five hundred microliters of cortex, striatum and hippocampus homogenates were incubated with 10 increasing concentration of [^{3}H]-YM-09151-2 (PerkinElmer, Milan, Italy, specific activity: 85.5 Ci/mmol) ranging between 0.015 nM and 10 nM, at a final volume of 1.5 ml. The non-specific binding was carried out by using L-745,870 30 μM and/or sulpiride 10 μM (Helmeste et al., 1996, 1997; Seeman et al., 1993; Tang et al., 1997; Tarazi et al., 1998). Given that [^{3}H]-YM-09151-2 may bind to sigma receptors (Ujike et al., 1996), 1-phenyl-2-propylaminopentane (PPAP) 100 nM was used to block them in some assays. To determine the amount of sigma sites bound by [^{3}H]-YM-09151-2, we performed several binding assays with increasing radioligand concentrations (0.01–10 nM) and PPAP 500 nM to define the non-specific binding. Furthermore, to evaluate the different potency of [^{3}H]-YM-09151-2 binding to D_2/D_3 and D_4 receptors, we carried out some assays with this ligand (concentration range: 0.015–10 nM) and raclopride 300 nM or L-745,870 300 nM. In these cases sulpiride 10 μM was used in these cases to define the non-specific binding and PPAP 100 nM to block sigma receptors.

The incubation with [^{3}H]-YM-09151-2 was performed for 2 h at 22 °C. The separation of bound and free ligand was carried out by vacuum filtration through GF/C fiber filters (Whatman, UK), which were pre-soaked in polyethyleneimine (2%) to minimize the non-specific binding to filters. Radioactivity was counted by a liquid scintillation counter (Packard LS-1600).

For the pharmacological characterization of the binding, the homogenates were incubated with increasing concentrations of L-745,870, raclopride, PPAP and 0.5 nM of the radioligand.

2.4. Data analysis and statistics

Equilibrium-saturation binding data, the maximum binding capacity (B_{max}, fmol/mg tissue) and the dissociation constant (K_d, nM) were analyzed by means of iterative curve-fitting computer programmes EBDA (McPherson, 1985).

The potency of the different compounds in displacing the [^{3}H]-YM-09151-2 binding was expressed as inhibition constant (K_i) values, calculated from the IC_{50} (concentration of drug causing 50% inhibition of the specific binding), using the Cheng and Prusoff equation (1973). Each determination was performed in triplicate, but not in all areas, for the lack of sufficient brain tissue.

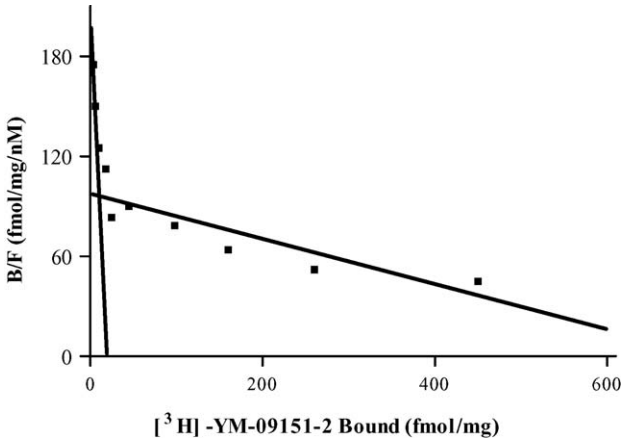

Fig. 1. Example of Scatchard analysis of the ^{3}H-YM-09151-2 binding to the prefrontal cortex.

3. Results

3.1. [^{3}H]-YM-09151-2 binding to the prefrontal cortex and striatum with L-745,870 to define the non-specific binding

The Scatchard analysis of the [^{3}H]-YM-09151-2 binding to the prefrontal cortex showed the presence of high and low affinity binding sites (Fig. 1). The B_{max} (mean ± SD, fmol/mg tissue) and K_d (mean ± SD, nM) values of the two sites were, respectively, 12 ± 5 and 0.091 ± 0.0099, and 710 ± 241 and 6.51 ± 1.42 (Table 1). The pharmacological competition studies with L-745,870 and raclopride did not lead to the expected diphasic curves, but rather to a monophasic curve with high K_i values (236 ± 33 nM for L-745,870 and 1590 ± 170 nM for raclopride): this effect might be attributed to the interference of receptors other than the D_2-like ones. The displacement curve of PPAP was also monophasic, with a very low K_i (10 ± 3 nM).

The situation in the striatum was similar, as high and low affinity binding sites were detected in this area (the B_{max} and K_d values of both sites at this level are reported in Table 1).

3.2. [^{3}H]-YM-09151-2 binding to the prefrontal cortex and striatum with sulpiride to define the non-specific binding and PPAP to block sigma receptors

In this case, in both areas, the Scatchard analysis revealed that the binding was specific and saturable, but only a single population of binding sites was detected (Fig. 2, panel a). The B_{max} was 9.4 ± 3 and the K_d 0.21 ± 0.08 in the first area, and 97 ± 15 and 0.07 ± 0.005 in the striatum (Table 2A).

The pharmacological competition studies with raclopride and L-745,870 led to no curve in the prefrontal cortex, probably for the low density of DA receptors in this area. On the contrary, in the striatum a one phase-displacement curve was obtained with both L-745,870 (K_i = 925 ± 140 nM) and raclopride (K_i = 11.4 ± 3 nM) (Fig. 3, panel a and b).

Table 1
B_{max} (mean ± SD, fmol/mg tissue) and K_d (mean ± SD, nM) values of the ^{3}H-YM-09151-2 binding to the prefrontal cortex and striatum with L-745,870 to define the non-specific binding.

	B_{max}		K_d	
	Hi	Lo	Hi	Lo
Prefrontal cortex	12 ± 5	710 ± 241	0.091 ± 0.009	6.51 ± 1.42
Striatum	103 ± 21	645 ± 30	0.06 ± 0.008	7.11 ± 1.12

Hi: high-affinity site; Lo: low affinity site.