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Absence of detectable striatal dopamine D_4 receptors in drug-treated schizophrenia

Gavin P. Reynolds *, Sarah L. Mason

Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN, UK

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Abstract

The difference between saturable binding of [^{3}H]emonapride (to D_2, D_3 and D_4 receptors) and [^{125}I]epidepride (to D_2 and D_3 receptors) was used to determine dopamine D_4 receptors in putamen taken post-mortem from antipsychotic-treated schizophrenic subjects and matched controls. Despite an overall increase in D_2/D_3 receptor density in schizophrenia, reflecting prior antipsychotic drug treatment, striatal D_4 receptors were not significantly detectable in either controls or schizophrenic subjects.

Keywords: Dopamine D_4 receptor; Schizophrenia; Antipsychotic

The report of an increase in striatal dopamine D_4 receptors in schizophrenia (Seeman et al., 1993) has fuelled interest in specific D_4 antagonists as potential antipsychotic agents. D_4 receptors were determined by subtracting saturable binding of [^{3}H]raclopride to D_2 and D_3 sites, from that of [^{3}H]emonapride (nemonapride, YM 09591-2), defining D_2, D_3 and D_4. However, using raclopride displacement to search for a D_4 component of the D_2-like receptors defined by [^{3}H]emonapride, we were unable to identify any D_4 sites (Reynolds and Mason, 1994) and concluded that the 70% elevation in striatal [^{3}H]emonapride binding in schizophrenia is due to an excess of D_2 or D_3 receptors and seen only in patients receiving antipsychotic drugs. However, Seeman et al. (1993) suggest that the D_4 increase they observe is independent of drug treatment, while Schoots et al. (1995), also using differential binding of [^{3}H]raclopride and [^{3}H]emonapride, report that chronic haloperidol treatment induces an apparent increase in dopamine D_4 receptors in rat striatum.

In order to resolve these discrepancies and determine whether striatal D_4-like receptors are increased in drug-treated schizophrenic patients, we have employed a new ligand, [^{125}I]epidepride, which, like raclopride, has over 100-fold selectivity for the D_2 and D_3 sites over D_4, and an affinity for D_2 and D_3 sites that is approximately 100-fold higher than raclopride (Kessler et al., 1993). Thus we have used the differential binding approach of Seeman et al. (1993), substituting [^{125}I]epidepride for [^{3}H]raclopride to define the non-D_4 component of D_2-like receptors.

Putamen was taken post mortem from control subjects (4 male, 2 female; mean age 61.8 ± 19.5 S.D. years) and schizophrenic patients (3 male, 2 female; 69.6 ± 16.4 years) and stored frozen at -70°C until analysis. All schizophrenics had received chronic treatment with antipsychotic drugs until within a few days of death. Tissue was homogenized in pH 7.4 buffer (containing 50 mM Tris-HCl, 1 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$ and 120 mM NaCl), washed twice by centrifugation at $48\,000 \times g$ and resuspension, and incubated at 1 mg/ml in triplicate at 37°C in a final volume of 0.25 ml for 35 min with [^{3}H]emonapride, or 0.2 ml for 75 min with [^{125}I]epidepride. Incubations were terminated by filtration and wash (for 12 s) with ice-cold buffer using a Skatron cell harvester, prior to scintillation counting of bound [^{3}H]emonapride or gamma counting of bound [^{125}I]epidepride. Inclusion of sulpiride at 50 μM was used to determine non-specific binding. Density of all D_2-like (i.e. D_2, D_3 and D_4) receptors was determined in each sample by Scatchard analysis of [^{3}H]emonapride bind-

* Corresponding author. Tel. 0114 282 4662, fax 0114 276 5413.

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Table 1
Density of D_2-like dopamine receptors in human putamen from five drug-treated schizophrenic subjects and six controls

	Receptor densities	
	Controls	Schizophrenics
D_2/D_3	14.8±4.1	29.0±1.7
$D_2/D_3/D_4$	16.7±5.5	29.3±3.3
'D_4' component	1.9±3.1	0.4±3.8

Values given are means±S.D. in pmol/g tissue obtained from saturable [^{125}I]epidepride (D_2/D_3) or [^{3}H]emonapride ($D_2/D_3/D_4$) binding.

ing over eight concentrations of 20 pM–2.4 nM, while the sum of D_2 and D_3 receptors in the same tissue homogenate was determined similarly using [^{125}I]epidepride over eight concentrations of 2.5 pM–320 pM.

Table 1 shows the expected elevation in D_2-like receptors, defined by [^{3}H]emonapride binding, in the tissue from schizophrenic subjects (Seeman et al., 1993; Reynolds and Mason, 1994). However, the results using [^{125}I]epidepride indicate that this increase is essentially fully ascribable to the D_2 and D_3 receptors. The small differences between the two mean densities may reflect small concentrations of D_4 sites, although these differences are not significantly above zero in either group (t-test; $P > 0.1$ in each case) and are certainly not increased in the schizophrenic patients. This is consistent with the 100-fold higher level of D_2 compared to D_4 mRNA in striatum (Van Tol et al., 1991). It may be that other subtle differences in the selectivity of these radioligands may contribute to the inequality of the detected receptor densities; emonapride binds to 5-HT_{1A} receptors ($K_i = 4.5$ nM) which may make a small contribution to its binding here (Assié et al., 1993).

Given the low levels of expression of D_4 receptor mRNA in the human striatum (Matsumoto et al., 1995), it is hardly surprising that the receptor itself is not consistently detectable in this region by radioligand binding. Thus we find no evidence for an increase in striatal dopamine D_4 receptors, due either to prior antipsychotic drug treatment or to the disease process, in patients with schizophrenia. Confirmation of this result awaits the identification of specific radioligands for the D_4 site.

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