ELSEVIER

European Journal of Pharmacology 286 (1995) R3–R5

ejp

Rapid communication

Schizophrenia: elevation of dopamine D_4-like sites, using [^{3}H]nemonapride and [^{125}I]epidepride

Philip Seeman [a,b,*], Hong-Chang Guan [a], Hubert H.M. Van Tol [a,b,c]

[a] *Department of Pharmacology, Medical Sciences Building, University of Toronto, Toronto M5S 1A8, Canada*
[b] *Department of Psychiatry, Medical Sciences Building, University of Toronto, Toronto M5S 1A8, Canada*
[c] *Molecular Neurobiology Laboratory, Clarke Institute of Psychiatry, University of Toronto, 250 College St., Toronto M5T 1R8, Canada*

Received 9 October 1995; accepted 13 October 1995

Abstract

We here report a three-fold elevation of dopamine D_4-like sites in schizophrenia, using [^{3}H]nemonapride to measure dopamine D_2 and D_3 receptors and D_4-like sites, and using [^{125}I]epidepride to measure D_2 and D_3 sites in ten control and nine schizophrenia post-mortem brain putamen tissues. This result differs from a recent report which did not detect significant D_4-like sites in control or schizophrenia putamen (Reynolds and Mason, 1995, Eur. J. Pharmacol. 281, R5). The present finding agrees with other reports wherein an elevation in D_4-like sites was found in schizophrenia, using [^{3}H]nemonapride for D_2, D_3 and D_4-like sites, but [^{3}H]raclopride for D_2 and D_3 sites. The nature of these D_4-like sites is not known.

Keywords: Schizophrenia; Dopamine receptor; Dopamine D_4-like site

The hypothesis of dopamine overactivity in schizophrenia is partly based on the fact that antipsychotic drugs block dopamine D_2-like receptors in direct relation to their clinical antipsychotic potencies (Seeman and Van Tol, 1994). In addition, dopamine D_2-like receptors are elevated in post-mortem schizophrenia brain tissues, including D_2 and D_3 receptors and D_4-like sites (Seeman et al., 1993; Murray et al., 1995; Sumiyoshi et al., 1995). (Because these elevated D_4-like sites have not yet been pharmacologically characterized, these sites have been referred to as D_4-like.) Although the elevation of D_4-like sites in schizophrenia had been questioned by Reynolds and Mason (1994), it was subsequently shown (Seeman and Van Tol, 1995) that the competition method of Reynolds and Mason could not discriminate the separate components of [^{3}H]nemonapride [1] binding to cloned D_2 and D_4 receptors.

Recently, Reynolds and Mason (1995) reported that they could not detect significant D_4-like sites in control or schizophrenia putamen, using [^{3}H]nemonapride to measure D_2 and D_3 receptors and D_4-like sites, and using [^{125}I]epidepride to measure D_2 and D_3 receptors. Their results differed from those wherein [^{3}H]nemonapride was used to measure D_2, D_3 and D_4-like sites, and [^{3}H]raclopride for D_2 and D_3 sites (Seeman et al., 1993).

In order to resolve these latter two conflicting studies, we examined putamen samples from post-mortem human brain controls (4 females, 6 males; 70.2 ± 3.2 years) and schizophrenia patients (5 females, 4 males; 67 ± 7 years), using the method of Reynolds and Mason (1995). As with the patients reported by Reynolds and Mason (1995), the present tissues were from patients who had received antipsychotic drugs up to several days before death.

Human frozen striata (Canadian Brain Tissue Bank, Toronto, and the Cambridge Brain Bank, UK) were blotted and weighed frozen. Buffer (50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, 120 mM NaCl) was added to yield 2 mg tissue per ml. The suspension was homogenized (Polytron PT-10 (Brinkmann Instruments, Westbury, NY,

* Corresponding author. Departments of Pharmacology and Psychiatry, Medical Sciences Building, Room 4344, 8 Taddle Creek Road, University of Toronto, Toronto, Ont., Canada M5S 1A8. Tel.: 1-416-978-4891; fax: 1-416-971-2445.

[1] Formerly emonapride or YM-09151-2.

SSDI 0014-2999(95)00677-X

Table 1
Densities of dopamine D_2-like receptors in human putamen

Receptor sites	Ligand	Density, B_{max}, pmol/g tissue	
		Control ($n = 10$)	Schizophrenia ($n = 9$)
$D_2 + D_3 + D_4$-like	[^{3}H]Nemonapride	14.7 ±1.3	30.5 ± 2
$D_2 + D_3$	[^{125}I]Epidepride	9.5 ±0.9	14.7 ± 0.9
D_4-like component		5.17 ± 0.5	15.8 ± 2.1

USA) 5 s at setting 5) but not washed, because previous work (see Refs. in Seeman et al., 1993) found that two washes of homogenized tissues may result in a loss of 30–60% of dopamine receptors in human tissues. Incubation tubes received 0.5 ml buffer, 0.5 ml of [^{3}H]nemonapride (71–75 Ci/mmol; New England Nuclear, E.I. du Pont de Nemours and Co., Boston, MA, USA) (12 final concentrations from 1 pM to 1 nM) or [^{125}I]epidepride (2000 Ci/mmol, Amersham, UK, or 1300 Ci/mmol, gift from Dr. R. Kessler, Vanderbilt University, Nashville, TN, USA) (12 final concentrations from 2 pM to 300 pM), and 0.5 ml of the tissue homogenate. The tubes were incubated for 2 h at room temperature (20°C), after which the incubates were filtered, using a 12-well cell harvester (Titertek, Skatron, Lier, Norway) and buffer-presoaked glass fiber filters mats (No. 70343, Skatron, Sterling, VA, USA). After filtering the incubates, the filters were rinsed with buffer for 15 s (7.5 ml buffer). The filters were placed in scintillation minivials and monitored for tritium 6 h later (in a Packard 4660 scintillation spectrometer at 55% efficiency) or placed into plastic tubes for gamma particle monitoring in a gamma counter (LKB 1282). Nonspecific binding was defined as that in the presence of 1 μM haloperidol or 10 μM *S*-sulpiride. The density of binding sites, B_{max}, and the dissociation constant, K_D, were obtained by Scatchard analysis.

Although the densities of [^{3}H]nemonapride sites in this study (Table 1) agreed with those of Reynolds and Mason (1995), the densities of [^{125}I]epidepride sites did not. Table 1 shows that the D_4-like component was detectable in control tissues and was elevated three-fold in schizophrenia tissues, in agreement with other reports (Seeman et al., 1993; Murray et al., 1995; Sumiyoshi et al., 1995) wherein [^{3}H]raclopride (in the presence of guanine nucleotide) was used instead of [^{125}I]epidepride.

The present findings differ markedly from those reported by Reynolds and Mason (1995) who could not detect such D_4-like sites. Reynolds and Mason washed the tissues twice while we did not (see above). They defined nonspecific binding with 50 μM sulpiride, while we used 1 μM haloperidol (which we found gave results similar to sulpiride). Because the densities of [^{3}H]nemonapride sites here agreed with those of Reynolds and Mason (1995), while those for [^{125}I]epidepride did not, there may have been some technical difference in deriving the specific activity of [^{125}I]epidepride. For example, Doyle et al. (1984) found that fully iodinated radioligands need not be corrected for the specific activity if the molecule is destroyed upon decay (which is the case for [^{125}I]epidepride, as confirmed by Amersham Life Sciences, Chicago, USA, and by Drs. T. De Paulis and R. Kessler, personal communications).

The pharmacological nature of the elevated D_4-like sites is not known. Using tissues which have significant amounts of the D_4-like sites, preliminary findings with D_4-selective [^{3}H]ligands indicate that the actual amount of dopamine D_4 receptors in the human striatum is different and much lower than the amount of D_4-like sites (manuscript in preparation). Further research is needed to determine the nature of the D_4-like binding sites and why they are elevated in schizophrenia.

Acknowledgements

Supported by NARSAD (National Association for Research in Schizophrenia and Depression, USA) and the Medical Research Council of Canada. We thank Dr. R. Kessler, Dr. T. De Paulis and N. Scott Mason for consultations and their gift of [^{125}I]epidepride.

References

Doyle, V.M., F.R. Bühler and E. Bürgisser, 1984, Inappropriate correction for radioactive decay in fully iodinated adrenergic radioligands, Eur. J. Pharmacol. 99, 353.

Murray, A.M., T.M. Hyde, M.B. Knable, M.M. Herman, L.B. Bigelow, J.M. Carter, D.R. Weinberger and J.E. Kleinman, 1995, Distribution of putative D_4 dopamine receptors in postmortem striatum from patients with schizophrenia, J. Neurosci. 15, 2186.

Reynolds, G.P. and S.L. Mason, 1994, Are striatal dopamine D_4 receptors increased in schizophrenia?, J. Neurochem. 63, 1576.

Reynolds, G.P. and S.L. Mason, 1995, Absence of detectable striatal dopamine D_4 receptors in drug-treated schizophrenia, Eur. J. Pharmacol. 281, R5.

Seeman, P. and H.H.M. Van Tol, 1994, Dopamine receptor pharmacology, Trends Pharmacol. Sci. 15, 264.

Seeman, P. and H.H.M. Van Tol, 1995, Dopamine D_4-like receptor elevation in schizophrenia: cloned D_2 and D_4 receptors cannot be discriminated by raclopride competition against [^{3}H]nemonapride, J. Neurochem. 64, 1413.

Seeman, P., H.-C. Guan and H.H.M. Van Tol, 1993, Dopamine D_4 receptors are elevated in schizophrenia, Nature 365, 441.

Sumiyoshi, T., C.A. Stockmeier, J.C. Overholser, P.A. Thompson and H.Y. Meltzer, 1995, Dopamine D_4 receptors and effects of guanine nucleotides on [^{3}H]raclopride binding in postmortem caudate nucleus of subjects with schizophrenia or major depression, Brain Res. 681, 109.