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Decrease in σ but no increase in striatal dopamine D_4 sites in schizophrenic brains

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Abstract

[^{3}H]Nemonapride differentially defines σ and dopamine receptor sites depending upon assay conditions. In post-mortem schizophrenic brain tissues, [^{3}H]nemonapride-labeled σ receptor binding is decreased compared to matched normal controls. No striatal dopamine D_4/D_4-like receptor differential was observed between the schizophrenic or control tissues, using the [^{3}H]nemonapride minus [^{3}H]raclopride subtraction method.

Keywords: σ Receptor; Dopamine D_4 receptor; Schizophrenia

Initial binding studies of [^{3}H]nemonapride to post-mortem schizophrenic brain tissue have suggested an increase in dopamine D_4 receptor densities (Seeman et al., 1993; Murray et al., 1995; Sumiyoshi et al., 1995). Reynolds and Mason (1995), however, were unable to confirm this. Recently, we reported that a large proportion of [^{3}H]nemonapride binding in striatum and other areas represents σ receptors (Helmeste et al., 1996). Several of the previous post-mortem schizophrenic studies have used assay conditions which would have included both σ and dopamine receptors in their analysis of dopamine D_4 receptor binding (Murray et al., 1995; Seeman et al., 1995). A positive differential representing σ receptors could then result when the subtraction method is used. This has led to some confusion as to which component, if any, is actually altered in post-mortem brains of patients suffering from schizophrenic psychosis. To try to dissect out the dopaminergic and σ receptor components of [^{3}H]nemonapride binding, we have analyzed 3 different brain regions of schizophrenics and controls, using assay conditions that could clearly distinguish σ from dopamine receptor sites. The analysis of a potential dopamine D_4 receptor component in [^{3}H]nemonapride binding was done by the subtraction method of Seeman et al. (1993).

This method uses subtraction of [^{3}H]raclopride's B_{max} (dopamine D_2 and D_3 receptor binding) from [^{3}H]nemonapride's B_{max} (dopamine D_2, D_3 and D_4 receptor binding when defined with 10 μM sulpiride), to give a difference representing dopamine D_4 receptor binding (dopamine D_4 receptor differential). This differential was originally described as labeling dopamine D_4 receptors (Seeman et al., 1993) but has more recently been described as a dopamine 'D_4-like' receptor component (Seeman et al., 1995), making the nature of this site unclear. The σ receptor component of [^{3}H]nemonapride binding (1 nM) in striatum, frontal cortex and cerebellum, was defined by 1 μM of the σ receptor ligand PPAP, (*R*(−)-*N*-(3-phenyl-*n*-propyl)-1-phenyl-2-aminopropane hydrochloride), as described previously (Helmeste et al., 1996).

Collection of brain samples was previously described (Akbarian et al., 1993). Schizophrenic ($n = 6$) and control ($n = 6$) samples were matched for age, sex and autolysis time (4 males and 2 females/group; age range = 21–85 years). The age-matching design takes care of potential age-dependent differences in dopamine D_4 receptors, which are unknown at present. Schizophrenics were diagnosed as chronic paranoid ($n = 3$) or chronic undifferentiated ($n = 3$) using DSM III-R criteria. One schizophrenic had never received neuroleptic medications, 4 had received neuroleptics [chlorpromazine ($n = 2$), haloperidol (1), thioridazine

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Table 1

(A) Dopaminergic binding in caudate (mean ± S.E.M.)			
[^{3}H]Nemonapride	Control ($n = 6$)	Schizophenics ($n = 6$)	
B_{max} (fmol/mg protein)	290 ± 27	428 ± 43	($P < 0.025$)
K_d (nM)	0.036 ± 0.003	0.111 ± 0.05	(N.S.)
[^{3}H]Raclopride	Control ($n = 6$)	Schizophrenics ($n = 5$) [b]	
B_{max} (fmol/mg protein)	294 ± 30	470 ± 66	($P < 0.05$)
K_d (nM)	2.45 ± 0.17	4.30 ± 2.01	(N.S.)
Dopamine 'D_4' receptor differential [a]	−4 ± 10	−15.8 ± 30.2	(N.S.)
(B) [^{3}H]Nemonapride σ binding (fmol/mg protein; mean ± S.E.M.)			
	Control ($n = 6$)	Schizophrenics ($n = 6$)	
Frontal cortex	180 ± 36	108 ± 26	(N.S.)
Caudate	159 ± 25	73 ± 21	($P < 0.05$)
Cerebellum	208 ± 40	123 ± 42	(N.S.)

[a] Dopamine 'D_4' receptor differential equals [^{3}H]nemonapride B_{max} minus [^{3}H]raclopride B_{max}.

[b] One schizophrenic sample failed to yield a usable [^{3}H]raclopride Scatchard despite repeated assays, due to the low specific binding and scatter of points (hence, $n = 5$, not 6 for this group).

Striatal tissue represented the head of the left caudate nucleus. Frontal cortex samples were from the left lateral prefrontal cortex (Brodmann's area 10). Cerebellar samples represented the left cerebellar hemisphere (posterolateral aspect).

Binding of tissue homogenates to [^{3}H]nemonapride (0.004–3 nM, 86.0 Ci/mmol; DuPont NEN, Boston, MA, USA) or [^{3}H]raclopride (0.2–20 nM, 74.0 Ci/mmol; DuPont NEN) was done in polypropylene tubes for 4 h at room temperature (22°C) for [^{3}H]nemonapride and 2 h (22°C) for [^{3}H]raclopride, before separation of bound from free ligand by vacuum filtration through GF/B glass fiber filters (Whatman, UK) (presoaked in 0.2% polyethyleneimine to minimize non-specific binding to filters).

Gpp(NH)p (sodium salt) (5′-guanylylimidodiphosphate) was not added to the assay, since it did not enhance [^{3}H]raclopride binding in our washed tissue. Two-tailed t-test for significance. N.S., not statistically significant.

(2), thiothixene (1)] and the 6th had received antidepressant (fluoxetine) and cardiac medications.

Assay conditions were as previously described (Helmeste et al., 1996), with laboratory investigators being blind to the disease status of the samples analyzed.

Both [^{3}H]nemonapride and [^{3}H]raclopride receptor densities as defined with 10 μM (−)sulpiride, were elevated in the caudate of schizophrenic samples compared to controls (Table 1A). Sulpiride, which has no affinity for σ receptors, defines the dopaminergic receptor component of [^{3}H]nemonapride binding.

No significant dopamine D_4/D_4-like receptor component could be consistently seen in either control or schizophrenic samples (Table 1A). The σ receptor component of [^{3}H]nemonapride binding was decreased in the caudate of schizophrenics. Frontal cortex and cerebellar σ receptor binding were also reduced, but reached statistical significance only when paired t-tests were used (Table 1B). In the caudate, schizophrenic samples showed a negative correlation between dopaminergic and σ receptor binding (correlation coefficient = −0.78). A larger sample size will be needed to address its significance.

In summary, we have not been able to demonstrate a dopamine D_4/D_4-like receptor differential in schizophrenic striatum, even though both [^{3}H]nemonapride and [^{3}H]raclopride binding densities are elevated in schizophrenics. Since the σ receptor component of [^{3}H]nemonapride binding showed dramatic reductions in schizophrenics compared to controls, the differential would be reduced, not elevated, in schizophrenic brains, should a haloperidol or butaclamol baseline be used. A reduction in cerebellar σ receptor binding has been previously observed in haloperidol-treated schizophrenics, but not in schizophrenics receiving other neuroleptics (Reynolds et al., 1991). Only one of our schizophrenics had received haloperidol (which has affinity for both σ and dopamine D_2 receptors), yet the reduction in σ receptor binding appeared to be a generalized phenomenon. It would now be useful to expand this study to include a larger medication-free schizophrenic brain sample and to examine the various subgroups of this heterogeneous disorder, to confirm that an elevation of striatal dopamine receptors is accompanied by decreases in σ receptor binding in schizophrenia.

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