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Role of dopamine D_2, D_4 and serotonin$_{2A}$ receptors in antipsychotic and anticataleptic action

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Busatto and Kerwin (1997) ably summarize research on whether the combined block of dopamine D_2 and serotonin$_{2A}$ receptors may provide more effective antipsychotic action with less Parkinsonism than by just blocking dopamine D_2 receptors alone.

Although all antipsychotic drugs occupy high levels of dopamine D_2 receptors under clinical conditions (Seeman, 1996; Seeman *et al.*, 1996a,b), it is unclear whether the additional blockade of serotonin$_{2A}$ receptors may help to alleviate psychosis and whether it may help to minimize antipsychotic-induced Parkinsonism.

One of the main reasons for this uncertain role of serotonin$_{2A}$ receptors in antipsychotic action stems from the uncertain values for the dissociation constants of the various antipsychotic drugs at the serotonin$_{2A}$ receptors. These values vary considerably among different laboratories. This variation is largely a result of the fact that the apparent dissociation constant (or inhibition constant) of an antipsychotic drug depends on the radioligand used to label the receptor (Seeman 1995, 1996; Seeman and Van Tol, 1995; Seeman *et al.*, 1996a,b).

For example, clozapine at the dopamine D_2 receptor has an inhibition constant of 388 ± 73 nM using [^{3}H]nemonapride, 186 ± 44 nM using [^{3}H]spiperone, and 84 ± 17 nM using [^{3}H]raclopride. Haloperidol at the dopamine D_2 receptor has an inhibition constant of 9.6 ± 1.5 nM using [^{3}H]nemonapride, 2.9 ± 0.4 nM using [^{3}H]spiperone and 0.67 ± 0.06 nM using [^{3}H]raclopride. These neuroleptic dissociation constants are related to the tissue/buffer partition coefficients of the radioligands. Thus, by extrapolating the relation between the neuroleptic inhibition constant and the tissue/buffer partition, one obtains the real inhibition or dissociation constant of the neuroleptic in the absence of any competing radioligand. This extrapolated radioligand-independent inhibition constant exactly agrees with the dissociation constant of the neuroleptic obtained by direct measurement using the radioactive form of the neuroleptic, such as [^{3}H]clozapine or [^{3}H]haloperidol (Seeman 1995; Seeman and Van Tol, 1995).

Using this experimental approach, the real dissociation constants for the neuroleptics discussed by Busatto and Kerwin (1997) are as in Table 1.

As partly outlined in Table 1, the so-called atypical neuroleptics fall into two groups. One group consists of those which bind loosely to the dopamine D_2 receptor (clozapine, melperone, perlapine, remoxipride and seroquel), all of which have true dissociation constants between 30 and 90 nM. These high values make them readily displaceable by the high endogenous dopamine in the striatum, thus minimizing their ability to elicit Parkinsonism (further details are given in Seeman, 1995; Seeman and Van Tol, 1995; Seeman *et al.*, 1996a,b).

Table 1 Real dissociation constants (nM) of antipsychotic drugs

Drug	D_2[a,b]	D_4[a,c]	5-HT_{2A}[d]
Chlorpromazine	0.66 ± 0.05	1.15 ± 0.04	1.8 ± 0.2
Clozapine	44 ± 8	1.6 ± 0.4	3.5 ± 0.4
Fluphenazine	0.32 ± 0.03	50 ± 10	3.2 ± 0.5
Haloperidol	0.35 ± 0.05	0.84 ± 0.5	46 ± 10
Iloperidone	3.5 ± 0.4	2.5 ± 0.3	0.17 ± 0.03
Isoclozapine	6 ± 0.06	5.8 ± 0.08	1.8 ± 0.2
Isoloxapine	6 ± 1	4.5 ± 1	3.7 ± 0.5
Loxapine	5.2 ± 0.03	7.8 ± 1.5	1.8 ± 0.3
Melperone	88 ± 30	410 ± 70	150
Molindone	6 ± 3	2400	5800
Olanzapine	3.0 ± 0.2[a]	1.6 ± 0.2[a]	2.3 ± 0.5
Perlapine	60 ± 10	30 ± 10	13
Risperidone	0.3 ± 0.1	0.25 ± 0.1	0.21 ± 0.05
Ritanserin	10 ± 4	30 ± 5	0.54 ± 0.1
Seroquel	78 ± 28	3000	74
Sertindole	0.95 ± 0.4	0.85 ± 0.2	0.3 ± 0.03
Thioridazine	0.4 ± 0.12	1.5 ± 0.5	1.1 ± 0.2
Trifluperazine	0.96 ± 0.2	44 ± 6	7.4 ± 1.5

[a]Updated from Seeman *et al.* (1996a,b).
[b]Using human cloned dopamine D_{2long} receptors (in COS 7 cells) or D_{2short} receptors (in GH4C1 cells), both of which gave identical values.
[c]Using human cloned dopamine $D_{4.2}$, $D_{4.4}$ or $D_{4.7}$ receptors, all of which gave identical values.
[d]Unpublished data, using human cloned serotonin 5-HT_{2A} receptors (Allelix Biopharmaceuticals Inc.).

The second group of atypical neuroleptics are those which bind more tightly to the dopamine D_2 receptor with values of the order of 1 nM for their true dissociation constants. This includes sertindole, thioridazine, olanzapine and iloperidone. These compounds, however, have high affinity for both dopamine D_4 receptors and serotonin$_{2A}$ receptors.

Hence, if dopamine D_4 receptors or serotonin$_{2A}$ receptors alleviate the Parkinsonism or catalepsy arising from the blockade of D_2 receptors, there should be a relation between the catalepsy doses and the ratio of the dissociation constants

at D_2 and at either one of the other receptors. This is examined in Fig. 1.

The data in Fig. 1 illustrate that the catalepsy doses correlate with the neuroleptic selectivities for the serotonin$_{2A}$ receptor and also correlate with the neuroleptic selectivities for the dopamine D_4 receptor. (The term 'selectivity' is here used to indicate the neuroleptic dissociation constant at 5-HT$_{2A}$ or at D_4, relative to the dissociation constant at the D_2 receptor.) The correlation coefficient for the catalepsy–serotonin receptor relation is 0.48 [Fig. 1 (a)] which is not statistically significant at the $p<0.05$ level. The correlation coefficient for the catalepsy–D_4 receptor relation is 0.77 [Fig. 1 (b)] which is statistically significant at the $p<0.02$ level. The correlations suggest that the atypical action of these neuroleptics may reside in either the combined action on the D_2 and 5-HT$_{2A}$ receptors, or in the combined action on the D_2 and D_4 receptors, or in the combined action on all three receptors.

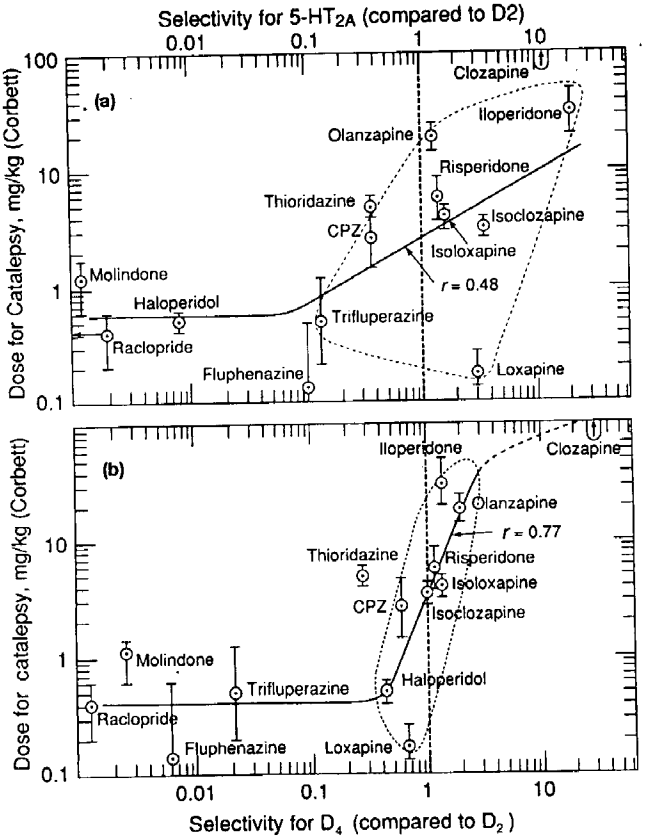

Figure 1 (a) The neuroleptic doses for eliciting catalepsy in 50% of the rats (Seeman *et al.*, 1996a) versus the ratio of the neuroleptic radioligand-independent dissociation constants for the dopamine D_2 receptor and the serotonin 5-HT$_{2A}$ receptors (ratios derived from Table 1). (b) Catalepsy doses versus the ratio of the neuroleptic radioligand-independent dissociation constants for the dopamine D_2 and D_4 receptors (ratios derived from Table 1). Thus, the horizontal axis indicates the neuroleptic selectivity for the dopamine D_4 receptor relative to that for the dopamine D_2 receptor. Neuroleptics having low affinity for the dopamine D_2 receptors (remoxipride, perlapine, seroquel and melperone), which have radioligand-independent dissociation constants exceeding 30 nM, are omitted in both (a) and (b), based on the concept that these loosely bound neuroleptics are atypical by virtue of being readily displaced by endogeneous dopamine. The inclusion of these four neuroleptics with very low affinity for D_2 would vitiate the above correlations. The correlation coefficient is given for those drugs which are encircled by a dashed line and omitting thioridazine because of its uniquely potent anticholinergic action. The correlation coefficient of 0.77 (b) is statistically significant at the $p<0.02$ level.

In order to resolve the question of whether the 5-HT$_{2A}$ receptor or the dopamine D_4 receptor contributes more effectively to the low level of Parkinsonism, it will require new neuroleptics with higher selectivities for either the 5-HT$_{2A}$ receptor or the dopamine D_4 receptor.

A further difficulty with the serotonin$_{2A}$ hypothesis is that isoclozapine is selective for the serotonin$_{2A}$ receptor, yet elicits marked catalepsy. In addition, loxapine is also selective for the serotonin$_{2A}$ receptor, yet causes intense catalepsy in rats and a significant amount of Parkinsonism in patients.

As indicated in Table 1, moreover, ritanserin has a 10 nM dissociation constant for the D_2 receptor. Thus, depending on the clinical dose used, one would expect ritanserin to occupy D_2 receptors (with low affinity) and to allay psychotic symptoms. At low doses, however, ritanserin would be selective for serotonin$_{2A}$ receptors, occupying about one dopamine D_2 receptor for every 10 serotonin$_{2A}$ receptors occupied. Nevertheless, the value of 10 nM for ritanserin at the D_2 receptor may account for the modest antipsychotic action of ritanserin (Meibach, 1989; Duinkerke *et al.*, 1993; Heck *et al.*, 1994; Weisel *et al.*, 1994). Although Weisel *et al.* found that the doses of 10–30 mg of ritanserin did not affect the binding of [^{11}C]raclopride to the D_2 receptors in three patients, ritanserin binds with low affinity (K of 10 nM) and would be expected to have a low occupancy of the D_2 receptors, similar to the situation with clozapine (Karbe *et al.*, 1991; Farde *et al.*, 1992; Louwerens *et al.*, 1993; Conley *et al.*, 1996; Nordström *et al.*, 1996). Moreover, it has also been found that ritanserin, in contrast to clozapine, does not mitigate against the extrapyramidal effects of haloperidol, but rather elicits a D_2-like dystonia in haloperidol-sensitized primates (Casey, 1991). Hence, it is likely that doses of ritanserin higher than 30 mg/day would be significantly antipsychotic via D_2 blockade.

An alleviation of antipsychotic-induced catalepsy is produced by 8-hydroxy-dipropylaminotetralin, a serotonin 5-HT$_{1A}$ agonist (Invernizzi *et al.*, 1988; Broekkamp *et al.*, 1988; Hicks, 1990; Wadenberg and Ahlenius, 1991; Wadenberg, 1992; Casey, 1992; Neal-Beliveau *et al.*, 1993). Indeed, it has recently been found that clozapine does act as a partial agonist at the human cloned serotonin 5-HT$_{1A}$receptor (Newman-Tancredi *et al.*, 1996). This may be the basis for the anticataleptic or anti-Parkinsonian action of clozapine. However, much research remains to be done to confirm that this particular mechanism is shared by other neuroleptics which cause low levels of Parkinsonism.

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References

Broekkamp C L E, Oosterloo S K, Berendsen H H G, van Delft A M L (1988) Effect of metergoline, fenfluramine, and 8-OHDPAT on catalepsy induced by haloperidol or morphine. Naunyn Schmiedeberg's Arch Pharmac *338*: 191–195

Busatto G F, Kerwin R W (1997) Perspectives on the role of serotonergic mechanisms in the pharmacology of schizophrenia. J Psychopharmacol *11*: 5–14

Casey D E (1991) The effect of a serotonin S2 antagonist, ritanserine, and an anticholineric benztropine on haloperidol-induced dystonia in nonhuman primates. 30th Meeting, American College of Neuropsychopharmacology (Abstracts) p. 127

Casey D E (1992) The effect of 8-OH-DPAT on haloperidol-induced dystonia in nonhuman primates. Am Coll Neuro-psychopharmac *32*: 109 (Abstract)

Conley R, Zhao M, Wong D, Tamminga C (1996) [^{11}C]NMSP receptor occupancy by clozapine and haloperidol in schizophrenic subjects. Biol Psychiat *39*: 513

Duinkerke S J, Botter P A, Jansen A A I, Van Dongen P A M, Van Haaften A J, Boom A J, Van Laarhoven J H M, Busard H L S M (1993) Ritanserin, a selective 5-$HT_{2/1C}$ antagonist, and negative symptoms in schizophrenia. Br J Psychiat *163*: 451–455

Farde L, Nordström A-L, Wiesel F-A, Paul S, Halldin C, Sedvall G (1992) Positron emission tomographic analysis of central D_1 and D_2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Archs Gen Psychiat *49*: 538–544

Heck A H, Post P, Daubenton F, Vahlne J-O, Olbrich R (1994) Ritanserin, a selective serotonin antagonist as add-on treatment in schizophrenia. Neuropsychopharmacology *10 (Suppl. 3)*: 207S

Hicks P B (1990) The effect of serotonergic agents on haloperidol-induced catalepsy. Life Sci *47*: 1609–1615

Invernizzi R W, Cervo L, Samanin R (1988) 8-Hydroxy-2-(di-*N*-propylamino)tetralin, a selective serotonin$_{1A}$ receptor agonist, blocks haloperidol-induced catalepsy by an action on raphe nuclei medianus and dorsalis. Neuropharmacology *27*: 515–518

Karbe H, Wienhard K, Hamacher K, Huber M, Herholz K, Coenen H H, Stöcklin G, Lövenich A, Heiss W D (1991) Positron emission tomography with (^{18}F)methylspiperone demonstrates D_2 dopamine receptor binding differences of clozapine and haloperidol. J Neural Transm *86*: 163–173

Louwerens J W, Buddingh J A, Zijlstra S, Pruim J, Korf J, Paans A M J, Vaalburg W, Slooff CJ (1993) Dopamine (D_2)-receptor occupancy in clozapine-treated patients as measured by positron emission tomography using ^{18}FESP. In Brunello N, Mendlewicz J, Racagni G (eds), New generation of antipsychotic drugs: novel mechanisms of action, Vol. 4. Karger, Basel, pp. 130–135

Meibach R C (1989) The role of 5-HT_2 receptor antagonism in the treatment of schizophrenia. 28th Meeting American College of Neuropsychopharmacology (Abstracts) p. 12

Neal-Beliveau B S, Joyce J N, Lucki I (1993) Serotonergic involvement in haloperidol-induced catalepsy. J Pharmac Exp Therap *265*: 207–217

Newman-Tancredi A, Chaput C, Verriele L, Millan M J (1996) Clozapine is a partial agonist at cloned, human serotonin 5-HT_{1A} receptors. Neuropharmacology *35*: 119–121

Nordström A-L, Farde L, Nyberg S, Karlsson P, Halldin C, Sedvall G (1996) D_1, D_2, and 5-HT_2 receptor occupancy in relation to clozapine serum concentration: A PET study of schizophrenic patients. Am J Psychiat *152*: 1444–1449

Seeman P (1995) Therapeutic receptor-blocking concentrations of neuroleptics. Int Clin Psychopharmac *10 (Suppl. 3)*: 5–13

Seeman P (1996) Clozapine withdrawal: serotonergic or dopaminergic mechanisms? Archs Gen Psychiat, in press

Seeman P, Van Tol H H M (1995) Deriving the therapeutic concentrations for clozapine and haloperidol: The apparent dissociation constant of a neuroleptic at the dopamine D_2 or D_4 receptor varies with the affinity of the competing radioligand. Eur J Pharmac *291*: 59–66

Seeman P, Corbett R, Van Tol H H M (1996a) Dopamine and serotonin receptors: Amino acid sequences, and clinical role in neuroleptic Parkinsonism. Jap J Pharmacol *71*: 187–204

Seeman P, Corbett R, Nam D, Van Tol H H M (1996b) Atypical neuroleptics have low affinity for dopamine D_2 receptors or are selective for D_4. Neuropsychopharmacology, in press

Wadenberg M-L (1992) Antagonism by 8-OH-DPAT, but not ritanserin, of catalepsy induced by SCH 23390 in the rat. J Neural Transm *89*: 49–59

Wadenberg M L, Ahlenius S (1991) Antipsychotic-like profile of combined treatment with raclopride and 8-OH-DPAT in the rat: enhancement of antipsychotic-like effects without catalepsy. J Neural Transm *83*: 43–53

Weisel F-A, Nordström A-L, Farde L, Eriksson B (1994) An open clinical and biochemical study of ritanserin in acute patients with schizophrenia. Psychopharmacology *114*: 31–38