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Chronic, but not acute, dosing of antipsychotic drugs alters neurotensin binding in rat brain regions

[1]P.E. Holtom, [2]P.L. Needham, *,[1]G.W. Bennett & [2]S. Aspley

[1]School of Biomedical Sciences, Queens Medical Centre, Nottingham, NG7 2UH and [2]Knoll Limited Research and Development, Nottingham, NG1 1GF

1 The present study compared high affinity neurotensin (NT) binding in rat brain following acute or chronic treatment with the classical antipsychotic, haloperidol, and the newer antipsychotic drugs, clozapine and zotepine.

2 Drugs were given orally, as an acute treatment (1 dose) or chronically (21 day dosing) and binding to the NT high affinity receptor was examined in three brain regions; striatum, nucleus accumbens/olfactory tubercle and frontal cortex.

3 Acute dosing with either vehicle, haloperidol, clozapine or zotepine produced no significant changes in NT binding from controls (naïve rats).

4 Chronic (21 day) dosing resulted in an increase in the K_D and B_{max} of high affinity receptors in the striatum following haloperidol, but not clozapine, zotepine or vehicles. In contrast, the newer antipsychotics, clozapine and zotepine but not haloperidol or vehicles, significantly altered NT binding in the nucleus accumbens/olfactory tubercle by decreasing the K_D and B_{max}.

5 Further differentiation between the two newer antipsychotic drugs occurred in the frontal cortex. Clozapine had no significant effect on NT binding, whereas zotepine significantly reduced the K_D of the high affinity receptor with no alteration in B_{max}.

6 The antipsychotic drugs tested did not interact directly with the NT high affinity receptor. Therefore, they must be acting indirectly *via* an alternative receptor mechanism to alter NT high affinity binding. In accordance with previously reported NT/dopamine receptor interactions, this would suggest cross-talk between these systems.

7 Overall, these data demonstrate that chronic, but not acute, administration of antipsychotic drugs alters NT binding in the rat brain. In addition, anatomical differences in NT binding arise according to the antipsychotic drug under test. This may be predictive of drug side-effect profile, antipsychotic efficacy or atypicality.

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Abbreviations: NT, neurotensin; NT-LI, neurotensin-like immunoreactivity; NTR-1, neurotensin receptor 1; NTR-2, neurotensin receptor 2

Introduction

The tridecapeptide neurotensin (NT) was first isolated from bovine hypothalamus (Carraway & Leeman, 1973) and has since been located heterogeneously throughout the central nervous system (Emson *et al*. 1982). In the mid-1980s, it was demonstrated that NT had a neurotransmitter/neuromodulatory role in the CNS (Iversen *et al*., 1980) and evidence suggested that NT was colocalized with dopamine particularly in limbic and cortical dopaminergic structures (Hökfelt *et al*., 1984). Furthermore, when NT was injected intracerebroventricularly (i.c.v.) it was found to cause behaviours in rodents similar to clinically prescribed antipsychotic drugs, such as hypothermia and decreased basal locomotor activity (Nemeroff, 1980). In addition, NT injected directly into the nucleus accumbens attenuated hyperlocomotion usually seen in response to amphetamine (Ervin *et al*., 1981), which is a standard test for assessing the potential antipsychotic efficacy of a compound (Ljungberg & Ungerstedt, 1985).

Currently available antipsychotic medications can be subdivided into two main categories: conventional drugs such as haloperidol, which treat the positive symptoms of schizophrenia quite adequately, but with a relatively high incidence of extrapyramidal side-effects (EPS), and newer antipsychotic drugs such as clozapine and zotepine, which have proven efficacy against both positive and negative symptoms of schizophrenia and induce minimal or no EPS (Kane *et al*., 1988; Petit *et al*., 1996). Furthermore, the newer antipsychotic drugs, clozapine and zotepine, have also been shown to improve cognition in schizophrenic patients (Meyer-Lindenberg *et al*., 1997).

Interest in the similarities between NT and antipsychotic drug action in animals has led to investigations that link NT systems to the administration of antipsychotic drugs in the clinic. It was observed that a subset of schizophrenic patients prior to drug treatment had significantly lower levels of NT-like immunoreactivity (NT-LI) in their cerebrospinal fluid (CSF) compared to normals or drug-naïve schizophrenic patients (Garver *et al*., 1991; Sharma *et al*., 1997). When this subset was given antipsychotic treatment, the level of NT-LI in the CSF rose to a plateau, the time course of which was coincident with the onset of therapeutic efficacy of the drug used (Haloperidol; 2–4 weeks).

The present study aimed to assess whether these previously reported temporal changes in CSF NT-LI in patients are reflected in rats at the receptor level by determining the effects

*Author for correspondence at: School of Biomedical Sciences (E floor), Queens Medical Centre, Nottingham, NG7 2UH

of acute and chronic dosing with a range of antipsychotics on NT binding. Neurotensin has two receptors in the adult brain (Kitabgi *et al*., 1987), one which has a low affinity for NT, termed neurotensin receptor-2 NTR-2 (Chalon *et al*., 1996), which has been shown to be involved in the formation of ligand-induced glial cell-surface receptor clusters that do not undergo subsequent internalization, the function of which has yet to be determined (Nouel *et al*., 1997). The second receptor has a high affinity for NT and is called neurotensin receptor-1 (NTR-1; Tanaka *et al*., 1990) and is located presynaptically in the striatum and postsynaptically in the nucleus accumbens (Schotte *et al*., 1988). NTR-1 is generally regarded as the receptor that modulates the action of NT in the brain (Kitabgi *et al*., 1987) and the present study has looked at this site in terms of the effect of antipsychotic drugs on NT binding.

Methods

Male CD rats (150–250 g; Charles River) housed in pairs on a 12 : 12 h light dark cycle (lights on 07.00 h with chow and water *ad libitum*) received either acute (1 dose) or chronic (21 day) oral treatment with antipsychotics at doses corresponding to 2 or $10 \times ED_{50}$ to antagonize amphetamine-induced hyperlocomotion in the rat (Haloperidol 0.246 and 1.23 mg kg^{-1}; clozapine 7.72 and 38.6 mg kg^{-1}; zotepine 1.02 and 5.1 mg kg^{-1}, respectively; data on file, Knoll Pharmaceuticals, Nottingham). Separate animals were dosed orally with the corresponding vehicles (haloperidol = vehicle 1; 5% [w v^{-1}] lactic acid in 5% [w v^{-1}] glucose, or clozapine and zotepine = vehicle 2; 1% [w v^{-1}] tartaric acid) or were naïve to drug treatment. Four hours after the last dose, the animals were killed and the striatum, nucleus accumbens/olfactory tubercle (combined) and frontal cortex were removed (Heffner *et al*., 1980), snap frozen in liquid nitrogen and stored at −80°C until use in the binding assay. Tissue was homogenized in 40 vols ice-cold Tris buffer (50 mM; pH 7.4), then centrifuged ($22{,}619 \times g$, 4°C, 3×10 min). The resultant pellet was resuspended to a concentration of 12.5 mg ml^{-1} (w v^{-1}) in buffer (50 mM Tris, 0.05% BSA, 0.03% bacitracin, 0.1 mM EDTA, pH 7.4) and a 12-point saturation analysis (final volume 500 μl) was performed using [^{3}H]-NT (0.04–20 nM) in the presence of 1 μM levocabastine to define the high affinity NT receptor (Kitabgi *et al*., 1987). Non-specific binding was defined by 10 μM cold NT. A 40 min incubation at 37°C was followed by filtration on Skatron pre-soaked filters (0.3% [v v^{-1}] polyethyleneamine; Bruns *et al*., 1983) and liquid scintillation analysis of bound [^{3}H]-NT. Six to seven saturation binding experiments were performed for all groups.

For the analysis of direct effects of drugs or vehicles on NT receptors tissues were prepared as above from drug naïve animals. Control groups had a sub-maximal radioligand concentration added (10 nM) in the presence of levocabastine (1 μM) and buffer to define the high affinity receptor or cold NT to define non-specific binding. The test groups had either vehicle 1 or 2, or haloperidol, clozapine or zotepine (1, 0.1 and 0.001 μM) in the buffer to act as possible displacing agents. The highest concentration tested was estimated to exceed any possible residual drug concentration in the membrane preparation, thereby excluding the possibility of direct residual drug effects. Incubation time and filtration techniques were as above. Six displacement experiments were performed for each drug or vehicle.

Statistical analysis

All binding was analysed using the EBDA/LIGAND programme (Macpherson, 1987). All saturation binding data was analysed using a one-way ANOVA followed by Tukey's *post-hoc* test. Displacement binding data was analysed using a paired *t*-test.

Chemicals used

Neurotensin, clozapine and haloperidol were obtained from Research Biochemicals International. Levocabastine was a kind gift from Janssen Pharma. Other chemicals were obtained from Sigma Ltd.

Results

Acute dosing with either the low or high doses of haloperidol, clozapine, zotepine or respective vehicles (vehicle 1 for haloperidol, vehicle 2 for clozapine and zotepine) produced no significant differences in NT receptor binding, either in receptor number or affinity, when compared to age and weight matched control drug naïve rats (Table 1).

In contrast, chronic dosing with haloperidol, clozapine or zotepine significantly altered NT binding in comparison to their respective vehicle and this varied according to drug used and tissue area (Figures 2–4). Vehicle 1 and vehicle 2 did not significantly alter NT binding from control (drug naïve) animals. The classical antipsychotic, haloperidol, significantly increased both the K_D and B_{max} ($P<0.05$ and $P<0.01$ at both doses, respectively) of the NT receptor at both the low and high dose in the striatum (Figure 2A,B) when compared to its own vehicle control, but had no significant effects in either the nucleus accumbens/olfactory tubercle (Figure 3A,B) or frontal cortex (Figure 4A,B). The changes in both K_D and B_{max} in the striatum following haloperidol treatment were not due to poor definition of the saturation binding plateau as can be seen in a

Table 1 Effects of acute antipsychotic drug treatment on high affinity NT receptor binding

	Striatum				*Nucleus accumbens*				*Frontal cortex*			
	Lower dose		*Higher dose*		*Lower dose*		*Higher dose*		*Lower dose*		*Higher dose*	
	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}
C	1.9±0.17	7.3±0.95	2.0±0.13	7.9±0.79	1.5±0.22	5.9±0.61	1.5±0.21	5.8±0.41	1.4±0.11	3.8±0.31	1.4±0.13	3.7±0.3
V1	2.0±0.12	8.7±0.65	2.1±0.21	8.2±0.64	1.4±0.17	5.6±0.51	1.4±0.17	5.1±0.62	1.5±0.09	3.3±0.09	1.5±0.19	3.5±0.09
V2	1.8±0.21	7.9±0.81	1.9±0.22	8.0±0.91	1.6±0.24	5.9±0.75	1.6±0.19	5.2±0.39	1.4±0.15	3.6±0.23	1.5±0.15	3.6±0.21
HAL	2.2±0.19	9.3±0.74	2.0±0.12	8.6±0.81	1.5±0.07	5.1±0.38	1.5±0.23	5.4±0.54	1.4±0.13	3.5±0.21	1.4±0.14	3.7±0.15
CZ	1.6±0.23	8.3±0.77	1.8±0.28	8.0±0.67	1.5±0.17	5.6±0.75	1.5±0.09	5.6±0.63	1.6±0.0.9	3.2±0.13	1.5±0.08	3.2±0.33
ZOT	2.0±0.22	9.3±0.80	2.0±0.13	8.1±0.92	1.4±0.16	5.3±0.63	1.3±0.24	5.5±0.72	1.4±0.12	3.5±0.12	1.4±0.12	3.6±0.10

Values are mean±s.e.mean; K_D in nM, B_{max} in fmol mg^{-1} protein, $n = 6–7$. C = control (no treatment); HAL = haloperidol (0.246 or 1.23 mg kg^{-1}); CZ = clozapine (7.72 or 38.6 mg kg^{-1}); ZOT = zotepine (1.02 or 5.1 mg kg^{-1}); V1 = vehicle 1 for HAL (5% [w v^{-1}] lactic acid in 5% [w v^{-1}] glucose); V2 = vehicle 2 for CZ and ZOT (1% [w v^{-1}] tartaric acid).

representitive curve in Figure 1. In contrast to the effects of haloperidol, the newer antipsychotics clozapine or zotepine had no effect on NT binding in the striatum (Figure 2A,B), but significantly decreased the K_D value of the NT receptor at both the low and high doses in the nucleus accumbens/olfactory tubercle, (clozapine, $P<0.05$ and $P<0.01$ at each dose, respectively; zotepine, $P<0.01$ at both doses; Figure 3A,B), and significantly decreased the B_{max} value at the higher dose of antipsychotic ($P<0.01$ and $P<0.05$, for clozapine and zotepine, respectively, Figure 3B) when compared to the vehicle control. However, in the frontal cortex, haloperidol and clozapine had no significant effect on NT binding (Figure 4A,B), whereas, zotepine significantly decreased the K_D value at both the high and low dose ($P<0.01$ for both, Figure 4A, B), but did not significantly alter the B_{max} (Figure 4A,B).

Administration of either vehicle 1, vehicle 2 or haloperidol, clozapine or zotepine (1, 0.1 and 0.001 μM) to tissue in the presence of 10 nM [^{3}H]-NT and 1 μM levocabastine did not cause significant displacement of bound radioligand when compared to control binding in all tissue regions (Table 2).

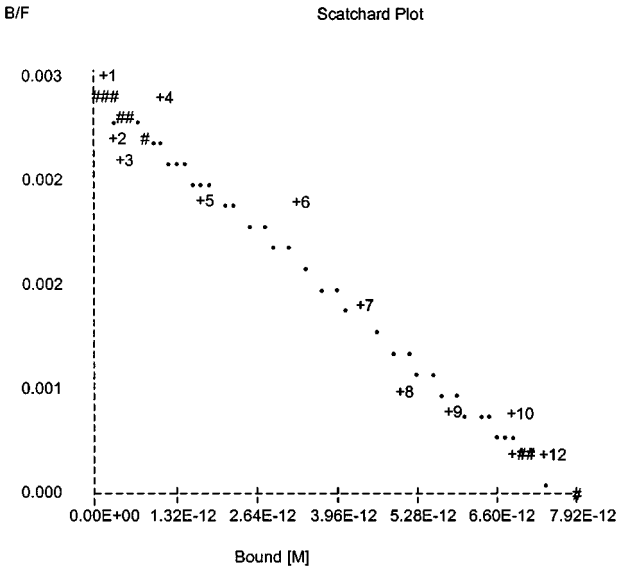

Figure 1 Scatchard plot depicting the effect of chronic high dose haloperidol (1.23 mg kg^{-1}, p.o.) on neurotensin binding. $K_D = 2.91 \pm 0.15$ nM and $B_{max} = 7.21 \pm 1.04$ fmol mg^{-1} protein.

Discussion

Following chronic antipsychotic drug treatment, a subset of schizophrenic patients with previously low levels of CSF NT-LI display an increase in CSF NT-LI coincident with the onset of therapeutic efficacy of the treatment (Widerlov *et al*., 1982; Lindström *et al*., 1988; Garver *et al*., 1991; Breslin *et al*., 1994; Sharma *et al*., 1997). In the present study, similar delayed actions, this time on high affinity NT receptor levels were seen in rodents treated with antipsychotic drugs. The effects of clinically relevant acute and chronic oral doses of a range of antipsychotic drugs on NT receptor binding were examined in three brain regions.

The present results showing no effect in response to acute administration of antipsychotic drugs, are consistent with the CSF NT-LI findings and together these data might help to explain why antipsychotic treatments do not reach their therapeutic potential for a number of weeks. The present binding data in response to chronic treatment with antipsychotic drugs also demonstrates fundamental differences in NT binding between the typical and newer antipsychotic drugs, both anatomically and functionally. Haloperidol, the classical antipsychotic drug used in this study, had an exclusive action on NT receptors in non-limbic structures (lowered the affinity and increased receptor number). Conversely, the newer antipsychotic drugs used, clozapine and zotepine, had no effect

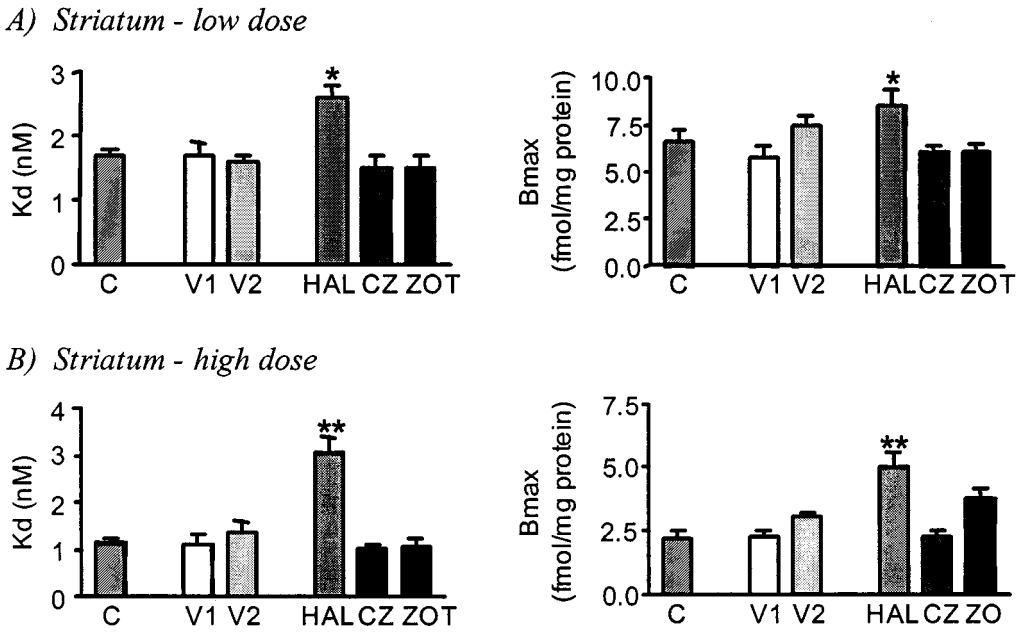

Figure 2 Effect of chronic (21 day) (A) low dose haloperidol (HAL; 0.246 mg kg^{-1}, p.o.), clozapine (CZ; 7.72 mg kg^{-1}, p.o.) and zotepine (ZOT; 1.02 mg kg^{-1}, p.o.), and (B) high dose HAL (1.23 mg kg^{-1}, p.o.), CZ (38.6 mg kg^{-1}, p.o.) and ZOT (5.1 mg kg^{-1}, p.o.) on NT binding in the striatum compared to vehicles (V1 = vehicle 1 for haloperidol (5% [w v^{-1}] lactic acid in 5% [w v^{-1}] glucose); V2 = vehicle 2 for clozapine and zotepine (1% [w v^{-1}] tartaric acid)) and drug naïve animals (C). Data are K_D (nM) and B_{max} (fmol mg^{-1} protein), mean $\pm$ s.e.mean, $n = 6-7$ for all groups. *$P<0.05$, **$P<0.01$ vs relevant vehicle, ANOVA followed by Tukey's *post-hoc* test.

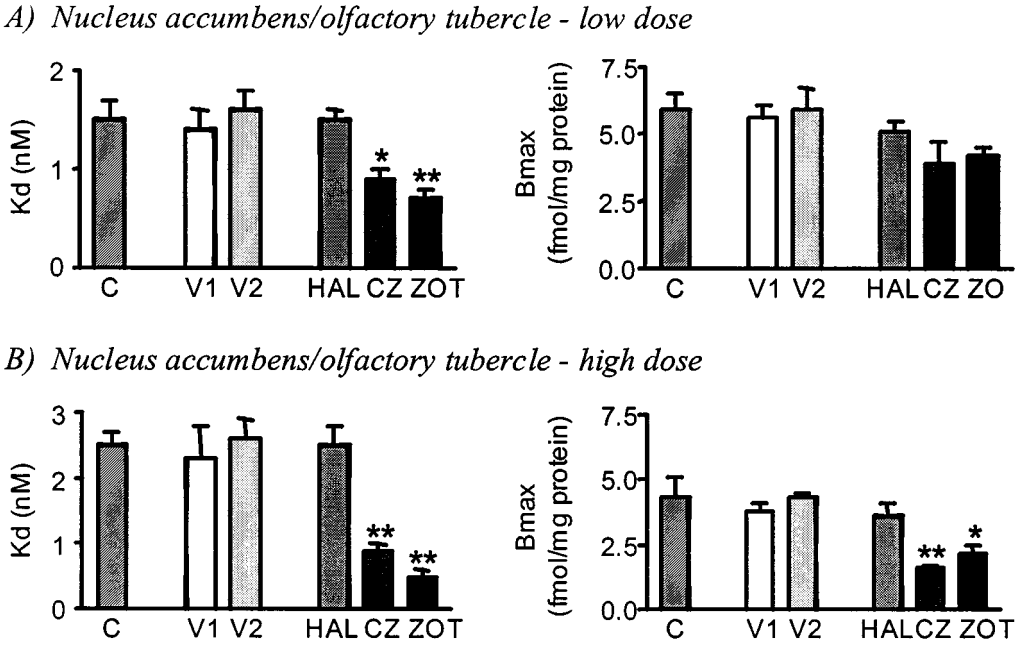

Figure 3 Effect of chronic (21 day) (A) low dose haloperidol (HAL; 0.246 mg kg^{-1}, p.o.), clozapine (CZ; 7.72 mg kg^{-1}, p.o.) and zotepine (ZOT; 1.02 mg kg^{-1}, p.o.), and (B) high dose HAL (1.23 mg kg^{-1}, p.o.), CZ (38.6 mg kg^{-1}, p.o.) and ZOT (5.1 mg kg^{-1}, p.o.) on NT binding in the nucleus accumbens/olfactory tubercle compared to vehicles (V1 = vehicle 1 for haloperidol (5% [w v^{-1}] lactic acid in 5% [w v^{-1}] glucose); V2 = vehicle 2 for clozapine and zotepine (1% [w v^{-1}] tartaric acid)) and drug naïve animals (C). Data are K_D (nM) and B_{max} (fmol mg^{-1} protein), mean ± s.e.mean, $n = 6-7$ for all groups. $^*P<0.05$, $^{**}P<0.01$ vs relevant vehicle, ANOVA followed by Tukey's *post-hoc* test.

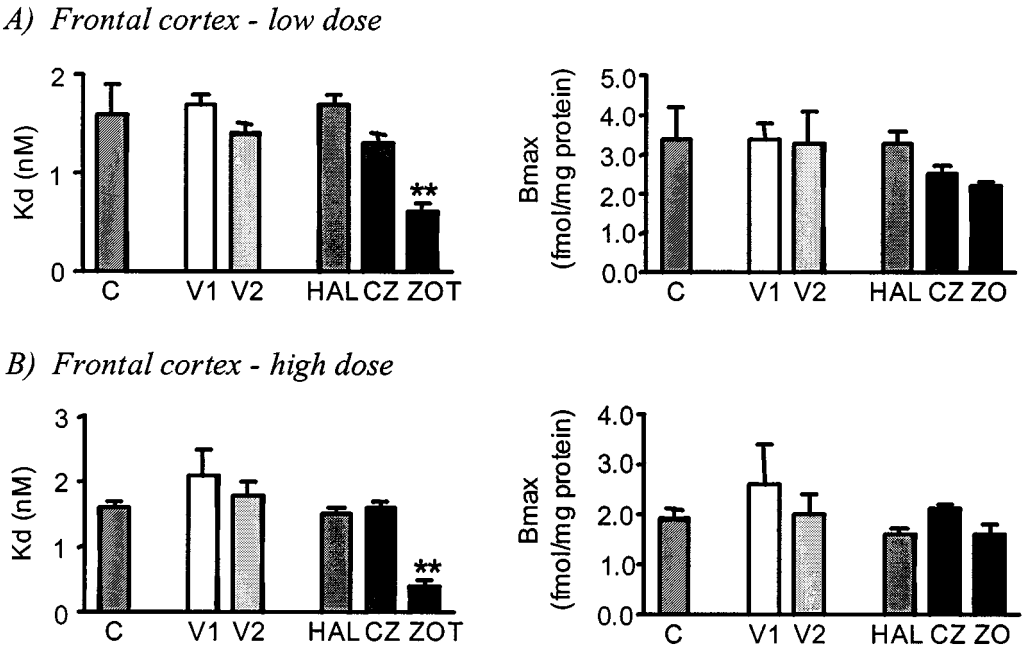

Figure 4 Effect of chronic (21 day) (A) low dose haloperidol (HAL; 0.246 mg kg^{-1}, p.o.), clozapine (CZ; 7.72 mg kg^{-1}, p.o.) and zotepine (ZOT; 1.02 mg kg^{-1}, p.o.), and (B) high dose HAL (1.23 mg kg^{-1}, p.o.), CZ (38.6 mg kg^{-1}, p.o.) and ZOT (5.1 mg kg^{-1}, p.o.) on NT binding in the frontal cortex compared to vehicles (V1 = vehicle 1 for haloperidol (5% [w v^{-1}] lactic acid in 5% [w v^{-1}] glucose); V2 = vehicle 2 for clozapine and zotepine (1% [w v^{-1}] tartaric acid)) and drug naïve animals (C). Data are K_D (nM) and B_{max} (fmol mg^{-1} protein), mean ± s.e.mean, $n = 6-7$ for all groups. $^*P<0.05$ vs relevant vehicle, ANOVA followed by Tukey's *post-hoc* test.

in the striatum, but significantly altered NT receptor function in sub-cortical limbic structures (i.e. nucleus accumbens/olfactory tubercle; increased receptor affinity and decreased receptor number). Moreover, there was a differentiation between the effects of the newer antipsychotic drugs, since only zotepine significantly altered NT receptor function in the frontal cortex (increased receptor affinity alone).

It is generally accepted that there are both clinical and pharmacological differences between conventional neuroleptic drugs such as haloperidol and the newer antipsychotic drugs which have an atypical profile, such as clozapine and zotepine. Assuming that the changes in NT receptor binding parameters observed in the present rat study after chronic treatment occur in human subjects, these effects may contribute to the clinical

Table 2 Displacement of high affinity NT receptor binding by antipsychotic drugs *in vitro*

Treatment	*Striatum*	*Nucleus accumbens*	*Frontal cortex*
V1	100.12±6.56	100.05±4.12	101.03±4.03
V2	102.36±4.29	100.04±5.99	100.00±2.19
Clozapine (1 μM)	99.50±5.24	97.59±7.01	99.26±9.34
Clozapine (0.1 μM)	100.95±5.43	105.09±7.99	100.00±4.27
Clozapine (0.001 μM)	105.04±7.52	103.44±4.34	99.48±2.99
Haloperidol (1 μM)	98.23±2.92	99.88±2.18	97.60±2.30
Haloperidol (0.1 μM)	100.30±4.91	98.05±5.67	98.98±3.83
Haloperidol (0.001 μM)	99.26±4.04	105.56±4.11	97.67±2.43
Zotepine (1 μM)	96.51±5.01	107.41±3.36	100.28±9.12
Zotepine (0.1 μM)	105.04±8.94	99.76±6.09	98.99±2.15
Zotepine (0.001 μM)	102.79±4.14	100.52±4.50	98.71±4.18

Displacement is a percentage of control total binding at 10 nM [^{3}H]-NT. Values are per cent mean±s.e.mean, $n=6$ for each group. V1 = vehicle 1 for haloperidol (5% [w v^{-1}] lactic acid in 5% [w v^{-1}] glucose); V2 = vehicle 2 for clozapine and zotepine (1% [w v^{-1}] tartaric acid).

observations. The incidence in patients of extrapyramidal symptoms (EPS) in which antipsychotic drugs act on caudate dopamine systems is well documented (Casey & Keepers, 1988; Keepers *et al*., 1983; Adler *et al*., 1989) and one advantage of the newer antipsychotic drugs is that they have a much lower comparative incidence of EPS than drugs like haloperidol (Petit *et al*., 1996; Kane *et al*., 1988). The finding that the action of haloperidol on NT receptor binding is restricted to the striatum raises the possibility that the manifestation of EPS is related in part to changes in NT systems in this brain region.

There is also clinical evidence that newer antipsychotic drugs improve the cognitive performance of psychotic patients (Meyer-Lindenberg *et al*., 1997), an aspect of schizophrenic symptomology that is not controlled by haloperidol (Gallhofer *et al*., 1996) and these actions are likely to be mediated by neurotransmitters in cortical and subcortical limbic structures (Riddle & Roberts, 1978). In support of this, recent studies have shown that clozapine and zotepine both alter dopamine efflux as measured by intracerebral microdialysis in cortical brain regions, an action that is not shared by the conventional drug, haloperidol (Volonte *et al*., 1997; Rowley *et al*., 1998a; 2000). Furthermore, we have evidence pre-clinically to suggest that the new antipsychotic, zotepine, has potential antidepressant activity (Needham *et al*., 1997), an action that may be related to the fact that of the drugs tested it was only zotepine that altered NT receptor binding in the cortex.

Interest in the mechanism of action of antipsychotic drugs has lead to significant research into the binding properties of leading therapeutic agents. It is widely acknowledged that most therapeutically active antipsychotic drugs target and block dopamine neurotransmission *via* D_2-like receptors (Seeman, 1987). Therefore, it would be useful to consider interactions between NT and dopamine as being possible underlying reasons for the changes in NT binding seen in the present study.

It has been shown that dopamine agonists and antagonists differentially alter NT tissue levels. Moreover, it has recently been demonstrated that stimulation of different dopamine receptors, eg D_1 vs D_2 causes opposite effects, increasing or decreasing respectively, striatal NT tissue levels (Hanson & Keefe, 1999). Administration of D_2-like antagonists, which may include neuroleptic agents such as haloperidol, causes increases in striatal (Goedert *et al*., 1985; Merchant *et al*., 1989) and nucleus accumbens (Brun *et al*., 1995) NT concentrations. Furthermore, it has been demonstrated that there are distinct regional differences between haloperidol and clozapine administration on measurements of NT-like immunoreactivity (Hanson *et al*., 1997). *In vivo* studies have also demonstrated a reciprocal relationship whereby infusions of NT increase extracellular dopamine levels in the striatum (Chapman *et al*., 1992) and the nucleus accumbens (Blaha *et al*., 1990).

Evidence has emerged that different members of the D_2-like family have opposing roles on NT mRNA production, particularly in the nucleus accumbens (Diaz *et al*., 1994). Furthermore, both acute (Merchant, 1994) and chronic (Bolden-Watson *et al*., 1993; Merchant *et al*., 1994) administration of antipsychotic drugs has been shown to elevate NT mRNA levels. However, there are regional differences between the effects of typical (haloperidol) and atypical (clozapine) antipsychotic drugs on NT mRNA levels (Merchant *et al*., 1994; Bolden-Watson *et al*., 1993) similar to that seen when measuring NT tissue levels (Hanson *et al*., 1997). These differences may account for the regional specificity seen when measuring NT binding in the presence of chronic typical and atypical antipsychotic drugs.

It has been demonstrated in this study that there are significant changes in both K_D and B_{max} values of NT receptor binding particularly in striatal and subcortical limbic regions. This phenomenon has been observed in studies by other groups who suggest that these changes may be due to interactions between NT and dopaminergic receptor systems. Agnati *et al*. (1985), demonstrated that dopamine caused a change in NT receptor function (decreased affinity, increased receptor number) in subcortical limbic membranes, which can be compared to the present changes with the dopamine receptor antagonists, clozapine and zotepine (increased the affinity and decreased the receptor number of NT high affinity sites). Lesioning studies in the striatum with 6-hydroxy dopamine have demonstrated that there is a close link between dopamine and NT receptor function (Fuxe *et al*., 1986), whereby striatal lesions produced similar alterations in NT receptor function to administration of chronic haloperidol in the present study. Herve *et al*. (1986) have demonstrated previously that destruction of dopamine cell groups using 6-hydroxydopamine lesions results in decreased numbers of NT binding sites in the striatum and mesencephalon. Furthermore, when the long-acting neuroleptic, pipotiazine palmitic ester was used to produce chronic blockade of dopamine neurotransmission, there was a resulting increase in the density of postsynaptic NT binding sites in the straitum (Herve *et al*., 1986). This suggests that there is a close relationship between dopamine and NT systems in the rat brain.

In addition, there is evidence to suggest that there are also alterations in NT receptor binding after long-term antipsychotic treatment in humans (Uhl & Kuhar, 1984) and that this is

via an indirect method which is confirmed in the present study where antipsychotic drugs did not alter NT receptor binding directly (Table 2). This lack of direct effect of the drugs on NT binding also indicates that the results our or *ex vivo* binding studies cannot be accounted for by residual drug within the membranes preparations from treated animals.

Additional evidence of receptor/receptor interactions between dopamine and NT is seen by examining the effect of NT on dopamine receptor binding. *In vitro* NT indirectly reduces the affinity of dopamine D_1 and D_2 agonist binding, but not the receptor number in the striatum (Miyoshi *et al*., 1989; Tanganelli *et al*., 1993) and D_2 agonist and D_3 receptor binding in subcortical limbic regions (Agnati *et al*., 1983; Von Euler & Fuxe, 1987; Li *et al*., 1993; 1995; Liu *et al*., 1994). The modulatory action on D_2 agonist binding is most prevalent at relatively low concentrations of NT (3 nM) and disappears when the concentration of NT rises to $\sim$30 nM, which would suggest a feedback mechanism working between NT and dopamine receptors (Fuxe *et al*., 1992). This would mean that decreased NT receptor function in the striatum would cause an increase in dopamine receptor function in this area, while an increase in NT receptor function in the subcortical limbic regions would cause a decrease in dopamine receptor function in these regions.

The rich pharmacology displayed by antipsychotic agents would make it naïve to suggest that dopamine is the only neurotransmitter that has sites which are capable of modulating NT receptor function. Other neurotransmitter systems, such as noradrenaline (NA) are known to modulate NT receptor function in the frontal cortex (Trovero *et al*., 1991), and it has previously been demonstrated that zotepine causes a marked increase in extracellular NA levels *in vivo* when compared to clozapine (Rowley *et al*., 1998b). In addition, NT modulates other neurotransmitters in the frontal cortex such as 5-HT (Heaulme *et al*., 1998), and zotepine displays significant affinity at 5-HT binding sites (Needham *et al*., 1996). Therefore, this may be an alternative mechanism by which zotepine exerts a different action to clozapine in the frontal cortex.

In summary, the present results suggest that changes in NT binding contribute to the differences seen in the side-effect profiles of antipsychotic drugs. Additionally, the changes in NT binding demonstrated here coupled with the clinical findings of temporal changes in NT-LI (Sharma *et al*., 1997; Garver *et al*., 1991) also support the contention that antipsychotic efficacy is mediated in part by changes in the NT system.

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