ELSEVIER

Life Sciences 69 (2001) 1257–1268

Life Sciences

Extended treatment with typical and atypical antipsychotic drugs Differential effects on the densities of dopamine D_2-like and $GABA_A$ receptors in rat striatum

Brian Dean[a,b,*], Tabasum Hussain[a,b], Elizabeth Scarr[a], Geoffrey Pavey[a], David L. Copolov[a,b]

[a]*The Rebecca L. Cooper Research Laboratories, The Mental Health Research Institute, Parkville, Victoria 3052, Australia*

[b]*Department of Psychological Medicine, Monash Medical Centre, Clayton, Victoria 3168, Australia*

Received 16 October 2000; accepted 14 February 2001

Abstract

In situ radioligand binding and quantitative autoradiography have been used to measure the density of striatal D_1-like, D_2-like, and $GABA_A$ receptors in rats treated with haloperidol at 0.01 or 0.1 mg/kg/day or chlorpromazine, olanzapine or clozapine at 0.1 or 1.0 mg/kg/day for 1, 3 or 7 months. [^{3}H]SCH23390 binding to D_1-like receptors was not changed by any drug treatments. There were significant increases in [^{3}H]nemonapride binding to D_2-like receptors at different time points due to treatment with haloperidol, chlorpromazine and olanzapine. By contrast, treatment with clozapine and olanzapine caused a time-dependent decrease in [^{3}H]muscimol binding to the $GABA_A$ receptor. These data suggest that treatment with atypical antipsychotic drugs, but not typical antipsychotic drugs, affect striatal GABAergic neurons. In addition, it would appear that clozapine might be unique in that it does not increase dopamine-D_2 like receptor density at doses which would be predicted to have antipsychotic effects in humans. The extent to which such changes are involved in the therapeutic effects of drugs such as olanzapine and clozapine remains to be determined.

Keywords: Antipsychotic drugs; Dopamine receptors; $GABA_A$ receptors

Introduction

The need to define the mechanisms by which antipsychotic drugs produce their beneficial effects and unwanted side-effects has led to intensive study of changes in molecular architec-

* Corresponding author. The Rebecca L. Cooper Research Laboratories, The Mental Health Research Institute of Victoria, Locked Bag 11, Parkville, Victoria, 3052, Australia. Tel.: 613-9389-2940; fax: 613-9387-5061.

E-mail address: B.Dean@papyrus.mhri.edu.au (B. Dean)

0024-3205/01/$ – see front matter
PII: S0024-3205(01)01214-0

ture of the rat brain following antipsychotic drug treatment. Early studies showed that rats treated for up to one month with typical antipsychotic drugs, such as haloperidol, had increased striatal dopamine D_2-like (DA-D_2) receptors [1,2] . This finding led to the proposal that long-term treatment with antipsychotic drugs in humans could lead to an increase DA-D_2 receptors, causing receptor supersensitivity with this resulting in the onset of tardive dyskinesia (TD). To explore this hypothesis further, rats were treated longer with antipsychotic drugs which showed that striatal DA-D_2 receptors remained increased in rats treated for up to a year with antipsychotic drugs [3–6] but that treatment for long periods did not increase DA-D_2 receptors in mesolimbic areas of the rat brain [7]. Moreover, it was shown that the ability of typical antipsychotic drugs to increase striatal DA-D_2 receptors was dose-dependent [8] and that the increases in DA-D_2 receptor density caused by antipsychotic drugs were slow to revert to non-treated levels after the cessation of drug treatment [9]. In contrast to treatment with typical antipsychotic drugs, treating rats with the atypical antipsychotic drugs clozapine (for up to 4 weeks) [10–14] or thioridazine (for 2 weeks) [14] did not result in an increase in the levels of striatal DA-D_2 receptors. Longer studies have shown that treating rats for up to a year with clozapine [6,7] or sulpiride [7] did not increase levels of DA-D_2 receptors. From these studies it was hypothesised that atypical antipsychotic failed to cause TD in humans because they do not cause receptor supersensitivity [15].

By contrast to studies on the DA-D_2 receptor, findings on the effects of antipsychotic drug treatment on DA-D_1 receptors do not appear to be consistent. For example, treatment with haloperidol for 21 weeks has been reported to decrease the density of striatal DA-D_1 receptors [16] whereas treatment with haloperidol [17–19] or fluphenazine [18,19] for up to 10 months had no effect on DA-D_1 receptors. It has also been reported that treatment with clozapine for 1 month increases the density of striatal DA-D_1 receptors [12,20]. This finding contrasts with other reports that treatment with clozapine for 1 month [13] or 1 year [7] does not change DA-D_1 receptors. The inconsistencies in these studies mean it is unclear if changes in DA-D_1 receptors are likely to be important in the actions of antipsychotic drugs.

We have recently reported a significant decrease in the density of $GABA_A$ receptors in the rat hippocampus and temporal cortex following treatment with clozapine and olanzapine but not haloperidol or chlorpromazine [21]. Our data differ from those in the striatum where treatment with haloperidol for 1 [20] and 10 [19] months increased the density of $GABA_A$ receptors. The differences in these data could be due to drug or brain region dependent factors, both of which would be potentially important in understanding the effects of antipsychotic drugs. To resolve some of these issues it was necessary for us to measure $GABA_A$ receptors treated under the same regimen as those in which we demonstrated a decrease in $GABA_A$ receptors in the hippocampus.

Existing studies have shown that antipsychotic drug treatment produces complex and regionally different changes in DA-D_2, DA-D_1 and $GABA_A$ receptors in the rat brain. Most importantly, clozapine may be uniquely effecting some mechanism that results in an increase in DA-D_1 receptor and a decrease $GABA_A$ receptor density. Alternatively, there may be an important role for the GABAergic systems in the mechanism by which clozapine exerts its unique therapeutic effects. Olanzapine is a new antipsychotic drug that has a similar pharmacological profile to clozapine [22] and therefore should have similar effects to those

of clozapine. If that is the case then it could be strongly argued that the apparent effects of clozapine on the dopamine/GABA systems are a function of its pharmacology profile and not due to affects on some unidentified mechanism. To test this hypothesis we examined the effect of treating rats for up to 7 months with typical (haloperidol and chlorpromazine) and atypical (olanzapine and clozapine) antipsychotic drugs on striatal DA-D_2, DA-D_1 and $GABA_A$ receptors.

Methods

Materials

[^{3}H]SCH23390, [^{3}H]Microscales and Hyperfilm-^{3}H® were purchased from Amersham Australia Pty. Ltd., Sydney, Australia. [^{3}H]nemonapride and [^{3}H]muscimol were obtained from New England Nuclear via AMRAD Biotech, Melbourne, Australia. SR95531 and chlorpromazine were obtained from Research Biochemicals International, USA. Clozapine was kindly donated by Sandoz, Australia, and olanzapine was kindly donated by Eli Lilly and Company, USA. All other chemicals were purchased from Sigma Aldrich Pty, Ltd., Australia.

Animals and drug treatment regimes

Male Sprague-Dawley outbred rats with an initial body weight of 150–200g were divided into individual groups of 5 animals per drug treatment and treated with either haloperidol (0.01 or 0.1 mg/kg/day), chlorpromazine (0.1 or 1.0 mg/kg/day), clozapine (0.1 or 1.0 mg/kg/day), or olanzapine (0.1 or 1.0 mg/kg/day) for 1 month, 3 months or 7 months. The drug doses used in these studies were selected with reference to earlier studies in rats but using lower doses to give CNS receptor occupancy approximating to that achieved when treating humans with antipsychotic drugs [23]. All drugs were administered as part of the rats' daily drinking water containing ethanol (0.2ml/kg/day) to ensure the drugs remained in solution. The vehicle treatment groups (n=5) received drinking water containing ethanol (0.2ml/kg/day) in the absence of drugs.

To reduce the residual levels of antipsychotic drug in the brain tissue, administration of drugs was terminated 48 hours prior to the rats being sacrificed by cervical dislocation and immediate decapitation. The brains were then rapidly removed and frozen by immersion in isopentane on dry ice. The brains were then placed in storage at −70°C until used for autoradiography.

Tissue preparation and in situ *radioligand binding with autoradiography*

Coronal sections (20μm) of rat striatum were cut at – 20°C using a Reichert-Jung freezing microtome. As the sections were cut they were directly thaw-mounted onto chrome-alum gelatin coated microscope slides and stored at –70°C prior to use. Immediately prior to incubation all the tissue sections were removed from −70°C storage and air-dried at room temperature for approximately 60 minutes.

For all radioligands, binding was measured at a single concentration at least three times the Kd for each radioligand used and represents a single point saturation analysis study. In

such a study, a good estimate of the density of binding sites for each radioligand can be obtained by subtracting the density of radioligand binding sites in the presence of non-radioactive drug from that in its absence.

Measurement of dopamine D_1- and D_2-like receptors

For the measurement of dopamine receptors, all incubations were at room temperature using 50mM Tris HCl (pH 7.4) containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$ and 1mM $MgCl_2$ as the standard buffer. Dopamine D_1-like receptor density was measured as the binding of [^{3}H]SCH23390 (3nM) in the absence or presence of 10^{-6}M *cis*-flupenthixol [13,24] . The density of D_2-like receptors was measured as the difference between binding of [^{3}H]nemonapride (4nM) in the absence or presence of (+)butaclamol (10^{-6}M) [25,26]. After incubation, slide-mounted tissue sections for both D_1-like and D_2-like receptor studies were transferred through four successive 1 minute washes in ice-cold buffer (4°C), followed by a rapid rinse in ice-cold distilled water.

Measurement of $GABA_A$ receptors

$GABA_A$ receptors were measured as described previously [27]. Thus tissue sections were washed 3 times in ice-cold buffer at 4°C for 5 minutes, and then air-dried using a stream of cool air at room temperature. The density of $GABA_A$ receptors was taken as the difference between the binding of [^{3}H]muscimol (90nM) in the absence or presence of SR95531 (10^{-5}M) following a 60 minute incubation in 50mM Tris Citrate (pH 7.1) at 4°C. Following incubation the sections were transferred through a 1 minute wash in ice-cold buffer and then briefly dipped in ice-cold distilled water.

Image analysis

Following incubation and washes, tissue sections were air-dried at room temperature using a stream of cool air and then apposed to tritium-sensitive Hyperfilm-^{3}H® with a set of high and low [^{3}H]microscales in x-ray film cassettes. Exposure time varied depending on the specific activity of the radioligand and relevant density of radioligand binding sites. All autoradiographs were analysed to determine if there was regional variability of radioligand binding sites. The density of radioligand was initially measured as film optical density using a Northern Light Precision Illuminator, CCD video camera module, and Micro-Computer Imaging Device (MCID) (Imaging Research Inc., St Catherines, Ontario, Canada) M1 image analysis software. These measurements were then compared to a standard curve of optical densities generated from [^{3}H] microscales exposed to the same X-ray film and converted to fmol/mg estimated tissue equivalents (net weight)(ETE).

Statistics

All statistical analyses were carried out using GraphPad Prism Software Inc. Differences between radioligand binding to striatum from the different treatment groups were identified using a one-way ANOVA followed by a post hoc Bonferonni multiple comparison test to establish significant differences between individual groups.

Results

[^{3}H]SCH23390 binding

There were significant differences in [^{3}H]SCH23390 binding to striatum across the groups of animals treated for one month ($F = 3.298$, d.f. = 8,36, $p = 0.006$: Table 1, Figure 1). Importantly, there was no difference between any drug treated group and the vehicle treated animals. The significant differences in [^{3}H]SCH23390 binding was to striatum from rats treated with low dose haloperidol compared to that in striatum from those receiving low (<0.05) and high dose olanzapine ($p<0.05$). There were no significant differences in [^{3}H]SCH23390 binding to striatum between the groups of rats treated for 3 months ($F = 0.7204$, d.f. = 8,36, $p = 0.67$) or 7 months ($F = 1.994$, d.f. = 8,36, $p = 0.076$).

[^{3}H]nemonapride binding

There were significant differences in the density of [^{3}H]nemonapride binding to striatum from rats treated for 1 month ($F = 11.81$, d.f. = 8,36, $p<0.0001$: Table 1 & Figure 1). This difference was in part due to significant increases in [^{3}H]nemonapride binding to striatum

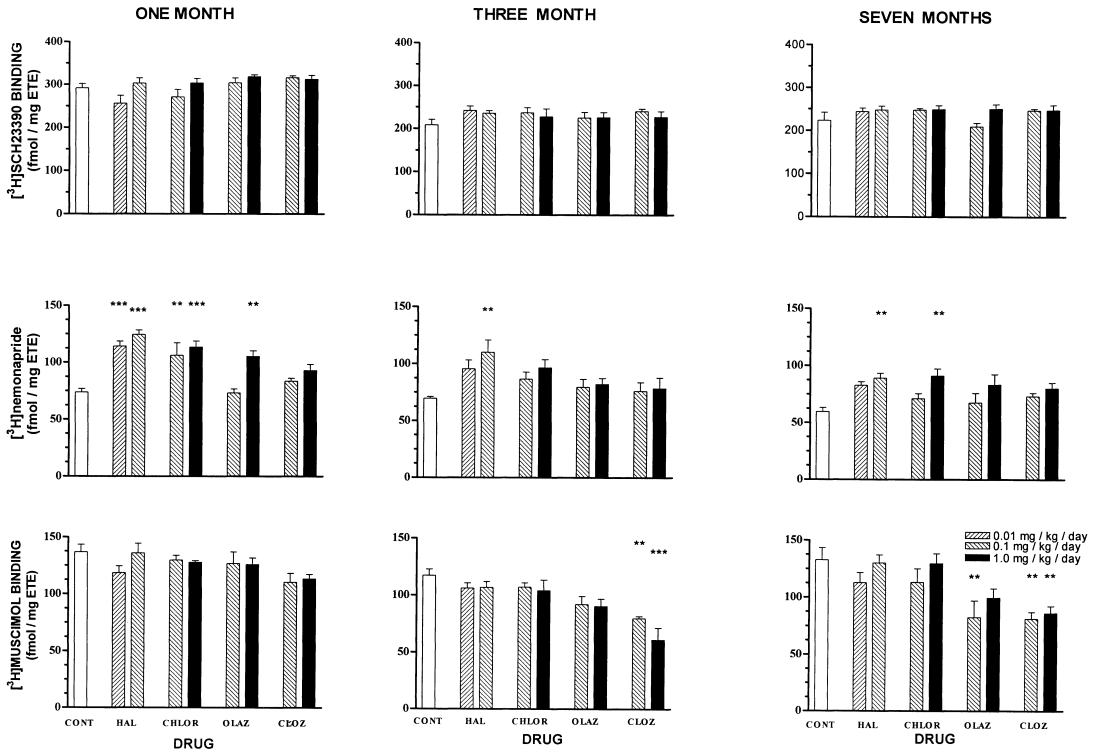

Fig. 1. The binding (mean ± SEM) of [^{3}H]SCH23390, [^{3}H]nemonapride and [^{3}H]muscimol to striatum from rats treated for 1, 3 or 7 months with two dose of haloperidol (HAL), chlorpromazine (CHLOR), olanzapine (OLAZ) or clozapine (CLOZ). Significant differences between binding to striatum from rats receiving vehicle and rats receiving antipsychotic drugs are indicated as * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Table 1
The binding (mean ± SEM) of [^{3}H]SCH 23390, [^{3}H]nemonapride and [^{3}H]muscimol to striatum from rats treated with two doses of haloperidol, chlorpromazine, olanzapine or clozapine for 1, 3, and 7 months

Treatment period (months)	Treatment	Low dose (0.1/0.01mg/kg/day)			High dose (1.0/0.1mg/kg/day)		
		[^{3}H]SCH23390	[^{3}H]nemonapride	[^{3}H]Muscimol	[^{3}H]SCH23390	[^{3}H]nemonapride	[^{3}H]Muscimol
1	Vehicle	292±10	74±3	137±6	292±10	74±3	137±6
	Haloperidol	257±18	114±4	118±6	304±12	124±4	136±8
	Chlorpromazine	271±17	106±11	130±4	304±10	114±5	128±2
	Olanzapine	304±11	73±4	127±10	319±5	105±5	126±6
	Clozapine	316±4	84±2	110±8	313±9	93±5	113±4
3	Vehicle	209±13	70±2	117±6	209±13	70±2	117±6
	Haloperidol	236±6	95±8	106±4	242±10	110±11	107±5
	Chlorpromazine	228±18	87±6	107±4	237±12	96±7	104±9
	Olanzapine	226±12	79±7	92±7	225±13	82±5	90±6
	Clozapine	228±13	76±8	80±2	240±6	79±9	61±10
7	Vehicle	223±19	60±4	133±11	223±19	60±4	133±11
	Haloperidol	244±9	83±3	113±9	247±9	89±4	130±7
	Chlorpromazine	247±4	71±4	113±12	249±9	91±6	130±9
	Olanzapine	210±8	68±8	83±14	251±10	83±9	100±8
	Clozapine	247±4	73±3	81±6	247±12	80±5	86±6

from rats treated with high ($p < 0.001$) and low ($p < 0.001$) doses of haloperidol, rats treated with low ($p < 0.01$) and high ($p < 0.001$) doses of chlorpromazine as well as rats treated with high dose olanzapine ($p<0.01$) compared to rats receiving vehicle. After 3 months treatment there was a significant difference in the density of [^{3}H]nemonapride binding to striatum across the treatment groups ($F = 2.832$, d.f. = 8, 36, $p = 0.015$). This was due to a significant increase in the density of [^{3}H]nemonapride binding to striatum from rats treated with high dose haloperidol ($p < 0.05$) compared to vehicle treated rats. At 7 months there was still a significant difference in [^{3}H]nemonapride binding to striatum across the groups ($F = 3.4$, d.f. = 8, 36, $p = 0.005$). This was due to a significant increase in [^{3}H]nemonapride binding to striatum from rats treated with high dose haloperidol ($p < 0.05$) or chlorpromazine ($p < 0.05$) in comparison to binding to the striatum from vehicle treated rats.

[^{3}H]muscimol binding

After 1 month of treatment there were no significant changes in [^{3}H]muscimol binding to striatum from any of the treatment groups ($F = 2.019$, d.f. = 8, 36, $p = 0.072$). After 3 months treatment there was a significant difference between [^{3}H]muscimol binding to striatum from the different treatment groups ($F = 7.063$, d.f. = 8, 36, $p < 0.0001$) This was in part due to a significant decrease in [^{3}H]muscimol binding to striatum from rats treated with the high ($p < 0.001$) and low ($p < 0.05$) doses of clozapine compared to animals receiving vehicle (Table 1 & Figure 1). The remaining difference was due to [^{3}H]muscimol binding to striatum being lower in animals treated with high dose clozapine compared to those that received the high or low dose of haloperidol ($p < 0.001$ and $p < 0.001$ respectively) or chlorpromazine ($p < 0.001$ and $p < 0.01$). After 7 months of drug treatment there was still a significant difference in [^{3}H]muscimol binding to striatum from the different groups ($F = 4.913$, d.f. = 8,36, $p = 0.0004$). This was in part due to a decrease in [^{3}H]muscimol binding to striatum from rats treated with the high ($p < 0.05$) dose of clozapine and the lower doses of clozapine ($p < 0.05$) and olanzapine ($p < 0.05$). In this instance the remaining differences were due to a significant decrease in [^{3}H]muscimol binding to striatum from rats receiving the low dose olanzapine or clozapine compared to those receiving the high doses of haloperidol ($p < 0.05$ and $p < 0.05$) or chlorpromazine ($p < 0.05$ and $p < 0.05$).

Discussion

The binding of [^{3}H]SCH23390, [^{3}H]nemonapride and [^{3}H]muscimol has been measured in striatum from rats treated with a variety of antipsychotic drugs for a number of treatment periods. A number of drug and time dependent differences in the outcome of antipsychotic drug treatments have been identified.

Dopamine receptors

There were no significant differences in [^{3}H]SCH23390 binding to striatum in any of the cohorts of the drug treated rats compared to that in the rats that received vehicle. Under the conditions used in this study, [^{3}H]SCH 23390 would predominantly bind to DA-D_1 receptors [28,29] . Hence our data suggest that treatment with antipsychotic drugs for up to 7 months does not significantly change the level of DA-D_1 receptors in the rat striatum compared to

that seen in untreated animals. This finding on striatal DA-D_1 receptors is in agreement with other studies which show no change in these receptors in rats treated for up to 1 year with antipsychotic drugs [6,7,13]. By contrast, our findings do not agree with a study that reported an increase in striatal DA-D_1 receptors following a 1 month treatment with haloperidol [16] or studies which suggest that treatment with clozapine increases the density of striatal DA-D_1 receptors [12,20]. Whilst further studies are necessary to fully resolve the effects of antipsychotic drug treatment on DA-D_1 receptors, our data would favor the hypothesis that antipsychotic drugs do not cause a change in DA-D_1 receptor density in rat striatum.

This study has shown a number of differences in the binding of [^{3}H]nemonapride to striatum from rats treated with antipsychotic drugs. As used in this study, [^{3}H]nemonapride has been shown to bind to both DA-D_2 and σ receptors [30,31]. However, we have shown that SKF 10047, a σ receptor antagonist [30], only displaced 10% of bound [^{3}H]nemonapride from human striatum suggesting that 90% of binding of the radioligand would be to DA-D_2 receptors. These data are consistent with reports that the density of σ receptors is very low in the rat striatum [32,33] and together indicate that [^{3}H]nemonapride would predominantly bind to DA-D_2 receptors in the striatum. Thus, our data show that there is an increase in DA-D_2 receptor density in rats that have received either dose of haloperidol or chlorpromazine and the high dose of olanzapine for 1 month. With regard to the typical antipsychotic drugs haloperidol and chlorpromazine, our data is in agreement with previous studies [6,11–13] that have shown that treating rats for one month with typical antipsychotic drugs up-regulate the DA-D_2 receptor. Moreover, our data is agreement with other studies [12,13,34,35] that show that treating rats with clozapine does not increase the density of DA-D_2 receptors in rat striatum. In addition we report that at the higher dose used, olanzapine caused a transient increase in the density of DA-D_2 receptors after treatment for 1 month. It is important to note that the recommended therapeutic daily dose of olanzapine is approximately 10 % of that for clozapine [15,36] therefore the effect we have observed at the higher dose of olanzapine may be attainable with a higher dose of clozapine. However, there is an association between the capacity of an antipsychotic drug to increase the density of DA-D_2 receptors in the rat striatum and their ability to produce extrapyramidal side effects in humans [37]. If this is true for olanzapine the use of this drug above the recommended dose [38] should be avoided as it could result in the onset of extrapyramidal side effects.

The results from treating rats for longer than one month show that the increase in DA-D_2 receptors caused by olanzapine are transient, not being detectable after 3 months. By contrast, as in an earlier study [7], increases in DA-D_2 receptors are still present after 7 months treatment with high dose typical antipsychotic drugs. Thus it would appear that the ability to "up-regulate" DA-D_2 receptors for long periods may be a function of the more selective DA-D_2 receptor antagonists. The rationale for classifying antipsychotic drugs as typical and atypical is that the atypical drugs cause fewer extra-pyramidal side effects [38]. From our data, another method of classifying atypical antipsychotic drugs might be their inability to cause long-term increases DA-D_2 receptor density in the rat striatum which would mean olanzapine clearly fulfills the criteria of an atypical antipsychotic drug.

$GABA_A$ receptors

This study has shown that treating rats for 3 months or more with clozapine, or for more than three months with high dose olanzapine, decreases [^{3}H]muscimol binding in the stria-

tum. Under the conditions used in this study [^{3}H]muscimol would be expected to bind predominately to the $GABA_A$ receptor [28]. Thus our data indicate that treatment with clozapine and, to a lesser extent olanzapine, causes a decrease in $GABA_A$ receptor density in the striatum. It has been shown that treating rats for 1 month with the same doses of clozapine and olanzapine, but not haloperidol or chlorpromazine, causes a decrease in $GABA_A$ receptors in hippocampus and temporal cortex [21]. This suggests that both clozapine and olanzapine down-regulate the $GABA_A$ receptor, however the time and dose-dependency of this effect may vary between brain regions. Our results agree with a previous study which showed that treating rats with haloperidol for 6 months did not change the density of $GABA_A$ receptors in the striatum [39] but differ from those of others that suggest that treatment with haloperidol increases striatal $GABA_A$ receptors [19,20]. Clearly the effects of antipsychotic drugs on the GABAergic system remain to be fully elucidated but our data suggests that treatment with atypical antipsychotic drugs decreases the levels of $GABA_A$ receptors, at least in the striatum and hippocampus.

Conclusions

We have shown that antipsychotic drugs have different effects on DA-D_2 receptors and $GABA_A$ receptors in rat brain. There is a close association between dopaminergic and GABAergic systems in the rat striatum [40] and therefore one could postulate that such changes could be inter-related and important in the therapeutic actions of antipsychotic drugs. However, our results would not support this hypothesis because the changes in DA-D_2 and $GABA_A$ receptors do not show any relationship and do not result from treatment with a single class of antipsychotic drug. Significantly, clozapine was the only drug that did not up-regulate DA-D_2 receptors but did down-regulate $GABA_A$ receptors within 3 months. However, as olanzapine and clozapine cause similar changes in dopaminergic and GABAergic markers in the rat striatum it would seem unlikely that these changes are associated with the unique outcomes arising from the use of clozapine which includes being effective in treatment resistant subjects and lessening negative symptoms [15].

Our data show that, of the drugs we studied, only the atypical antipsychotic drugs affected the density of $GABA_A$ receptors in the rat striatum. The atypical antipsychotic drugs used in this study, clozapine and olanzapine, differ from the typical antipsychotic drugs used in that they have a high affinity for serotonergic and muscarinic receptors [22]. Significantly, stimulation of the serotonergic system has been shown to increase GABA release [41] whereas stimulation of the cholinergic systems has been shown to decrease the level of GABA [42]. Therefore by blockading these pathways, clozapine and olanzapine should be able to decrease and increase the levels of GABA respectively. It has been shown that decreasing levels of $GABA_A$ receptor predominantly reflect increasing levels of GABA [43]. Thus, our data in the rat striatum would be most consistent with the hypothesis that atypical antipsychotic drugs modulate levels of $GABA_A$ receptors by inhibiting the muscarinic system, causing an increase in GABA release. This hypothesis is supported by a study showing problems in changing psychotic subjects from treatment with clozapine to an atypical antipsychotic drugs with no cholinergic activity, risperidone [44], because of a resulting cholinergic overdrive and GABA supersensitivity. Studies on the effect of atypical antipsychotic drugs without

cholinergic activity on levels of $GABA_A$ receptor in the rat would be worthwhile to confirm our hypothesis as the hypothesis may have significance when considering altering antipsychotic drug treatment regimes in humans.

Acknowledgments

BD is the holder of a NARSAD Young Investigator Award. TH was the recipient of a National Health and Medical Research Dora Lush Scholarship (957473).The authors would like to thank Renee Pearson, Lee Naylor, Fiona Parkin, and Susie Kitsoulis for their contributions towards the antipsychotic drug treatments of rats.

References

1. Burt DR, Creese I, and Snyder SH. Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. Science 1997;196:326–8.
2. Muller P and Seeman P. Brain neurotransmitter receptors after long-term haloperidol: dopamine, acetylcholine, serotonin, alpha-noradrenergic and naloxone receptors. Life Sci. 1977;21:1751–8.
3. Clow A, Jenner P, Theodorou A, and Marsden CD. Striatal dopamine receptors become supersensitive while rats are given trifluoperazine for six months. Nature 1979;278:59–61.
4. Owen F, Cross AJ, Waddington JL, Poulter M, Gamble SJ, and Crow TJ. Dopamine-mediated behaviour and 3H-spiperone binding to striatal membranes in rats after nine months haloperidol administration. Life Sci. 1980;26:55–59.
5. Dyer RG, Murugaiah K, Theodorou A, Clow A, Jenner P, and Marsden CD. During one year's neuroleptic treatment in rats striatal dopamine receptor blockade decreases but serum prolactin levels remain elevated. Life Sci. 1981;28:167–74.
6. Florijn WJ, Tarazi FI, and Creese I. Dopamine receptor subtypes: differential regulation after 8 months treatment with antipsychotic drugs. Pharmacol.Exp.Therapeut.1997;280:561–9.
7. Rupniak NM, Kilpatrick G, Hall MD, Jenner P, and Marsden CD. Differential alterations in striatal dopamine receptor sensitivity induced by repeated administration of clinically equivalent doses of haloperidol, sulpiride or clozapine in rats. Psychopharmacology (Berl) 1984;84:512–9.
8. Clow A, Theodorou A, Jenner P, and Marsden CD. Cerebral dopamine function in rats following withdrawal from one year of continuous neuroleptic administration. Eur.J.Pharmacol. 1980;63:145–57.
9. Murugaiah K, Theodorou A, Mann S, Clow A, Jenner P, and Marsden CD. Chronic continuous administration of neuroleptic drugs alters cerebral dopamine receptors and increases spontaneous dopaminergic action in the striatum. Nature 1982;296:570–2.
10. Seeger TF, Thal L, and Gardner EL. Behavioral and biochemical aspects of neuroleptic-induced dopaminergic supersensitivity: studies with chronic clozapine and haloperidol. Psychopharmacology (Berl) 1982;76: 182–7.
11. Wilmot CA and Szczepanik AM. Effect of acute and chronic treatments with clozapine and haloperidol on serotonin (5-HT2) and dopamine (D2) receptors in rat brain. Brain Res. 1989;487:288–98.
12. O'Dell SJ, La Hoste GJ, Widmark CB, Shapiro RM, Potkin SG, and Marshall JF. Chronic treatment with clozapine or haloperidol differentially regulates dopamine and serotonin receptors in the rat brain. Synapse 1990;6:146–53.
13. Wan DC, Dean B, Pavey G, and Copolov DL. Treatment with haloperidol or clozapine causes changes in dopamine receptors but not adenylate cyclase or protein kinase C in the rat forebrain. Life Sci. 1996;59: 2001–8.
14. Boyson SJ, McGonigle P, Luthin GR, Wolfe BB, and Molinoff PB. Effects of chronic administration of neuroleptic and anticholinergic agents on densities of D2 dopamine and muscarinic cholinergic receptors in rat striatum. J.Pharmacol.Exp.Ther. 1998;244:987–93.

15. Baldessarini RJ and Frankenburg FR. Clozapine: A novel antipsychotic agent. New Eng.J.Med. 1991;324: 746–54.
16. Sasaki T, Kennedy JL, and Nobrega JN. Regional brain changes in 3H]SCH 23390 binding to dopamine D1, receptors after long-term haloperidol treatment: lack of correspondence with the development of vacuous chewing movements. Behav.Brain Res. 1998;90:125–32.
17. Laruelle M, Jaskiw GE, Lipska BK, Kolachana B, Casanova MF, Kleinman JE, and Weinberger DR. D1 and D2 receptor modulation in rat striatum and nucleus accumbens after subchronic and chronic haloperidol treatment. Brain Res. 1992;575:47–56.
18. MacKenzie RG and Zigmond MJ. Chronic neuroleptic treatment increases D-2 but not D-1 receptors in rat striatum. Eur.J.Pharmacol. 1985;113:159–65.
19. See RE, Aravagiri M, and Ellison GD. Chronic neuroleptic treatment in rats produces persisting changes in GABAA and dopamine D-2, but not dopamine D-1 receptors. Life Sci. 1989;44:229–36.
20. See RE, Toga AW, and Ellison G. Autoradiographic analysis of regional alterations in brain receptors following chronic administration and withdrawal of typical and atypical neuroleptics in rats. J.Neural Transm.Gen.Sect. 1990;82:93–109.
21. Farnbach-Pralong D, Bradbury R, Copolov D, and Dean B. Clozapine and olanzapine treatment decreases rat cortical and limbic GABA(A) receptors. Eur.J.Pharmacol. 1998;349:R7–R8.
22. Bymaster FP, Calligaro DO, Falcone JF, Marsh RD, Moore NA, Tye NC, Seeman P, and Wong DT. Radioreceptor binding profile of the atypical antipsychotic olanzapine. Neuropsychopharmacology. 1996;14:87–96.
23. Kapur S., Wadenberg ML, Remington G. Are animal studies of antipsychotic drugs appropriately dosed? Lessons from bedside to bench. Can.J.Psychiatr. 2000;45:241–246.
24. Knable MB, Hyde TM, Murray AM, Herman MM, and Kleinman JE. A postmortem study of frontal cortical dopamine D1 receptors in schizophrenics, psychiatric controls, and normal controls. Biol.Psychiatry 1996;40:1191–9.
25. Murray AM, Hyde TM, Knable MB, Herman MM, Bigelow LB, Carter JM, Weinberger DR, and Kleinman JE. Distribution of putative D4 dopamine receptors in postmortem striatum from patients with schizophrenia. J.Neurosci. 1995;15:2186–91.
26. Marzella, P., Hill, C., Keks, N. A., Singh, B., and Copolov, D. L. The binding of both [3H]nemonapride and [3H]raclopride are increased in schizophrenia. Biological Psychiatr. 1997;42:648–54.
27. Dean B, Hussain T, Hayes W, Scarr E, Kitsoulis S, Hill C, Opeskin K, and Copolov DL. Changes in serotonin2A and GABA(A) receptors in schizophrenia: studies on the human dorsolateral prefrontal cortex. J.Neurochem. 1999;72:1593–99.
28. Cortes R, Gueye B, Pazos A, Probst A, and Palacios JM. Dopamine receptors in human brain: autoradiographic distribution of D1 sites. Neuroscience 1989;28:263–73.
29. Dawson TM, McCabe RT, Stensaas SS, and Wamsley JK. Autoradiographic evidence of [3H]SCH 23390 binding sites in human prefrontal cortex (Brodmann's area 9). J.Neurochem. 1987;49:789–96.
30. Helmeste D, Tang SW, Fang H, and Li M. Brain σ receptors labelled by [3H]nemonapride. Eur.J.Pharmacol. 1996;301:R1–R3.
31. Ujike H, Akiyama K, and Kuroda S. [3H]YM-09151-2 (nemonapride), a potent radioligand for both sigma 1 and sigma 2 receptor subtypes. Neuroreport 1996;7:1057–61.
32. Gundlach AL, Largent BL, and Snyder SH. Autoradiographic localization of sigma receptor binding sites in guinea pig and rat central nervous system with (+)3H-3-(3-hydroxyphenyl)-N-(1- propyl)piperidine. J.Neurosci. 1986;6:1757–70.
33. Zukin SR, Tempel A, Gardner EL, and Zukin RS. Interaction of [3H](-)-SKF-10,047 with brain sigma receptors: characterization and autoradiographic visualization. J.Neurochem. 1986;46:1032–41.
34. Lee T and Tang SW. Loxapine and clozapine decrease serotonin (S2) but do not elevate dopamine (D2) receptor numbers in the rat brain. Psychiatry Res. 1984;12:277–85.
35. Rupniak NM, Hall MD, Kelly E, Fleminger S, Kilpatrick G, Jenner P, and Marsden CD. (1985) Mesolimbic dopamine function is not altered during continuous chronic treatment of rats with typical or atypical neuroleptic drugs. J.Neural Transm. 1985;62:249–66.
36. Tollefson GD, Beasley CMJ, Tran PV, Street JS, Krueger JA, Tamura RN, Graffeo KA, and Thieme ME.

Olanzapine versus haloperidol in the treatment of schizophrenia and schizoaffective and schizophreniform disorders: results of an international collaborative trial Am.J.Psychiatry 1997;154:457–65.
37. Reynolds GP (1998) Receptor mechanisms of antipsychotic drug atypicality Eur. Psychiatr. 1998;13 (suppl. 1):5S–8S.
38. Kerwin RW. The New Atypical Antipsychotics: A lack of extrapyramidal side- effects and new routes in schizophrenia. Br.J.Psychiatry 1994;164:141–148.
39. Shirakawa O and Tamminga. CA Basal ganglia $GABA_A$ and dopamine D_1 binding site correlates of haloperidol-induced oral dyskinesias in rat. Exp.Neurol. 1994;127:62–69.
40. Garbutt JC and van Kammen DP. The interaction between GABA and dopamine: Implications for schizophrenia. Schizophr.Bull. 1983;9:336–53.
41. Meyer DK, Holland A, Lais A and Szabo B. Effects of p-chloroamphetamine on release of [3H]gamma-aminobutyric acid from slices of rat caudate-putamen. Eur.J.Pharmacol. 1991;196:189–195.
42. Smolders I, Bogaert L, Ebinger G, and Michotte Y. Muscarinic modulation of striatal dopamine, glutamate and GABA release, as measured with in vivo microdialysis. J.Neurochem. 1997;68:1942–1948.
43. Benes FM, Vincent SL, Alsterberg G, Bird ED and SanGiovanni JP. Increased $GABA_A$ receptor binding in superficial layers of cingulate cortex in schizophrenia. 1992;12:924–929.
44. Verghese C, DeLeon J, Nair C, Simpson GM. Clozapine withdrawal effects and receptor profiles of typical and atypical neuroleptics. Biol. Psych. 1996;39:135–138.