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Clozapine-induced dopamine levels in the rat striatum and nucleus accumbens are not affected by muscarinic antagonism

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Abstract

The effect of the muscarinic antagonist, scopolamine, was examined for a change in the increase in extracellular dopamine, dihydroxyphenyl acetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindolacetic acid (5-HIAA), induced by haloperidol or clozapine in the striatum and nucleus accumbens of anaesthetised and awake rats, monitored using in vivo cerebral microdialysis. Rats received scopolamine (1 mg kg^{-1}; s.c.) or vehicle followed by haloperidol (1 mg kg^{-1}; s.c.) or clozapine (20 mg kg^{-1}; s.c.). Dopamine, DOPAC, HVA and 5-HIAA overflow into striatal or accumbens perfusates was determined using high performance liquid chromatography with electrochemical detection (HPLC-ECD). Scopolamine failed to modify the clozapine- or haloperidol-induced efflux of dopamine or its metabolites in either the striatum or nucleus accumbens following systemic administration in anaesthetised or awake rats. Although pretreatment with scopolamine tended to produce a smaller increase in the clozapine-induced efflux of DOPAC in striatal perfusates than following clozapine treatment alone, this was not statistically significant. Furthermore, local infusion of scopolamine (100 μM) with clozapine (1 mM) via the microdialysis probe did not attenuate the elevated efflux of dopamine observed following clozapine alone, in either the striatum or nucleus accumbens, in anaesthetised rats. This treatment did prevent the clozapine-induced increase in DOPAC and HVA in the striatum but not the nucleus accumbens. Carbachol (50 μM) infused into the dorsolateral striatum or nucleus accumbens raised extracellular dopamine levels 200% and 150%, respectively above baseline. Our data suggest that the increased efflux of dopamine and its metabolites in the rat basal ganglia following clozapine administration is not significantly dependent upon the interaction of clozapine with muscarinic receptors.

Keywords: Clozapine; Dopamine; Muscarinic receptor; Microdialysis; Antipsychotics; Cholinergic

1. Introduction

The aetiology of schizophrenia has been shown to be intimately associated with dopaminergic neurotransmission. This primarily stems from the ability of all currently available antipsychotic agents to interfere with the action of dopamine at the dopamine D_2 receptor family. With few exceptions, however, blockade of these receptors results in the development of extrapyramidal movement disorders, generally associated with a relative overactivity of the cholinergic system within the basal ganglia (DeVeaugh-Geiss, 1982). Such Parkinsonian motor side effects are generally controlled by the concomitant administration of centrally acting muscarinic anticholinergic drugs (Baldessarini, 1990).

Of the few antipsychotic agents that do not induce movement disorders (atypical antipsychotics), the most notable is clozapine. Clozapine interacts with many brain neurotransmitter systems, several of which have been purported to confer its atypical antipsychotic action. One of these hypotheses concerns clozapine's affinity for muscarinic receptors (Miller and Hiley, 1974). While originally supposed to be an antagonist at muscarinic acetylcholine receptors, a recent examination of the efficacy of clozapine at muscarinic receptor subtypes has reported that in contrast to an antagonist activity at muscarinic M_1, M_2, M_3, and M_5 receptors expressed in CHO cells in vitro, clozapine acted as an agonist at muscarinic M_4 receptors (Zorn et al., 1994). This observation is of particular interest since the proposed locus for an anticholinergic drug in controlling extrapyramidal motor effects is in the striatum.

However, the extrapyramidal side effects of neuroleptics may not be fully explained by the cholinergic hypothesis. Recent evidence from catalepsy studies, a rodent model

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of neuroleptic-induced extrapyramidal side effects, implicates haloperidol-induced increases in GABA in the globus pallidus as being responsible for the motor side effects, clozapine did not enhance GABA levels within this structure (Chapman and See, 1996). Further, Delfs et al. (1995) showed that glutamate decarboxylase mRNA levels in the globus pallidus increased following haloperidol but decreased following clozapine administration. Indeed, the dose of haloperidol used induced catalepsy, which was reversed by scopolamine, together with the enhanced mRNA for glutamate decarboxylase. Thus, these data appear to suggest that the motor side effects from antipsychotic agents may be expressed via the striatopallidal pathway. In this context, haloperidol would enhance acetylcholine output in the striatum which would activate muscarinic M_1 receptors located on the GABAergic projection neurones to the globus pallidus (Harrison et al., 1996; Wang and McGinty, 1997) enhancing GABA neurotransmission since the dopamine D_2 receptors on these neurones would be blocked by haloperidol. Clozapine would have no effect on this pathway since clozapine would block the muscarinic M_1 receptor on the GABA projection neurone and thus, little excitation of this pathway would result.

The cellular and subcellular distributions of muscarinic receptor subtypes have only been examined in the rat brain. Within the striatum, the majority of neurones express muscarinic M_1 receptor protein (77.8%), muscarinic M_2 receptors were largely restricted to large interneurones (2.7%) and muscarinic M_3 receptors were not detectable in this region. Muscarinic M_4 receptor protein was expressed in about half of all neostriatal neurones (44.2%). Muscarinic M_2 receptors appear to function as the primary cholinergic autoreceptor in the striatum, although both muscarinic M_1 and M_4 receptors are also localised at axon terminals forming asymmetrical synapses in the dorsal striatum, presumably acting as presynaptic heteroreceptors on glutamatergic nerve terminals (Hersch and Levey, 1995; Smolders et al., 1997; Rawls and McGinty, 1998). A further differentiation of muscarinic M_1 and M_4 receptors are now evident as briefly mentioned above. The striatonigral pathway appears to express both dopamine D_1 receptors and muscarinic M_4 receptors while the striatopallidal pathway expresses dopamine D_2 and muscarinic M_1 receptors (Harrison et al., 1996; Wang and McGinty, 1997; Wang et al., 1997).

Nigrostriatal dopaminergic neurones are considered to exert an inhibitory influence on acetylcholine output by an action of dopamine on dopamine D_2 receptors (Stoof and Kebabian, 1982; Fujiwara et al., 1987), located on cholinergic interneurones (Le Moine et al., 1990). Consequently, in vitro and in vivo studies have shown that blockade of dopamine D_2 receptors by various neuroleptics results in increased striatal acetylcholine release (Stoof et al., 1979; Damsma et al., 1990a; Marien and Richard, 1990) and stimulation of dopamine D_2 receptors causes inhibition of striatal acetylcholine output (Stoof et al., 1979; Damsma et al., 1990a). Furthermore, using microdialysis, Damsma et al. (1990b) have shown that the selective dopamine D_1 agonist SKF 38393 increases, whereas the dopamine D_1 receptor antagonist SCH 23390 decreases, acetylcholine release (Consolo et al., 1987). In an interesting series of experiments, Damsma et al. (1991) and Imperato et al. (1993, 1994) have shown that haloperidol, (−)-sulpiride and clozapine increased acetylcholine output in the striatum. Moreover, the dopamine D_1 antagonist SCH 23390 was able to abolish the acetylcholine output of haloperidol and (−)-sulpiride, but only modestly reduced that due to clozapine. These data suggest that clozapine elevates acetylcholine output via a different mechanism from haloperidol and (−)-sulpiride.

Recently two independent groups have reported that increases in rat striatal dopamine metabolism (Rivest and Marsden, 1991) or dopamine levels (Meltzer et al., 1994) following clozapine administration could be attenuated by muscarinic antagonists.

In the present study, the effect of scopolamine on the modulation of extracellular levels of dopamine and its metabolites following clozapine or haloperidol administration were investigated in both anaesthetised and awake, freely moving rats. The aim of the present study was to investigate the hypothesis of Meltzer et al. (1994), that scopolamine attenuated clozapine-induced dopamine levels in the striatum of Sprague–Dawley rats to determine: (1) if this action of scopolamine on clozapine generalised to Wistar rats; (2) if this neurochemical marker of clozapine may be used to identify similar compounds to clozapine; (3) if this effect of scopolamine is modified by an anaesthetic. Furthermore, in addition to the Meltzer study, the aim was to identify the locus of an effect, by perfusing clozapine via reverse microdialysis in the striatum or nucleus accumbens in anaesthetised rats. Finally, carbachol infusions were investigated in specific brain regions to determine the action of a cholinergic agonist on extracellular dopamine levels.

2. Methods

2.1. Microdialysis procedure

2.1.1. Anaesthetised procedure

Male Wistar rats (300–400 g; Interfauna, UK) were maintained on a 12 h/12 h light/dark cycle with free access to food and water. Rats were anaesthetised with chloral hydrate (400 mg kg^{-1}; i.p. with supplementary doses of 60 mg kg^{-1} as required) and a concentric microdialysis probe (regenerated cellulose dialysis fiber, permeability limit 6000 Da, o.d. 225 μm, Harvard, UK) was implanted into either the dorsolateral striatum (coordinates: AP +0.7 mm, L −3.2 mm, DV −6.0 mm; 3 mm dialysis tip), nucleus accumbens (AP +2.2 mm, L +1.2 mm, DV

−7.8 mm; 2 mm dialysis tip) or medial prefrontal cortex (AP +3.2 mm, L +0.8 mm, DV −6.0 mm, 3 mm dialysis tip; all coordinates were relative to bregma suture, Paxinos and Watson, 1986) and cemented in place. The probe was continuously perfused with physiological buffer (composition in mM): NaCl 140, KCl 3, $CaCl_2$ 2.4, $MgCl_2$ 1.0, NaH_2PO_4 0.27, Na_2HPO_4 1.2, and glucose 7.4 (pH 7.4).

2.1.2. Awake procedure

In studies on awake rats, a guide cannula was implanted above the region of interest under hypnorm/hypnoval anaesthesia. Following 7 days of recovery, the dialysis probe was manually inserted into the guide on the day of the experiment.

2.2. HPLC-ECD analysis of dopamine and metabolites

Dopamine and its metabolites in perfusates were analysed as described by Zetterström et al. (1985). Briefly, the high performance liquid chromatography (HPLC) system utilised an HPLC column (4.6 × 150 mm ultrasphere C18, 5.0 μM particles, Beckman) and a mobile phase comprising 0.12 M NaH_2PO_4, 0.33 mM sodium octane sulphonate, 0.1 mM Na_2EDTA and 15% (v/v) methanol (final pH 3.8) at a flow rate of 1.3 ml min^{-1}. Electrochemical detection was by a glassy carbon electrode (BAS MF1000, held at +0.7 V with respect to a Ag/AgCl reference electrode) connected to a BAS LC-4B potentiometer. Following collection, dialysate samples (20 μl) were injected by an autosampler (Gilson 231) onto the analytical column. The approximate limit of detection for dopamine was 20 fmol/sample.

2.3. Experimental design

After implantation of the dialysis probe, a baseline period of 2 h was allowed for stabilisation of dopamine and metabolite output. Four or five 20 min samples were collected and averaged and referred to as baseline. After the baseline period, drugs were administered either subcutaneously or through the probe and their effects were followed for 2 h. In some experiments, the muscarinic antagonist scopolamine (1 mg kg^{-1}) or vehicle was injected subcutaneously 15 min before the antipsychotics. In the reverse dialysis studies, carbachol or clozapine plus scopolamine or artificial cerebrospinal fluid were perfused through the probe for the duration of the experiment (2 h). The systemic doses of the antipsychotics and scopolamine were chosen on the basis of the investigation by Meltzer et al. (1994) and pilot studies with carbachol and scopolamine. The dose utilized in this study for clozapine was the most commonly found dose in the literature for microdialysis and behavioural experiments. The dose of scopolamine was that used by Meltzer et al. (1994) as mentioned above, but this dose was also found to reverse the carbachol-induced increase in microdialysate dopamine levels.

2.4. Drugs

The drugs used were clozapine (Research Biochemicals Semat, UK) scopolamine hydrochloride (Sigma, Poole, Dorset, UK), haloperidol (Organon Laboratories, UK), and carbachol (Sigma, UK). For subcutaneous administration, clozapine and haloperidol (not corrected for salt) were dissolved in 100 μl of 0.4 M tartaric acid and made up to volume with 0.9% (w/v) NaCl. The pH was corrected to pH 7.0 with 1 M sodium hydroxide. Scopolamine was dissolved in 0.9% (w/v) NaCl. In the reverse dialysis experiments, clozapine was dissolved as above and made up to volume with perfusion buffer. The final pH of these solutions was pH 7.0 in order to ensure that the compound remained in solution. Vehicle controls of the same pH were included in these experiments.

2.5. Data analysis

Only rats that showed correct placement of dialysis probes were included in subsequent analysis. The amount of dopamine, DOPAC, HVA and 5-HIAA in each sample was expressed as a percentage of the amount in the four to five samples collected prior to drug administration. The mean of these four to five baseline samples was taken as 100% and all data are presented relative to this value. Each time point represents the mean ± S.E.M. of the results obtained from six to eight rats. Data was analysed before being transformed to percentage of baseline, by a two-way repeated measures hierarchical analysis of variance (ANOVA). To determine if scopolamine had any effect upon dopamine levels induced by either clozapine or haloperidol two-way repeated measures ANOVAs were used. This analysis did not include the two control groups for each of the antipsychotic drugs since this allowed an assessment of the main effect and interaction for the antipsychotic and antipsychotic and scopolamine groups. This removed the necessity to follow up the ANOVA with a post hoc analysis. Statistical significance was taken as $P < 0.05$. The details of the statistical analysis are contained in the figure legends.

3. Results

3.1. Effect of acute administration of antipsychotics and scopolamine on dopamine output in the striatum and nucleus accumbens of awake and anaesthetised rats

Clozapine and haloperidol were tested for their effect on striatal and accumbens dopamine efflux following pretreatment with scopolamine or vehicle in both anaesthetised and awake rats. Clozapine (20 mg kg^{-1}) administered subcutaneously increased dopamine output in both striatal and accumbens perfusates in anaesthetised and awake rats. The maximal increase in dopamine output occurred 20 min after clozapine administration for accum-

bens perfusates and 40 min after clozapine administration for striatal perfusates. After these times, the augmented dopamine efflux gradually decreased but remained significantly above baseline throughout the experimental epoch of 2 h (Fig. 1a–d). Similar to clozapine, in both striatal and accumbens perfusates, haloperidol (1 mg kg^{-1}; s.c.) administration produced increased dopamine output in anaesthetised and awake rats (Fig. 2a–d). Scopolamine (1 mg kg^{-1}; s.c.) did not modify basal striatal or accumbens dopamine efflux in awake or anaesthetised rats (Figs. 1 and 2). Pretreatment with scopolamine (1 mg kg^{-1}; s.c.) did not modulate the clozapine- or haloperidol-induced increase in dopamine levels in striatal or accumbens perfusates in awake or anaesthetised rats (Fig. 1a–d; Fig. 2a–d).

3.2. Effect of acute administration of antipsychotic drugs and scopolamine on extracellular levels of DOPAC, HVA and 5-HIAA

In addition to dopamine, monoamine metabolites were measured in striatal and accumbens perfusates following

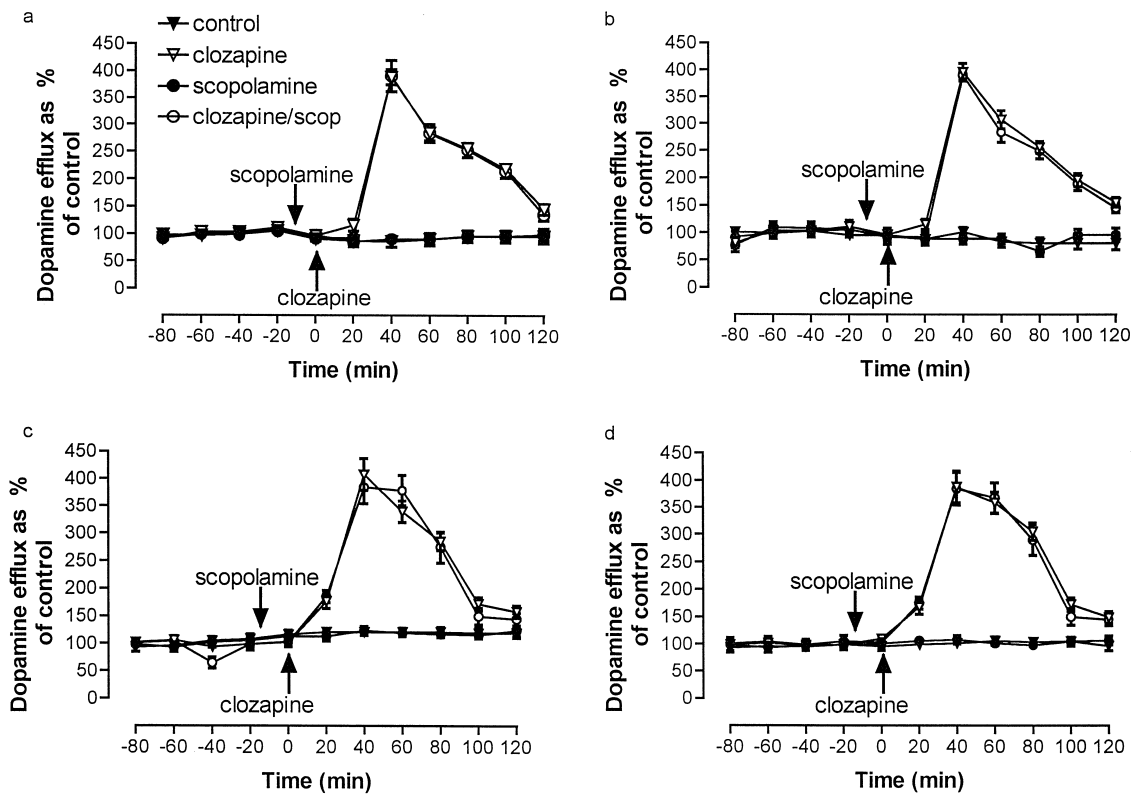

Fig. 1. Effect of clozapine (20 mg kg^{-1}; s.c.), scopolamine (1 mg kg^{-1}; s.c.), vehicle control or clozapine and scopolamine on extracellular dopamine levels in various brain regions of awake or anaesthetised rats. (a) The striatum of awake rats ($n = 6$). The average basal level of dopamine in the striatum was 0.10 ± 0.03 pmol/20 μl. Two-way ANOVA with repeated measures demonstrated a significant effect of drug treatment [$F(3,20) = 14.68$; $P < 0.01$], a significant effect of time [$F(10,200) = 15.88$; $P < 0.01$] and a significant interaction [$F(30,200) = 45.65$; $P < 0.001$] clearly indicating that clozapine and clozapine plus scopolamine increased dopamine levels over time. A further repeated measures ANOVA between the clozapine and clozapine plus scopolamine treatment group found no significant difference between these two groups [$F_{main}(1,10) = 0.01$; $P > 0.05$, $F_{time}(10,100) = 186.93$; $P < 0.001$, $F_{interaction}(10,100) = 1.25$; $P > 0.05$]. Scopolamine did not produce an effect on its own. (b) The striatum of anaesthetised rats ($n = 6$). The average basal level of dopamine in the striatum was 0.12 ± 0.03 pmol/20 μl. Two-way ANOVA with repeated measures demonstrated a significant effect of drug treatment [$F(3,20) = 13.16$; $P < 0.01$], a significant effect of time [$F(10,200) = 14.92$; $P < 0.01$] and a significant interaction [$F(30,200) = 42.15$; $P < 0.001$]. A further repeated measures ANOVA between the clozapine and clozapine plus scopolamine treatment group found no significant difference between these two groups [$F_{main}(1,10) = 0.05$; $P > 0.05$, $F_{time}(10,100) = 152.79$; $P < 0.001$, $F_{interaction}(10,100) = 1.16$; $P > 0.05$]. Scopolamine did not produce an effect on its own. (c) The nucleus accumbens of awake rats ($n = 6$). The average basal level of dopamine in the nucleus accumbens was 0.07 ± 0.02 pmol/20 μl. Two-way ANOVA with repeated measures demonstrated a significant effect of drug treatment [$F(3,20) = 11.42$; $P < 0.01$], a significant effect of time [$F(10,200) = 14.62$; $P < 0.01$] and a significant interaction [$F(30,200) = 41.86$; $P < 0.001$]. A further repeated measures ANOVA between the clozapine and clozapine plus scopolamine treatment group found no significant difference between these two groups [$F_{main}(1,10) = 0.11$; $P > 0.05$, $F_{time}(10,100) = 168.14$; $P < 0.001$, $F_{interaction}(10,100) = 1.48$; $P > 0.05$]. Scopolamine did not produce an effect on its own. (d) The nucleus accumbens of anaesthetised rats ($n = 6$). The average basal level of dopamine in the nucleus accumbens was 0.05 ± 0.09 pmol/20 μl. Two-way ANOVA with repeated measures demonstrated a significant effect of drug treatment [$F(3,20) = 13.86$; $P < 0.01$], a significant effect of time [$F(10,200) = 15.56$; $P < 0.01$] and a significant interaction [$F(30,200) = 40.34$; $P < 0.001$]. A further repeated measures ANOVA between the clozapine and clozapine plus scopolamine treatment group found no significant difference between these two groups [$F_{main}(1,10) = 0.16$; $P > 0.05$, $F_{time}(10,100) = 147.98$; $P < 0.001$, $F_{interaction}(10,100) = 1.12$; $P > 0.05$]. Scopolamine did not produce an effect on its own.

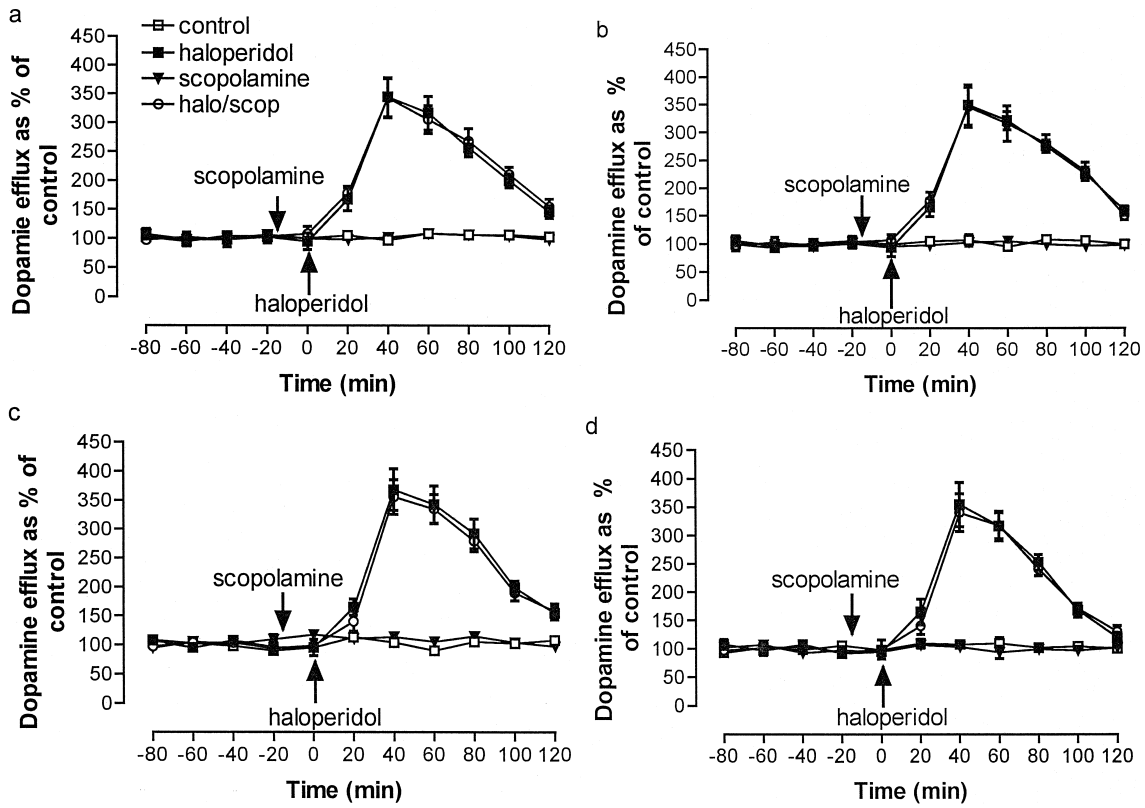

Fig. 2. Effect of haloperidol (1 mg kg^{-1}; s.c.), scopolamine (1 mg kg^{-1}; s.c.), vehicle control or haloperidol and scopolamine on extracellular dopamine levels in various brain regions of awake or anaesthetised rats. (a) The striatum of awake rats ($n = 6$). The average basal level of dopamine in the striatum was 0.11 ± 0.01 pmol/20 μl. Two-way ANOVA with repeated measures demonstrated a significant effect of drug treatment [$F(3,20) = 12.67$; $P < 0.01$], a significant effect of time ($F(10,200) = 16.98$; $P < 0.01$) and a significant interaction [$F(30,200) = 39.34$; $P < 0.001$]. A further repeated measures ANOVA between the haloperidol and haloperidol plus scopolamine treatment group found no significant difference between these two groups [$F_{main}(1,10) = 0.85$; $P > 0.05$, $F_{time}(10,100) = 171.13$; $P < 0.001$, $F_{interaction}(10,100) = 0.99$; $P > 0.05$]. Scopolamine did not produce an effect on its own. (b) The striatum of anaesthetised rats ($n = 6$). The average basal level of dopamine in the striatum was 0.09 ± 0.02 pmol/20 μl. Two-way ANOVA with repeated measures demonstrated a significant effect of drug treatment [$F(3,20) = 11.54$; $P < 0.01$], a significant effect of time [$F(10,30) = 15.88$; $P < 0.01$] and a significant interaction [$F(30,200) = 53.76$; $P < 0.001$]. A further repeated measures ANOVA between the haloperidol and haloperidol plus scopolamine treatment group found no significant difference between these two groups [$F_{main}(1,10) = 0.34$; $P > 0.05$, $F_{time}(10,100) = 121.13$; $P < 0.001$, $F_{interaction}(10,100) = 0.19$; $P > 0.05$]. Scopolamine did not produce an effect on its own. (c) The nucleus accumbens of awake rats ($n = 6$). The average basal level of dopamine in the nucleus accumbens was 0.06 ± 0.02 pmol/20 μl. Two-way ANOVA with repeated measures demonstrated a significant effect of drug treatment [$F(3,20) = 10.39$; $P < 0.01$], a significant effect of time [$F(10,200) = 11.62$; $P < 0.01$] and a significant interaction [$F(30,200)$]. A further repeated measures ANOVA between the haloperidol and haloperidol plus scopolamine treatment group found no significant difference between these two groups [$F_{main}(1,10) = 0.62$; $P > 0.05$, $F_{time}(10,100) = 143.17$; $P < 0.001$, $F_{interaction}(10,100) = 0.19$; $P > 0.05$]. Scopolamine did not produce an effect on its own. (d) The nucleus accumbens of anaesthetised rats ($n = 6$). The average basal level of dopamine in the nucleus accumbens was 0.05 ± 0.02 pmol/20 μl. Two-way ANOVA with repeated measures demonstrated a significant effect of drug treatment [$F(3,20) = 11.79$; $P < 0.01$], a significant effect of time [$F(10,30) = 16.59$; $P < 0.01$] and a significant interaction [$F(30,200) = 147.98$; $P < 0.001$]. A further repeated measures ANOVA between the haloperidol and haloperidol plus scopolamine treatment group found no significant difference between these two groups [$F_{main}(1,10) = 0.25$; $P > 0.05$, $F_{time}(10,100) = 134.34$; $P < 0.001$, $F_{interaction}(10,100) = 0.48$; $P > 0.05$]. Scopolamine did not produce an effect on its own.

antipsychotic drug administration. Clozapine and haloperidol each caused a marked increase in extracellular dopamine metabolites in both brain regions examined in anaesthetised and awake rats. 5-HIAA levels were also increased in striatal and accumbens perfusates following clozapine or haloperidol administration. However, there was not as large an increase in 5-HIAA levels as compared with the dopamine metabolites following antipsychotic administration. Scopolamine (1 mg kg^{-1}; s.c.) did not alter dopamine metabolite or 5-HIAA levels in either striatal or accumbens perfusates in anaesthetised or awake rats. Furthermore, there was no notable change in clozapine- or haloperidol-induced dopamine metabolite or 5-HIAA levels in perfusates following scopolamine pretreatment. Tables 1 and 2 summarise the drug-induced changes in monoamine metabolite levels.

3.3. Effect of carbachol infusions on dopamine output in the striatum and nucleus accumbens in anaesthetised rats: a reverse dialysis study

Preliminary concentration-effect data (not shown) were obtained using various concentrations of carbachol perfused through the microdialysis probe implanted in the

dorsolateral striatum and the effect upon dopamine in striatal perfusates was identified. From these pilot studies 50 μM carbachol was chosen to pharmacologically probe the striatum and nucleus accumbens. Carbachol increased dopamine, DOPAC and HVA in striatal and accumbens perfusates. When carbachol (50 μM) was co-perfused with scopolamine (100 μM) in the striatum, no increase in the dopamine content of the striatal perfusates were observed. Furthermore, systemic administration of scopolamine (1 mg kg^{-1}) blocked the carbachol-induced efflux of dopamine. Fig. 3a–d summarises these data.

3.4. Effect of clozapine (1 mM) on dopamine output in the striatum and nucleus accumbens of anaesthetised rats: a reverse dialysis study

Clozapine (1 mM) was perfused through the microdialysis probe in either the striatum or nucleus accumbens for 2 h during the sampling period. In the striatum, 1 mM clozapine induced an increase in the levels of dopamine in striatal perfusates which slowly decreased throughout the following 120 min, but remained significantly above baseline (Fig. 4a). Similar increases in dopamine efflux were obtained following perfusion of clozapine through the dialysis probe when implanted in the nucleus accumbens. Thus, increased dopamine levels were observed in the initial accumbens perfusates, which gradually declined towards baseline throughout the 2 h sampling period (Fig. 4b). Scopolamine infused in the striatum or nucleus accumbens did not alter basal levels of dopamine or the dopamine metabolites (Fig. 4a and b). When clozapine and scopolamine (100 μM) were co-infused through the microdialysis probe in the striatum or nucleus accumbens, scopolamine did not alter the clozapine-induced increase in dopamine levels in striatal or accumbens perfusates (Fig. 4a and b).

An infusion of clozapine (1 mM) into the striatum produced a $250 \pm 16\%$ of baseline maximal effect on DOPAC efflux 100 min after the initiation of the infusion. This increase dramatically declined to $120 \pm 8\%$ of baseline during the next 20 min (Fig. 4c). Clozapine administration via the probe into the nucleus accumbens produced a maximal increase in DOPAC ($200 \pm 15\%$) 20 min after the initiation of the clozapine infusion, which declined over the 2 h infusion period to $120 \pm 8\%$ of baseline (Fig. 4d).

Interestingly, when clozapine was co-infused with scopolamine in the striatum, the increase in DOPAC efflux induced by clozapine alone was significantly reduced. An increase in perfusate DOPAC 80 min after the initiation of the infusion produced an increased level of $125 \pm 8\%$ of baseline (Fig. 4c). This reduction by scopolamine of the ability of clozapine to elevate DOPAC was not observed in the nucleus accumbens (Fig. 4d).

HVA levels in both striatal and accumbens perfusates were increased following perfusion with clozapine. However, the increased levels did not reach statistical significance in the striatum until 20 min into the perfusion period ($148 \pm 14\%$) and 40 min for the accumbens ($150 \pm 14\%$). In both brain regions 60 min into the perfusion period with clozapine, the HVA levels in perfusates reached a plateau which was maintained for the remainder of the experiment ($180 \pm 15\%$). When scopolamine was co-infused with clozapine in the striatum, scopolamine reduced the clozapine-induced HVA efflux. Scopolamine co-infusion in the nucleus accumbens had no effect on HVA output (data not shown).

Table 1
Effect on dopamine metabolite and 5-HIAA levels in either the dorsolateral striatum or nucleus accumbens following haloperidol (1 mg kg^{-1}; s.c.) administration in anaesthetised or awake rats either alone or following pretreatment with scopolamine (scop; 1 mg kg^{-1}; s.c.)

Metabolite	Striatum				Nucleus Accumbens			
	Anaesthetised		Awake		Anaesthetised		Awake	
	Alone	Scop	Alone	Scop	Alone	Scop	Alone	Scop
DOPAC	238[b]	229[b]	250[b]	239[b]	229[b]	218[b]	246[b]	239[b]
HVA	221[b]	267[b]	241[b]	234[b]	234[b]	229[b]	265[b]	271[b]
5-HIAA	156[a]	139[a]	155[a]	142[a]	149[a]	158[a]	147[a]	153[a]

Data is expressed as area under the curve as a percentage of vehicle control ($n = 6$).
[a] $P < 0.05$.
[b] $P < 0.01$ vs. control.
One-way ANOVA followed where appropriate with post hoc analysis with the multiple range Newman–Keuls test.

Table 2
Effect on dopamine metabolite and 5-HIAA levels in either the dorsolateral striatum or nucleus accumbens following clozapine (20 mg kg^{-1}; s.c.) administration in anaesthetised or awake rats either alone or following pretreatment with scopolamine (scop; 1 mg kg^{-1}; s.c.)

Metabolite	Striatum				Nucleus Accumbens			
	Anaesthetised		Awake		Anaesthetised		Awake	
	Alone	Scop	Alone	Scop	Alone	Scop	Alone	Scop
DOPAC	245[b]	252[b]	262[b]	240[b]	239[b]	228[b]	253[b]	229[b]
HVA	231[b]	240[b]	239[b]	251[b]	241[b]	256[b]	260[b]	265[b]
5-HIAA	141*	129*	150*	159*	163*	164*	164*	172*

Data is expressed as area under the curve as a percentage of vehicle control ($n = 6$).
[a] $P < 0.05$.
[b] $P < 0.01$ vs. control.
One-way ANOVA followed where appropriate with post hoc analysis with the multiple range Newman–Keuls test.

4. Discussion

The present study has confirmed the ability of clozapine and haloperidol to increase the efflux of dopamine and associated metabolites in both the dorsolateral striatum and nucleus accumbens in vivo following either systemic injection or local infusion.

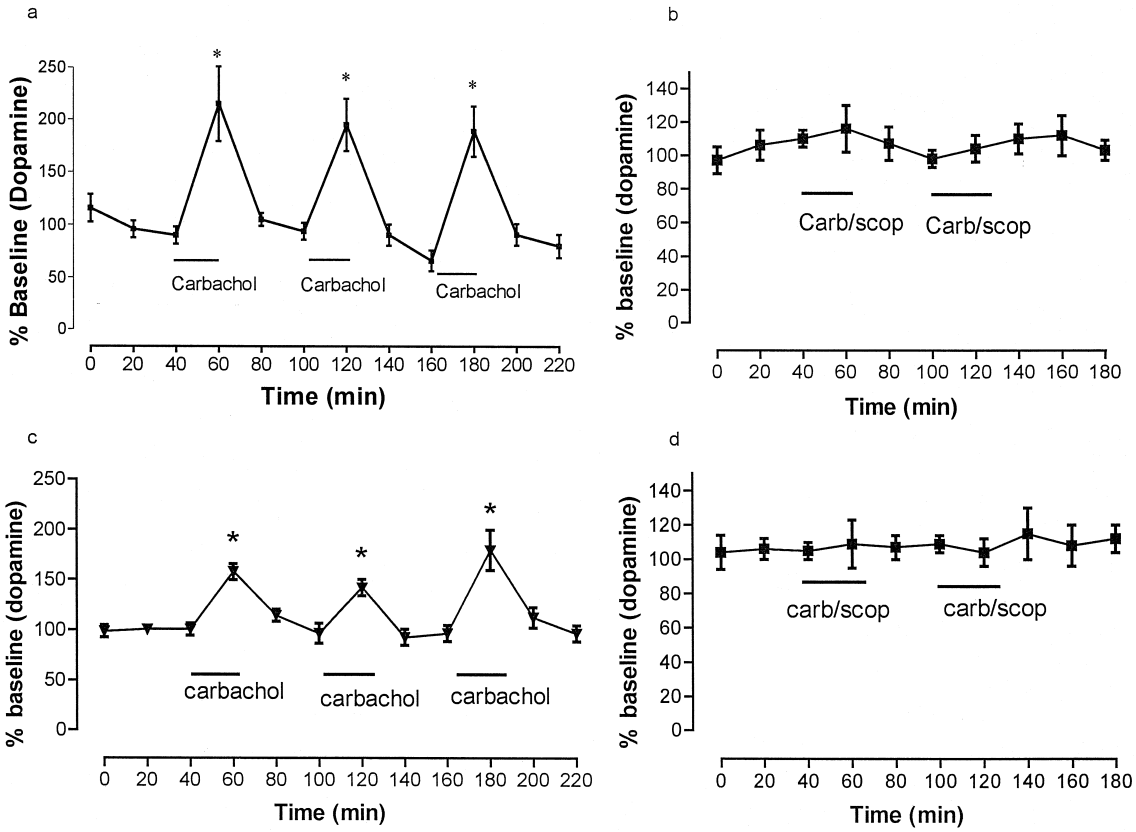

Fig. 3. Effect on dopamine levels in the striatum and nucleus accumbens of reverse dialysis of carbachol (50 μM) and acsf or scopolamine (100 μM). (a) The action of carbachol in the striatum ($n = 4$). Carbachol infusions were initiated as indicated by the bar for 20 min each stimulus period. Carbachol significantly increased dopamine levels during the stimulus period. (b) The action of scopolamine on carbachol induced dopamine levels. Scopolamine significantly antagonised the ability of carbachol to induced dopamine release in the striatum. (c) The action of carbachol in the nucleus accumbens ($n = 4$). Carbachol infusions were initiated as indicated by the bar for 20 min each stimulus period. Carbachol significantly increased dopamine levels during the stimulus period. (d) The action of scopolamine on carbachol induced dopamine levels in the nucleus accumbens. Scopolamine significantly antagonised the ability of carbachol to induced dopamine release.

The principle finding from the present study was the inability of scopolamine to decrease the clozapine-induced dopamine efflux in the striatum and nucleus accumbens when both agents were administered under the various conditions investigated. However, scopolamine attenuated the clozapine-induced increase in DOPAC efflux when both drugs were perfused in the striatum but not in the nucleus accumbens. Systemic scopolamine administration did not decrease clozapine-induced DOPAC efflux. Since it is difficult to ascribe functional significance to metabolite levels, it makes the interpretation of these data difficult. Nevertheless, the metabolic pathways for dopamine are known and metabolite levels do reflect known pharmacological mechanism(s) of action for certain drugs. Microdialysis of extracellular levels reflect an amalgamation of synthesis, release, reuptake and metabolism and without corroborative data it can be difficult to discuss the mechanism of differences between treatment groups especially for metabolites. However, since dopamine recovered by dialysis presumably reflects synaptic events and dopamine is biologically active, it can be comfortably stated that changes in metabolite levels reflect on-going changes in dopamine neurotransmission. Therefore, at the very least the metabolite data, taken together, may suggest that the decrease in DOPAC and HVA levels in the absence of a change in dopamine efflux after co-perfusion of scopolamine and clozapine may be the result of a reduction in dopamine turnover. Alternatively, it could be interpreted as a decrease in the synthesis of dopamine. Nevertheless, scopolamine applied alone in the striatum, nucleus accumbens or administered systemically, did not affect dopamine, DOPAC or HVA extracellular levels in any of the specific brain regions examined. These data, at the dose level examined for scopolamine, suggests that there is no tonic muscarinic influence upon basal dopaminergic tone in the striatum or nucleus accumbens. Rivest and Marsden (1991) using voltammetry reported similar effects of muscarinic antagonists on clozapine-induced DOPAC efflux in the rat striatum. These investigators found that scopolamine (i.p.) or atropine (i.c.v.) partially blocked the clozapine-induced increase in DOPAC in the striatum and to a lesser extent in the nucleus accumbens following systemic administration of clozapine (30 mg kg^{-1}). Surprisingly, Meltzer et al. (1994) using microdial-

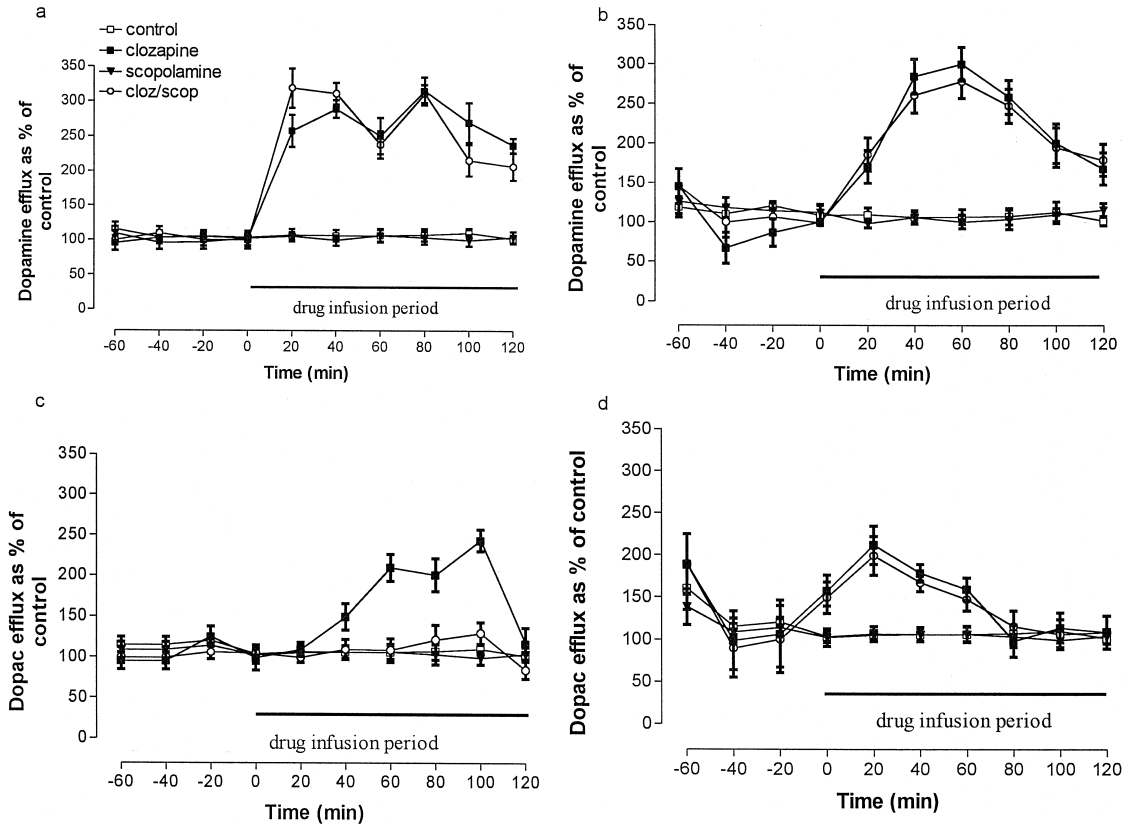

Fig. 4. Reverse dialysis study investigating the effect of clozapine (1 mM), scopolamine (100 μM), vehicle control or clozapine and scopolamine on extracellular dopamine and DOPAC levels in various brain regions of the anaesthetised rat. (a) The effect of a clozapine infusion on dopamine levels in the striatum of anaesthetised rats ($n = 6$). The average basal level of dopamine in the striatum was 0.11 ± 0.02 pmol/20 μl. Two-way ANOVA with repeated measures demonstrated a significant effect of drug treatment [$F(3,20) = 9.19$; $P < 0.01$] a significant effect of time [$F(10,200) = 13.82$; $P < 0.01$] and a significant interaction [$F(10,200) = 102.98$; $P < 0.001$]. A further repeated measures ANOVA between the clozapine and clozapine plus scopolamine treatment group found no significant difference between these two groups [$F_{main}(1,10) = 0.15$; $P > 0.05$, $F_{time}(10,100) = 121.18$; $P < 0.001$, $F_{interaction}(10, 100) = 0.97$; $P > 0.05$]. Scopolamine did not produce an effect on its own. (b) The effect of a clozapine infusion on dopamine levels in the nucleus accumbens of anaesthetised rats ($n = 6$). The average basal level of dopamine in the nucleus accumbens was 0.07 ± 0.02 pmol/20 μl. Two-way ANOVA with repeated measures demonstrated a significant effect of drug treatment [$F(3,20) = 8.16$; $P < 0.01$], a significant effect of time [$F(10,30) = 13.12$; $P < 0.01$] and a significant interaction [$F(10,200) = 167.65$; $P < 0.001$]. A further repeated measures ANOVA between the clozapine and clozapine plus scopolamine treatment group found no significant difference between these two groups [$F_{main}(1,10) = 0.19$; $P > 0.05$, $F_{time}(10,100) = 111.18$; $P < 0.001$, $F_{interaction}(10,100) = 0.59$; $P > 0.05$]. Scopolamine did not produce an effect on its own. (c) The effect of an infusion of clozapine on DOPAC levels in the striatum of anaesthetised rats ($n = 6$). The average basal level of DOPAC in the striatum was 2.06 ± 0.12 pmol/20 μl. Two-way ANOVA with repeated measures demonstrated a significant effect of drug treatment [$F(3,20) = 8.42$; $P < 0.01$], a significant effect of time [$F(10,30) = 13.52$; $P < 0.01$] and a significant interaction [$F(10,200) = 115.98$; $P < 0.001$]. A further repeated measures ANOVA between the clozapine and clozapine plus scopolamine treatment group found a significant difference between these two groups [$F_{main}(1,10) = 6.98$; $P < 0.001$, $F_{time}(10,100) = 101.71$; $P < 0.001$, $F_{interaction}(10,100) = 9.75$; $P < 0.001$]. Scopolamine did not produce an effect on its own. (d) The effect of an infusion of clozapine on DOPAC levels in the nucleus accumbens of anaesthetised rats ($n = 6$). The average basal level of DOPAC in the nucleus accumbens was 1.04 ± 0.10 pmol/20 μl. Two-way ANOVA with repeated measures demonstrated a significant effect of drug treatment [$F(3,20) = 10.86$; $P < 0.01$], a significant effect of time [$F(10,30) = 13.19$; $P < 0.01$] and a significant interaction [$F(30,200) = 185.98$; $P < 0.001$]. Further analysis found no difference between clozapine and clozapine plus scopolamine treatment [$F_{main}(1,10) = 0.05$; $P > 0.05$, $F_{time}(10,100) = 151.16$; $P < 0.001$, $F_{interaction}(10,100) = 0.12$; $P > 0.05$]. Scopolamine did not produce an effect on its own.

ysis did not find a clozapine-induced increase in DOPAC levels in the nucleus accumbens. However, their earlier work, using similar protocols and drug dosages, found that clozapine increased DOPAC levels in the accumbens (Ichikawa and Meltzer, 1991). It is difficult to reconcile their more recent finding (Meltzer et al., 1994) that pretreated with scopolamine, followed by clozapine, produced a very weak increase in DOPAC levels when one also considers their two previous studies.

Previously, Memo et al. (1988) found that scopolamine, in a dose-dependent manner, was able to decrease basal tissue levels of DOPAC and HVA in the prefrontal cortex and hippocampus in Sprague–Dawley rats. This result suggests that in some brain regions cholinergic mechanisms exert a tonic influence upon dopaminergic metabolism. Indeed, our data demonstrate the ability of a muscarinic agonist to increase the efflux of dopamine and associated metabolites in both the dorsolateral striatum and

nucleus accumbens, but not in medial prefrontal cortex (data not shown). In the present study, we have shown the ability of a muscarinic acetylcholine agonist to increase dopamine extracellular levels in both the striatum and nucleus accumbens, but the inability of a muscarinic acetylcholine antagonist to block the dopamine elevating action of clozapine, in specific brain regions that demonstrate an interplay between cholinergic and dopaminergic neurotransmission.

When D_2 dopamine receptors are blocked, dopamine efflux is increased, resulting in stimulation of dopamine D_1 receptors and increased output of acetylcholine in the striatum. Consequently, a muscarinic antagonist may be able to reduce some biochemical measures of dopaminergic neurotransmission by reducing the postsynaptic action of antipsychotic-induced acetylcholine efflux. Imperato et al. (1993) modestly reduced the increase in clozapine-induced acetylcholine output with SCH 23390, suggesting that clozapine increases acetylcholine efflux via an additional mechanism which may include presynaptic muscarinic M_2 receptor blockade. Muscarinic M_1 and M_4 receptors are located presynaptically within the striatum as presynaptic heteroreceptors on glutamatergic terminals (Hersch and Levey, 1995). In this context, Zorn et al. (1994) suggest that clozapine may have an agonist activity at M_4 acetylcholine receptors. However, activation of muscarinic M_1 or M_4 receptors will decrease glutamatergic neurotransmission (Hersch and Levey, 1995; Smolders et al., 1997; Rawls and McGinty, 1998) and may result in a decrease in dopamine efflux in the striatum via a direct action on striatal glutamatergic nerve terminals and via a transynaptic mechanism involving disinhibition through decreased GABAergic transmission in the striatonigral pathway. This mechanism results in enhanced GABA tone in the thalamus which inhibits glutamate in the cortex and decreases glutamate neurotransmission in the striatum.

In contrast to the earlier finding of Meltzer et al. (1994), our study in conscious rats using the same drug protocol, failed to note a change in dopamine efflux in either the striatum or nucleus accumbens when either systemic clozapine or haloperidol was administered after scopolamine. It was noted that striatal perfusates from scopolamine and clozapine pretreated animals had a tendency towards lower levels of DOPAC than clozapine plus vehicle treated animals. This effect was not statistically significant.

Meltzer et al. (1994) have previously suggested that the clozapine-induced increase in dopamine efflux is attributable solely to a muscarinic agonist action of clozapine which requires an intact cholinergic system. The idea that a decrease in clozapine-induced dopamine levels following pretreatment with scopolamine appears to be at odds with the current understanding of the striatonigral–thalamus–cortex neurocircuitry of the basal ganglia, if one posits a transsynaptic mechanism following systemic administration of clozapine. One final possibility may be that the increase in clozapine-induced acetylcholine interacts with muscarinic M_4 receptors, which attenuates GABA release in the striatonigral pathway and consequently increases activity dependent dopamine. The present study involving reverse microdialysis of clozapine and scopolamine through the microdialysis probe into the striatum does not, however, support this hypothesis. However, at the doses utilised in both this and Meltzer's study, clozapine is known to interact with dopamine receptors (Protais et al., 1994). Indeed, the dose of 20 mg kg^{-1} (i.p.) is 10 times its ED_{50} dose against dopamine agonist-induced behaviours (Protais et al., 1994). Currently, the accepted mechanism for D_2 antagonists to increase dopaminergic activity is via D_2 somatodendritic autoreceptors with a contribution from presynaptic nerve terminal autoreceptors (Wolf et al., 1987; Westerink and De Vries, 1989; Santiago and Westerink, 1991; Westerink et al., 1992; Pucak and Grace, 1994). Thus, it would be surprising if this D_2 component of clozapine did not act, at least in part, to increase dopamine extracellular levels as we found.

In summary, we did not reproduce the finding of Meltzer et al. (1994) that scopolamine prevents the clozapine-induced increase in dopamine efflux in the striatum. However, we identified a scopolamine-reversible component to clozapine-induced DOPAC and HVA elevation following local application of scopolamine and clozapine into the striatum. It was noted, however, that following systemic administration of scopolamine and clozapine, there was a trend towards lower levels of DOPAC in striatal and accumbens perfusates. These data are in partial agreement with Rivest and Marsden (1991) who observed a decreased clozapine-induced elevation of extracellular DOPAC after scopolamine pretreatment. Apart from minor differences in our dialysis technique from that of Meltzer et al. (1994), the main difference between the present study and Meltzer's investigation was the strain of rat used; Wistar rats and Sprague–Dawley rats, respectively. It is conceivable that strain differences exit which thus provide different results.

We conclude that the dopamine elevating action of clozapine in Wistar rats is primarily due to its dopamine D_2 receptor antagonist properties. The differences observed between the dorsolateral striatum and nucleus accumbens in the DOPAC response to clozapine and scopolamine following reverse dialysis may be attributed to the differences in the cholinergic innervation of these two structures. We suggest that further empirical investigations are required to address the complex dopaminergic and cholinergic interactions within the basal ganglia.

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