Muscarinic antagonists attenuate the increase in accumbens and striatum dopamine metabolism produced by clozapine but not by haloperidol

¹Robert Rivest & *Charles A. Marsden

¹Department of Pharmacology, Medical School, University of Sherbrooke, Quebec, Canada J1H-5N4 and *Department of Physiology and pharmacology, Medical School, Queen's Medical Centre, University of Nottingham, Nottingham, UK

1 The effect of the muscarinic antagonists, scopolamine and atropine, were examined on the increase in extracellular 3,4-dihydroxyphenylacetic acid (DOPAC) in the nucleus accumbens and the striatum induced by haloperidol and clozapine by use of *in vivo* differential pulse voltammetry with carbon fibre electrodes in anaesthetized rats.

2 Animals received saline (1 ml kg⁻¹, s.c.), scopolamine (1 mg kg⁻¹, o.p.) or atropine (20 µg, i.c.v.) followed 15 min later by saline (10 µl, i.c.v.), haloperidol (1 mg kg⁻¹, s.c.) or clozapine (30 mg kg⁻¹, i.p.) and extracellular DOPAC was simultaneously recorded in the nucleus accumbens and the striatum every 5 min for 60 min after drug administration.

3 Scopolamine or atropine alone had no effect on the DOPAC peak height but attenuated the increase in extracellular DOPAC induced by clozapine in both brain regions. Neither scopolamine nor atropine altered the haloperidol-induced increase in accumbens or striatal extracellular DOPAC.

4 The present results demonstrate that muscarinic antagonists attenuate the increase in accumbens and striatal dopamine metabolism *in vivo* produced by the atypical neuroleptic clozapine but not the haloperidol-induced increase in dopamine metabolism. The results indicate that central muscarinic receptors are involved in the actions on dopaminergic function of clozapine but not haloperidol.

Keywords: Neuroleptics; DOPAC; *in vivo* voltammetry; scopolamine; atropine

Introduction

The attenuation of psychotic symptoms with most neuroleptics is generally associated with the development of extrapyramidal side-effects (Marsden *et al.*, 1975). It is believed that the blockade of dopamine receptors located in the meso-accumbens and meso-cortical dopamine neurones produces the therapeutic effects of the neuroleptics while the extrapyramidal side-effects result from the increase in striatal cholinergic activity that follow the blockade of dopamine receptors in this brain region (Snyder *et al.*, 1974; Meltzer & Stahl, 1976). However, some neuroleptics (e.g. clozapine) are distinguished by having a lower incidence of extrapyramidal side-effects and have been named "atypical" neuroleptics (Herman & Pleasure, 1963; Gerlach & Simmelsgard, 1978).

Clozapine binds with high affinity to histamine and muscarinic receptor sites (Creese *et al.*, 1976; Seeman *et al.*, 1976; Miller & Hiley, 1974; Snyder *et al.*, 1974; Richelson & Nelson, 1984) and with less affinity to dopamine and 5-hydroxytryptamine receptor and α -adrenoceptor sites (Peroutka *et al.*, 1977; Fjalland *et al.*, 1977; Meltzer *et al.*, 1989). It has been suggested that blockade of muscarinic receptors by clozapine helps to prevent the extrapyramidal side-effects common with most neuroleptics (Snyder *et al.*, 1974).

There is evidence that clozapine can effectively prevent cholinergic mediated effects. Thus, clozapine will inhibit oxotremorine induced tremors and physostigmine-induced mortality in mice (Fjalland *et al.*, 1977) and antagonize acetylcholine induced hindgut contractions in the crayfish (Herrling & Misbach-Lesenne, 1982). The anti-cholinergic activity of clozapine was shown in a study where clozapine reversed the acetylcholine-induced contraction of guinea-pig isolated ileum, reduced tremors induced by oxotremorine in mice and

increased pupillary aperture in mice (Sayers & Bürki, 1976). Moreover, Racagni *et al.* (1976) reported that clozapine blocked the increase in acetylcholine content and the decrease in turnover rate induced by arecoline and oxotremorine in the striatum. In contrast, clozapine does not reduce contralateral torsion in guinea-pigs induced by injection of physostigmine into the right carotid artery (De Jonge & Funcke, 1962). It has been reported that clozapine (20 mg kg⁻¹, i.p.) does not increase *in vitro* acetylcholine turnover and release and *in vivo* release in the nucleus accumbens and the striatum (Costa *et al.*, 1978; Compton & Johnson, 1988) although a high dose of clozapine (50 mg kg⁻¹, i.v.) did increase the release of acetylcholine measured by means of push-pull cannulae in the striatum of gallamine-immobilized cat (Costa *et al.*, 1978). Thus while not every study demonstrated clozapine to possess anticholinergic properties, it is generally accepted that one of its actions involves the antagonism of muscarinic receptors.

On the other hand, there is also evidence to support the dopamine antagonist effects of clozapine. For example clozapine attenuates behavioural hyperactivity induced by peripheral or central administration of dopamine agonists in rat (Costall & Naylor, 1976; Chipkin & Latranyi, 1987; Tschanz & Rebec, 1988; Criswell *et al.*, 1989; McMillen *et al.*, 1989; Murray & Waddington, 1990). Moreover, acute administration of clozapine increases the firing rate of dopamine cells in the ventral tegmental area and the substantia nigra (Chiodo & Bunney, 1983) and the release and metabolism of dopamine in the nucleus accumbens and the striatum *in vivo* (Maidment & Marsden, 1987; Stamford *et al.*, 1988; Moghaddam & Bunney, 1990).

Since clozapine has high affinity for muscarinic receptors in the brain and because it is well established that cholinergic and dopaminergic systems interact in the striatum (Sethy & Van Woert, 1974; Le Moine *et al.*, 1990), we have examined whether the blockade of muscarinic receptors with scopolamine or atropine can alter the increase in extracellular 3,4-dihydroxyphenylacetic acid (DOPAC) in the nucleus accumbens and the striatum induced by clozapine and haloperidol.

¹ Author for correspondence at: Department of Psychology, Center for Studies in Behavioral Neurobiology, Concordia University, 1455 de Maisonneuve West, Montreal, Quebec, Canada H3G 1M8.

Extracellular DOPAC was simultaneously recorded in the two brain regions by use of differential pulse voltammetry with carbon fibre electrodes (Rivest *et al.*, 1991).

Methods

Animals

Male Wistar rats (250 and 280 g) were housed, 5 per cage, in a room (21°C) having a 12 h light/dark cycle. Food and water were available *ad libitum*.

Voltametric procedures

Working electrodes, made from one pyrolytic 12 µm carbon fibre, and the reference and auxiliary electrodes were prepared as previously described (Sharp *et al.*, 1984). To obtain sensitivity and adequate separation between ascorbic acid, catechol and indoles, the working electrodes were electrically pretreated by a method based on that of Gonon *et al.* (1980) and modified by Crespi *et al.* (1984). First, working, reference and auxiliary electrodes were immersed in a phosphate buffered saline solution (pH 7.4, 0.1 M) and connected to a polarograph (Princeton Applied Research 174A). A triangular waveform (0 to 3.0 V, 70 Hz) was applied for 10 s, then a continuous potential of +1.5 V, -0.9 V for 5 s each. This was followed by another continuous potential varying from 1.1 V to 1.5 V, depending on the sensitivity of the electrode. Sensitivity and selectivity of the electrodes were examined by performing one voltammetric recording in a phosphate buffered solution containing ascorbic acid (5×10^{-3} M), DOPAC (1×10^{-4} M) and 5-hydroxyindoleacetic acid (5-HIAA, 5×10^{-5} M). Electrodes showing poor separation, low sensitivity or high charging current were discarded. Differential pulse voltammetric parameters for the *in vitro* recording were as follows: potential range: -0.2 to 0.350 V, increase scan rate: 5 mV s^{-1} , pulse amplitude 50 mA, pulse frequency 2 s^{-1} .

Surgery and experimental procedure

Rats were anaesthetized with chloral hydrate (400 mg kg^{-1} , i.p.) which was maintained during the experiment by additional injections ($70 \text{ mg kg}^{-1} \text{ h}^{-1}$). Animals were mounted on a stereotaxic frame with the upper incisor bar set at 3.3 mm below the interaural line. A guide cannula (23 G) was stereotactically implanted 2 mm above the left lateral ventricle and fixed with dental cement (Espe Durelon). The coordinates used were A/P: -0.2; L: 1.4; V: 4.2, with reference to bregma (Paxinos & Watson, 1982). For the implantation of the voltammetric electrodes, one hole (approximately 2 mm diam) was made in the cranium above the striatum and one above the nucleus accumbens. The dura was carefully ruptured and removed with a hypodermic needle. One working electrode was stereotactically implanted in the medio-dorsal part of the striatum (A/P: +0.7, L: 3.0, V: 4.0 from the surface of the brain) and another into the nucleus accumbens (A/P: +1.7, L: 1.5, V: 7.0 from the surface of the brain) with reference to bregma (Paxinos & Watson, 1982). Auxiliary and reference electrodes were positioned on the surface of the brain. Electrodes were connected to the polarograph and the DOPAC oxidation peak was recorded every 5 min during a 60 min stabilisation period before the administration of any drugs. To detect DOPAC exclusively, the initial potential applied to the working electrode was set to -50 mV which corresponded with the end of the ascorbic acid peak and the scan was stopped at 200 mV corresponding to the beginning of the 5-HIAA + uric acid peak.

Saline (1 ml kg^{-1} , i.p.) or scopolamine (1 mg kg^{-1} , i.p.), atropine ($20 \mu\text{g}$, i.c.v.) were administered followed 15 min later by saline (i.c.v.) or haloperidol (1 mg kg^{-1} , s.c.) or clozapine (30 mg kg^{-1} , i.p.). Extracellular DOPAC was recorded every 5 min for the following 60 min.

Histology

The placement of the electrodes was checked histologically. Brains were removed, frozen and sectioned with a microtome, slices mounted on glass slides and stained with cresyl violet for examination. For i.c.v. injection, $2 \mu\text{l}$ of ink was injected by the same procedure as used for atropine. The brains were removed, sliced transversely at the injection sites and the dispersion of ink in the ventricle directly checked.

Statistics

The DOPAC peak was recorded every 5 min. Peak heights were measured and converted to a percentage of the basal level calculated from the mean of the last four peaks before drug administration. Differences between groups were assessed by analysis of variance followed by a Scheffe's F Test using Stat-view II Statistical Software, $*P < 0.05$.

Drugs

Scopolamine and atropine (Sigma) were dissolved in saline. Clozapine (Sandoz) was dissolved in saline-HCl 0.3 M and the pH adjusted to 4-5 with saline-NaOH 1 M. Haloperidol (Janssen) was obtained in an injectable ampoule (5 mg ml^{-1}) and was diluted with saline.

Results

Compared with saline (1 ml kg^{-1} , i.p.), scopolamine (1 mg kg^{-1} , i.p.) and atropine ($20 \mu\text{g}$, i.c.v.) had no effect on extracellular DOPAC in the nucleus accumbens or the striatum (Figure 1 and 2). Haloperidol (1 mg kg^{-1} , s.c.) increased extracellular striatal and accumbens DOPAC (Figure 1 and 2). Pretreatment with scopolamine 15 min before haloperidol produced a small reduction of the increase in dopamine metabolism induced by haloperidol in the nucleus accumbens (Figure 1) but the effect of scopolamine did not reach a significant level ($F = 3.7$, d.f. = 11, 121, $P < 0.08$). Figure 1 shows also that scopolamine did not alter the increase in extracellular DOPAC induced by haloperidol in the striatum ($F = 0.5$, d.f. = 11, 121, $P < 0.51$). The central injection of atropine ($20 \mu\text{g}$, i.c.v.) 15 min before haloperidol did not reduce the increase in extracellular DOPAC induced by haloperidol either in the nucleus accumbens ($F = 3.5$, d.f. = 9, 99, $P < 0.09$) or the striatum ($F = 0.1$, d.f. = 10, 110, $P < 0.68$). Clozapine (30 mg kg^{-1} , i.p.) also increased the DOPAC peak height in both the nucleus accumbens and the striatum (Figure 3) and pretreatment with scopolamine attenuated this increase in both regions (nucleus accumbens: $F = 17.7$, d.f. = 13, 143, $P < 0.001$; striatum: $F = 24.9$, d.f. = 13, 143, $P < 0.001$). Pretreatment with atropine ($20 \mu\text{g}$, i.c.v.) also attenuated the effect produced by clozapine in both brain regions (nucleus accumbens: $F = 34.7$, d.f. = 10, 110, $P < 0.001$; striatum: $F = 41.1$, d.f. = 10, 110, $P < 0.001$) (Figure 4).

Discussion

Peripheral or central administration of muscarinic antagonists attenuated the increase in accumbens and striatal extracellular DOPAC induced by the atypical neuroleptic clozapine but not by the typical neuroleptic haloperidol. The present results agree with a previous report suggesting that cholinergic mechanisms may be involved in the effects of clozapine on dopaminergic neurones (Chiodo & Bunney, 1985). In this study, chronic co-administration of haloperidol with a muscarinic antagonist produced a similar response on the firing of dopaminergic neurones in the ventral tegmental area and the substantia nigra to that observed after a chronic treatment with clozapine (Chiodo & Bunney, 1985).

The finding that the muscarinic antagonists attenuated the clozapine-induced increase in striatal and accumbens extra-

Figure 1 Effect of saline (—), scopolamine (Δ), haloperidol (■) and scopolamine + haloperidol (□) on extracellular DOPAC in the nucleus accumbens (a) and the striatum (b). Saline (1 ml kg^{-1} , i.p.) or scopolamine (1 mg kg^{-1} , i.p.) were injected followed 15 min later by saline (i.p.) or haloperidol (1 mg kg^{-1} , s.c.). DOPAC peak height was monitored every 5 min for 60 min. The basal extracellular DOPAC level was estimated from the mean of the last four peaks recorded before drug administration and are shown as 100%. Each value is the mean of the recordings made in 6 animals (7 for the group scopolamine + haloperidol); s.e.mean shown by vertical bars. The basal levels of DOPAC in the nucleus accumbens and the striatum were estimated from the DOPAC peak heights *in vitro* and were 41.2 ± 6.4 and 39.4 ± 7.8 ($\mu\text{M} \pm \text{s.d.}$) respectively ($n = 12$).

cellular DOPAC but had no effects on the haloperidol-induced increase may reflect the different profile of the two neuroleptics for affinity at various receptor sites. Clozapine has affinity for a wide range of receptor sites; muscarinic and histamine receptors as well as lower affinity for dopamine, nor-adrenaline and 5-hydroxytryptamine receptors (Miller & Hiley, 1974; Creese *et al.*, 1976; Seeman *et al.*, 1976; Perry *et al.*, 1983; Richelson & Nelson, 1984; Meltzer *et al.*, 1989). While the increase in dopamine metabolism induced by haloperidol probably involves a specific action at dopamine receptors, it is apparent from the present results that the same effect produced by clozapine is dependent upon the participation of muscarinic receptors. Furthermore, as the effects of clozapine on dopamine metabolism were reduced following intracerebroventricular administration of atropine, the muscarinic receptors involved are situated centrally. It is however possible that a certain quantity of atropine injected into the lateral ventricle leaked to the periphery and produced some effects in which both peripheral and central receptors may have been involved.

It remains to be determined what mechanisms operate in the muscarinic receptor involvement in the clozapine-induced increase in dopamine metabolism. With the doses used in the present study, both muscarinic antagonists markedly attenuated the clozapine-induced increase in extracellular DOPAC in the nucleus accumbens and the striatum. While the present findings suggest that muscarinic receptors play a significant role in the effects of clozapine, it is unlikely that the increase

Figure 2 Effect of saline (—), atropine (Δ), haloperidol (■) and atropine + haloperidol (□) on the extracellular DOPAC in the nucleus accumbens (a) and the striatum (b). Saline (1 ml kg^{-1} , i.p.) or atropine ($20 \mu\text{g}$, i.c.v.) were injected followed 15 min later by saline (i.p.) or haloperidol (1 mg kg^{-1} , s.c.). DOPAC peak height was monitored every 5 min for 60 min. The basal extracellular DOPAC level was estimated from the mean of the last four peaks recorded before drug administration and are shown as 100%. Each value is the mean of the recordings made in 6 animals ($n = 5$ for the atropine + saline group); s.e.mean shown by vertical bars.

in DOPAC induced by the neuroleptic depends entirely on the blockade of these receptors since neither scopolamine nor atropine increased DOPAC *per se*. Another reason why clozapine is probably not acting as a pure muscarinic antagonist is that while atropine increases acetylcholine but not dopamine release in the striatum (Westerink *et al.*, 1990), clozapine (50 mg kg^{-1} ; but not 20 mg kg^{-1} , i.v.) has been reported to increase the release of acetylcholine in the caudate nucleus measured using push-pull cannulae in the gallamine-immobilized cat (Stadler *et al.*, 1974) as well as increasing dopamine release and metabolism in the striatum of the rat (Maidment & Marsden, 1987; Stamford *et al.*, 1988; Moghaddam & Bunney, 1990). However clozapine (20 mg kg^{-1}) has no effect on acetylcholine release *in vitro* in the rat nucleus accumbens and striatum (Compton & Johnson, 1989). To our knowledge, it has not been determined whether clozapine alters acetylcholine release in the rat *in vivo* at the dose used in the present study.

One possibility is that clozapine increases DOPAC by mechanisms that involve the participation of both muscarinic and dopamine receptors. Therefore, clozapine may act on muscarinic receptors located on cholinergic as well as dopaminergic neurones since lesion studies with 6-hydroxydopamine have shown that muscarinic receptors may be located on dopaminergic nerve terminals in the striatum (De Belleruche *et al.*, 1979; Gurwitz *et al.*, 1980). It is feasible that clozapine antagonises muscarinic auto-receptors located on cholinergic terminals resulting in release of acetylcholine that acts on cholinergic receptors located on dopaminergic neurones

Figure 3 Effect of scopolamine on the increase in extracellular DOPAC induced by clozapine in the nucleus accumbens (a) and the striatum (b). Saline (1 ml kg^{-1} , i.p.) or scopolamine (1 mg kg^{-1} , i.p.) were injected followed 15 min later by clozapine (30 mg kg^{-1} , i.p.). DOPAC peak height was monitored every 5 min for 60 min. Groups are: saline + clozapine (■) and scopolamine + clozapine (□). The basal extracellular DOPAC level was estimated from the mean of the last four peaks recorded before drug administration and are shown as 100%. Each value is the mean of the recordings made in 6 animals ($*P < 0.05$, Scheffe's F Test) for the clozapine group and 9 animals for scopolamine + clozapine group; s.e.mean shown by vertical bars.

Figure 4 Effect of atropine on the increase in extracellular DOPAC induced by clozapine in the nucleus accumbens (a) and the striatum (b). Saline (1 ml kg^{-1} , i.p.) or atropine ($20 \mu\text{g}$, i.c.v.) were injected followed 15 min later by clozapine (30 mg kg^{-1} , i.p.). DOPAC peak height was monitored every 5 min for 60 min. The basal extracellular DOPAC level was estimated from the mean of the last four peaks recorded before drug administration and are shown as 100%. Groups are: saline + clozapine (■) and atropine + clozapine (□). Each value is the mean of 6 recordings with s.e.mean shown by vertical bars; $*P < 0.05$ (Scheffe's F Test).

to cause an increase in dopamine metabolism. In contrast, by blocking both M_1 and M_2 sites, atropine causes the release of acetylcholine but also blocks the effects of the neurotransmitter on dopamine cells and so has no effect on dopamine metabolism on its own. The effects of clozapine on DOPAC are antagonized by both atropine and scopolamine as under these conditions both M_1 and M_2 sites are antagonized. Further investigations are needed to examine this hypothesis.

An alternative explanation is that the increase in dopamine metabolism by clozapine resulted from the blockade of dopamine receptors but can be regulated by muscarinic function. According to this hypothesis, muscarinic antagonists should attenuate both the clozapine and haloperidol-induced increase in dopamine metabolism. In the present study, scopolamine and atropine caused a small but not significant reduction in the effect produced by haloperidol. In contrast to the present study, earlier reports have suggested that muscarinic antagonists can inhibit haloperidol-induced increases in dopamine metabolism *ex vivo* (Andén & Bédard, 1971; Andén, 1972; Westerink & Korf, 1975). Several factors may account for the

difference in the results between these studies and the present one. Thus, while the present study investigated changes in extracellular DOPAC concentrations in the nucleus accumbens and the striatum *in vivo*, the previous work reported changes in tissue dopamine and homovanillic acid concentrations in the nucleus accumbens and the striatum of rats and rabbits. Moreover, the drugs and doses differed as well as the time at which dopamine metabolism was measured following drug treatments.

The present study demonstrates that muscarinic receptors are involved in the increase in dopamine metabolism induced by the atypical neuroleptic clozapine but not by the typical neuroleptic haloperidol after acute administration. The results support the view that the affinity of clozapine for muscarinic receptors is important for the effect of the neuroleptic on dopaminergic systems and may account for the therapeutic profile of the drug.

This work was supported by the Wellcome Trust and by a studentship to R.R. by the FCAR of Quebec.

References

- ANDÉN, N.-E. (1972). Dopamine turnover in the corpus striatum and the limbic system after treatment with neuroleptic and anti-acetylcholine drugs. *J. Pharm. Pharmacol.*, **24**, 905-906.
- ANDÉN, N.-E. & BÉDARD, P. (1971). Influences of cholinergic mechanisms on the function and turnover of brain dopamine. *J. Pharm. Pharmacol.*, **23**, 460-462.
- CHIODO, L.A. & BUNNEY, B.S. (1983). Typical and atypical neuroleptics: differential effects of chronic administration on the activity of A9 and A10 midbrain dopaminergic neurons. *J. Neurosci.*, **3**, 1607-1619.
- CHIODO, L.A. & BUNNEY, B.S. (1985). Possible mechanisms by which repeated clozapine administration differentially affects the activity of two subpopulations of midbrain dopamine neurons. *J. Neurosci.*, **5**, 2539-2544.
- CHIPKIN, R.E. & LATRANYI, M.B. (1987). Similarity of clozapine and SCH 23390 in reserpinized rats suggests a common mechanism of

- action. *Eur. J. Pharmacol.*, **136**, 371–375.
- COMPTON, D.R. & JOHNSON, K.M. (1989). Effects of acute and chronic clozapine and haloperidol on *in vivo* release of acetylcholine and dopamine from striatum and nucleus accumbens. *J. Pharmacol. Exp. Ther.*, **248**, 521–530.
- COSTA, E., CHENEY, D.L., MAO, C.C. & MORONI, F. (1978). Action of antischizophrenic drugs on the metabolism of γ -aminobutyric acid and acetylcholine in globus pallidus, striatum and n. accumbens. *Fed. Proc.*, **37**, 2408–2414.
- COSTALL, B. & NAYLOR, R.J. (1976). A comparison of the abilities of typical neuroleptic agents and of thioridazine, clozapine, sulpiride and metoclopramide to antagonise the hyperactivity induced by dopamine applied intracerebrally to areas of the extrapyramidal and mesolimbic systems. *Eur. J. Pharmacol.*, **40**, 9–19.
- CREESE, I., BURT, D.R. & SNYDER, S. (1976). Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science*, **192**, 481–483.
- CRESPI, F., PARET, J., KEANE, P.E. & MORRE, M. (1984). An improved differential pulse voltammetry technique allows the simultaneous analysis of dopaminergic and serotonergic activities *in vivo* with a single carbon-fibre electrode. *Neurosci. Lett.*, **52**, 159–164.
- CRISWELL, H.E., MUELLER, R.A. & BREESE, G.A. (1989). Clozapine antagonism of D-1 and D-2 dopamine receptor-mediated behaviors. *Eur. J. Pharmacol.*, **159**, 141–147.
- DE BELLEROCHE, S. & GARDINER, I.M. (1982). Cholinergic action in the nucleus accumbens: modulation of dopamine and acetylcholine release. *J. Pharmacol.*, **75**, 359–365.
- DE JONGE, M.C. & FUNCKE, A.B.H. (1962). Sinistrotorsion in guinea pigs as a method of screening central anticholinergic activity. *Arch. Int. Pharmacodyn.*, **137**, 375–382.
- FJALLAND, B., CHRISTENSEN, A.V. & HYTTTEL, J. (1977). Peripheral and central muscarinic receptor affinity of psychotropic drugs. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **301**, 5–9.
- GERLACH, J. & SIMMELSGARD, H. (1978). Tardive dyskinesia during and following treatment with haloperidol, biperiden, thioridazine and clozapine. *Psychopharmacology*, **59**, 105–112.
- GONON, F., BUDA, M., CESPUGLIO, R., JOUVET, M. & PUJOL, J.-F. (1980). *In vivo* electrochemical detection of catechols in the neostriatum of anaesthetized rats: dopamine or DOPAC? *Nature*, **286**, 902–904.
- GURWITZ, D., KLOOG, Y., EGOZI, Y. & SOKOLOVSKY, M. (1980). Central muscarinic receptor degeneration following 6-hydroxydopamine lesion in mice. *Life. Sci.*, **26**, 79–84.
- HERMAN, E. & PLEASURE, H. (1963). Clinical evaluation of thioridazine and chlorpromazine in chronic schizophrenia. *Disease Nervous System*, **24**, 54–59.
- HERRLING, P.L. & MISBACH-LESENNE, B. (1982). Effects of clozapine in a selective muscarinic bioassay and on single cells of the rat hippocampus. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **320**, 20–25.
- LE MOINE, C., TISON, F. & BLOCH, B. (1990). D2 dopamine receptors gene expression by cholinergic neurons in the rat striatum. *Neurosci. Lett.*, **117**, 248–252.
- MAIDMENT, N.T. & MARSDEN, C.A. (1987). Acute administration of clozapine, thioridazine and metoclopramide increases extracellular DOPAC and decreases extracellular 5-HIAA, measured in the nucleus accumbens and striatum of the rat using *in vivo* voltammetry. *Neuropharmacology*, **26**, 187–193.
- MARSDEN, C.D., TARSY, D. & BALDESSARINI, R.J. (1975). Spontaneous and drug-induced movement disorders in psychotic patients. In *Psychiatric Aspects of Neurological Disease*, ed. Benson, D.F. & Blumer, D. p. 219. New York: Grune and Stratton.
- MCMILLEN, B.A., DAVANZO, E.A., SONG, A.H., SCOTT, S.M. & RODRIGUEZ, M.E. (1989). Effects of classical and atypical antipsychotic drugs on isolation-induced aggression in male mice. *Eur. J. Pharmacol.*, **160**, 149–153.
- MELTZER, H.Y. & STAHL, S.M. (1976). The dopamine hypothesis of schizophrenia: a review. *Schizophr. Bull.*, **2**, 19–76.
- MELTZER, H.Y., MATSUBARA, S. & LEE, J.C. (1989). Classification of typical and atypical drugs on the basis of dopamine D-1, D-2 and serotonin₂ pKi values. *J. Pharmacol. Exp. Ther.*, **251**, 238–246.
- MILLER, R.J. & HILEY, C.R. (1974). Anti-muscarinic properties of neuroleptics and drugs-induced parkinsonism. *Nature*, **248**, 596–597.
- MOGHADDAM, B. & BUNNEY, B. (1990). Acute effects of typical and atypical antipsychotic drugs on the release of dopamine from prefrontal cortex, nucleus accumbens, and striatum of the rat: an *in vivo* microdialysis study. *J. Neurochem.*, **54**, 1755–1760.
- MURRAY, A.M. & WADDINGTON, J.L. (1990). The interaction of clozapine with dopamine D₁ versus dopamine D₂ receptor-mediated function: behavioural indices. *Eur. J. Pharmacol.*, **186**, 79–86.
- PAXINOS, G. & WATSON, C. (1982). *The Rat Brain in Stereotaxic Coordinates*, New York: Academic press.
- PEROUTKA, S.J., U'PRICHARD, D.C., GREENBERG, D.A. & SNYDER, S.H. (1977). Neuroleptic drug interactions with norepinephrine alpha receptor binding sites in rat brain. *Neuropharmacology*, **16**, 549–556.
- PERRY, D.B., SIMON, P.R. & U'PRICHARD, D.C. (1983). Interaction of neuroleptic compounds at alpha 2-adrenergic receptor affinity states in bovine caudate nucleus. *Eur. J. Pharmacol.*, **95**, 315–318.
- RACAGNI, G., CHENEY, D.L., TRABUCCHI, M. & COSTA, E. (1976). *In vivo* actions of clozapine and haloperidol on the turnover rate of acetylcholine in rat striatum. *J. Pharmacol. Exp. Ther.*, **196**, 323–332.
- RICHELSON, E. & NELSON, A. (1984). Antagonism by neuroleptics of neurotransmitter receptors of normal human brain *in vitro*. *Eur. J. Pharmacol.*, **103**, 197–294.
- RIVEST, R., JOLICOEUR, F.B. & MARSDEN, C.A. (1991). Neurotensin causes a greater increase in the metabolism of dopamine in the accumbens than in the striatum *in vivo*. *Neuropharmacology*, **30**, 25–33.
- SAYERS, A.C. & BÜRKI, H.R. (1976). Anti-acetylcholine activities of psychoactive drugs: a comparison of the [³H] quinuclidinyl benzilate binding assay with conventional methods. *J. Pharm. Pharmacol.*, **28**, 252–253.
- SEEMAN, P., LEE, T., CHAU-WONG, M. & WONG, K. (1976). Anti-psychotic drug doses and neuroleptic/dopamine receptors. *Nature*, **261**, 717–719.
- SETHY, V.M. & VAN WOERT, M.H. (1974). Regulation of striatal acetylcholine concentration by dopamine receptors. *Nature*, **251**, 529–530.
- SHARP, T., MAIDMENT, N.T., BRAZELL, M.P., ZETTERSTROM, T., UNGERSTEDT, U., BENNETT, G.W. & MARSDEN, C.A. (1984). Changes in monoamine metabolites measured by simultaneous *in vivo* differential pulse voltammetry and intracerebral dialysis. *Neuroscience*, **12**, 1213–1221.
- SNYDER, S.H., GREENBERG, D. & YAMAMURA, H.I. (1974). Antischizophrenic drugs and brain cholinergic receptors. *Arch. Gen. Psychiatry*, **31**, 58–61.
- STADLER, H., LLOYD, K.G. & BARTHOLINI, G. (1974). Dopaminergic inhibition of striatal cholinergic neurons: synergistic blocking action of gamma-butyrolactone and neuroleptic drugs. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **283**, 129–134.
- STAMFORD, J.A., KRUK, Z.L. & MILLAR, J. (1988). Actions of dopamine antagonists on stimulated striatal and limbic dopamine release: an *in vivo* voltammetric study. *Br. J. Pharmacol.*, **94**, 924–932.
- TSCHANZ, J.T. & REBEC, G.V. (1988). Atypical antipsychotic drugs block selective components of amphetamine-induced stereotypy. *Pharmacol., Biochem. Behav.*, **31**, 519–522.
- WESTERINK, B.H.C., DE BOER, P. & DAMSMA, G. (1990). Dopamine-acetylcholine interaction in the striatum studied by microdialysis in the awake rat: some methodological aspects. *J. Neurosci. Methods*, **34**, 117–223.
- WESTERINK, B.H.C. & KORF, J. (1975). Influence of drugs on striatal and limbic homovanillic acid concentration in the rat brain. *Eur. J. Pharmacol.*, **33**, 31–40.

(Received January 24, 1991)

Revised May 9, 1991

Accepted May 17, 1991)