Br. J. Pharmacol. (1992), **105**, 703–707

Dopamine release and metabolism in the rat frontal cortex, nucleus accumbens, and striatum: a comparison of acute clozapine and haloperidol

[1]F. Karoum & M.F. Egan

Neuropsychiatry Branch, Intramural Research Program, National Institute of Mental Health, Neuroscience Research Center, Washington, D.C. 20032, U.S.A.

1 The effects of the typical and typical neuroleptic agents clozapine (CLZ) (2.5–20 mg kg^{-1}, i.p.) and haloperidol (Hal) (0.05–1.0 mg kg^{-1}), were compared on dopamine release and metabolism in the rat prefrontal cortex (PFC), nucleus accumbens (ACC) and striatum (ST). Dopamine release was estimated by measuring the steady-state concentration of 3-methoxytyramine (3-MT) and the level of 3-MT 10 min after pargyline (3-MT accumulation); dopamine metabolism was evaluated from the steady-state concentrations of its acidic metabolites.

2 Both drugs increased 3-MT accumulation in the PFC in a dose-dependent manner. In contrast to Hal, CLZ failed to increase 3-MT accumulation in the ACC or ST. The ST was the region most sensitive to Hal in terms of 3-MT accumulation and, by inference, dopamine release.

3 Both CLZ and Hal dose-dependently elevated the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in all 3 brain regions studied. The ACC appears to be the region most sensitive to these drugs in terms of changes in the levels of HVA.

4 The result of the present investigations suggest measurements of 3-MT production in the 3 brain regions analysed might be a useful and simple pharmacological tool in the search for atypical neuroleptic drugs with a selectivity of action for the cortical systems.

Keywords: Dopamine release and metabolism; neuroleptics; haloperidol; clozapine; cortex; nucleus accumbens; striatum

Introduction

The recent demonstration (Kane *et al.*, 1988) that clozapine (CLZ) helps a significant percentage of schizophrenic patients who have responded poorly to the typical neuroleptics has generated a great deal of interest. Clozapine has been called an 'atypical' neuroleptic because it produces few extrapyramidal side effects and does not appear to cause tardive dyskinesia (Berzewski *et al.*, 1969; Angst *et al.*, 1971; Honigfeld *et al.*, 1984; Meltzer, 1989; Farde *et al.*, 1989). Other properties that distinguish CLZ from typical neuroleptics such as haloperidol (Hal) is the failure to produce long-lasting elevation of plasma prolactin levels, failure to induce dopamine D_2 receptor supersensitivity in the striatum after chronic treatment, and inability to inhibit markedly apomorphine-induced circling behaviours in rats with unilateral lesions of their substantia nigra (Coward *et al.*, 1989).

Several hypotheses have been put forward as to why CLZ is capable of producing clinical improvement when other neuroleptics have failed. These notions involve the effects of CLZ on 5-hydroxytryptaminergic (Meltzer, 1989), cholinergic (Stille *et al.*, 1971; Snyder *et al.*, 1974) and adrenergic systems (Bartholini *et al.*, 1972; McMillan & Shore, 1978; Chiodo & Bunney, 1985) in the brain, especially in the limbic dopamine systems (Anden & Stock, 1973; Chiodo & Bunney, 1985). However, none of these systems apparently play key roles in mediating the behavioural effects of atypical neuroleptics in the experimental animal (Coward *et al.*, 1989). There is however, a number of recent reports that implicate 5-HT_3 receptors in animal models of psychosis (Costall *et al.*, 1988) and show a relatively high affinity of CLZ for these receptors (Barnes *et al.*, 1990). It is therefore conceivable that CLZ mediates some of its antipsychotic effects via 5-HT_3 receptors.

To examine how biochemically dopaminergic neuronal function is affected by neuroleptics, we have compared the effects of CLZ with those of a representative typical neuroleptic, Hal, on indices of dopamine release and metabolism in three brain regions implicated in mediating some of the antipsychotic and side effects of the drugs. These regions include the striatum (ST), nucleus accumbens (ACC) and prefrontal cortex (PFC). Three-methoxytyramine (3-MT) accumulation was used as a measure of dopamine release (Kehr, 1981; Waldmeier *et al.*, 1981; Wood & Altar, 1988) and dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were used as indices of dopamine metabolism and turnover (Westerlink, 1985). Since changes in 3-MT steady-state concentration generally follow changes in 3-MT accumulation (but not *vice versa*) (Wood & Altar, 1988), and since 3-MT steady-state is simpler to assay and perhaps more representative of the natural state of dopamine release (Kehr, 1981; Waldmeier *et al.*, 1981; Wood & Altar, 1988), we have also measured the concentration of 3-MT in the brain after Hal and CLZ.

Methods

Male, Sprague-Dawley rats (Zivic-Miller, Allison Park, PA, U.S.A.) weighing about 200 g were used. CLZ was donated by Sandoz Research Institute (East Hanover, N.J., U.S.A.), and Hal was obtained from McNeil Pharmaceuticals (Spring House, PA, U.S.A.). For the assessment of DOPAC and HVA dose-response to varying doses of the neuroleptics, rats were injected i.p. with the neuroleptic and killed by decapitation 60 min later. 3-MT accumulation was measured 10 min after i.p. injection of pargyline, 75 mg kg^{-1}. Details of the doses and time that the rats were killed are given in the appropriate tables. Rats were decapitated for the assay of dopamine, DOPAC and HVA, and killed by microwave irradiation for the assay of 3-MT. The microwave instrument (Radio Japan NJE 2603, Great Plains Laboratories, Norman, Oklahoma, U.S.A.) was focused on the head at an output power of 7 kW for 1.3 s. Brains were quickly removed, cooled on ice, and the prefrontal cortex (PFC), the nucleus accumbens (ACC), and the striatum (ST) dissected out as previously reported (Karoum *et al.*, 1990). Dissected brain tissue was frozen on

[1] Author for correspondence.

Table 1. 3-Methoxytyramine (concentrations) accumulation in rat brain following single doses of clozapine and haloperidol

Treatment	*Dose* [*n*]	*Frontal cortex*	*Nucleus accumbens*	*Striatum*
Saline	— [5]	1.18 (0.13)	4.40 (1.00)	6.7 (0.60)
Clozapine	2.5 [5]	1.23 (0.08)	4.46 (0.80)	5.2 (0.85)
	5.0 [5]	1.23 (0.08)	3.12 (0.28)	7.8 (0.47)
	10.0 [5]	1.79 (0.20)**	5.20 (0.53)	7.8 (0.47)
	20.0 [5]	2.93 (0.54)**	5.97 (1.15)	5.3 (1.17)
Saline	— [5]	1.26 (0.14)	4.40 (1.00)	6.6 (2.16)
Haloperidol	0.05 [5]	1.46 (0.09)	—	21.7 (3.87)**
	0.10 [10]	1.76 (0.06)**	7.80 (1.60)	13.7 (1.35)**
	0.20 [5]	1.92 (0.30)*	8.7 (1.49)*	14.7 (5.51)**
	0.40 [10]	3.88 (1.11)**	9.61 (0.87)**	11.7 (1.62)**
	1.00 [10]	1.76 (0.24)*†	8.16 (1.39)*	15.2 (1.18)**

Results are expressed as pmol mg^{-1} protein $10\,min^{-1}$; mean (s.e.mean). Treatments started at 0 time and pargyline was administered after 50 min. Rats were killed by microwave irradiation at 60 min.
* $P < 0.05$; ** $P < 0.02$ compared with saline-treated rats by unpaired t tests.
† $P < 0.05$ when compared with 0.40 haloperidol.

solid CO_2 and homogenized in 0.5 ml of 1 N HCl containing 50 ng of the deuterated 3-MT ([$^2H_3$]-3-MT) or 50 mg each of the following: dopamine ([$^2H_4$]-dopamine); DOPAC ([$^2H_5$]-DOPAC); and HVA ([$^2H_5$]-HVA). Five μl of the homogenate was removed for protein determination (Lowry *et al.*, 1951), the remaining part was centrifuged at 12,000 *g* and the clear supernatant separated and stored at −16°C until analysed.

The supernatants from the tissue homogenates were prepared for the mass fragmentography of DOPAC and HVA as previously described (Karoum, 1983). For the assay of 3-MT, 0.1 ml of the clear supernatant obtained from the tissue homogenate after centrifugation, was mixed into 0.1 ml of distilled water in a 2 ml polypropylene tube followed by 1.0 ml of ethylacetate and 0.3 ml of phosphate buffer (prepared by mixing 3 parts 0.5 M Na_3PO_4 and one part 0.5 M Na_2HPO_4). The mixture was vortex-mixed for 1 min, centrifuged at 12,000 *g* for 3 min, and about 0.8 ml of the ethylacetate transferred into a Microflex tube (Kontes, Vineland, N.J., U.S.A.). The volume of this extract was reduced to about 0.1 ml under a gentle steam of N_2. The extraction was repeated and 1.0 ml of ethylacetate transferred and combined with the first extract. The ethylacetate extract was then evaporated to dryness and the 3-MT converted to its pentafluoropropionate derivative as previously described (Karoum, 1983).

A model 4500 Finnigan gas chromatograph quadrupole mass spectrometer (Finnigan MAT, San Jose, CA, U.S.A.) was used for the assay of dopamine and its metabolites. Gas chromatograph separation was achieved on a 30 m fused silica capillary column bond coated with methyl:vinyl:silicone gum (SPB-5), (Supelco Inc., Bellefonte, PA, U.S.A.). Injection port pressure was maintained at 15 psi and a split ratio of 1:20 was used. The oven temperature was kept isothermally at 190°C. Negative chemical ionization was used for the mass fragmentography of 3-MT employing ions with mass-to-charge ratio (m/z) 311 and 314, respectively, for 3-MT and its deuterated isomer. For the assay of dopamine, DOPAC and HVA, electron impact ionization was employed and the ions detected were (compound/protonated ion/deuterated ion) as follows: (dopamine/428/431); (DOPAC/488/493); (HVA/356/361).

Results

3-Methoxytyramine concentration and rate of formation in the brain

The rates of accumulation of 3-MT in various brain regions following doses of Hal and CLZ are summarized in Table 1. Both Hal and CLZ significantly increased 3-MT accumulation in the PFC. The minimum effective doses for these drugs (doses that elevated 3-MT accumulations by other 30% in all 3 brain regions) were 0.1 and 10 mg kg^{-1}, respectively. However, 0.2 mg kg^{-1} of Hal appears to be the minimum dose that significantly elevates 3-MT levels in all 3 brain regions. The peak Hal effect on PFC 3-MT accumulation appeared to occur around 0.4 mg kg^{-1}; the accumulation at 1.0 mg kg^{-1} was significantly lower. The inverted 'U' response curve for Hal was observed in a total of 4 separate experiments (results not shown). The responses of the ACC and PFC to Hal were similar but not so clear. The ST was more responsive to Hal than were the other brain areas. At 0.05 mg kg^{-1}, 3-MT accumulation increased by over 3 fold in the ST while it remained unchanged in the PFC.

In contrast to the effect of Hal, CLZ did not increase 3-MT accumulation in the ACC or the ST (Table 1). This experiment was repeated on 5 other occasions. In 3 of these experiments, 10 mg kg^{-1} CLZ produced a slight but significant reduction in striatal 3-MT. In one experiment CLZ produced a marked and highly significant ($P < 0.01$) reduction in 3-MT accumulation at a dose of 20 mg kg^{-1}. 3-MT accumulation in the ACC was never affected. It thus appeared that CLZ selectively enhances dopamine release in the PFC but not in the ACC and ST.

As shown in Table 2, the changes in the steady-state concentration of 3-MT following 10 mg kg^{-1} CLZ or 0.4 mg kg^{-1} Hal are similar to those in 3-MT accumulation, except for the

Table 2 Steady-state concentrations of 3-methoxytyramine in rat brain, 60 min after intraperitoneal injections of clozapine (10 mg kg^{-1}) and haloperidol (0.4 mg kg^{-1})

Treatment	[*n*]	*Frontal cortex*	*Nucleus accumbens*	*Striatum*
Saline	[15]	0.39 (0.09)	1.24 (0.09)	1.29 (0.09)
Clozapine	[8]	0.43 (0.10)	1.09 (0.23)	1.25 (0.10)
Haloperidol	[5]	0.38 (0.04)	2.13 (0.25)**	2.31 (0.35)*

Results are expressed in pmol mg^{-1} protein; mean (s.e.mean).
* $P < 0.05$, ** $P < 0.02$; comparison with saline by unpaired t tests.

Table 3 3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentrations in rat brain following various doses of clozapine and haloperidol

		Frontal cortex		*Nucleus accumbens*		*Striatum*	
Treatment	*Dose* (mg kg^{-1})	*DOPAC*	*HVA*	*DOPAC*	*HVA*	*DOPAC*	*HVA*
Saline	—	1.3 (0.1)	3.5 (0.32)	51.2 (4.8)	29.0 (0.5)	65.4 (4.7)	49.3 (5.3)
Clozapine	2.5	1.1 (0.1)	3.7 (0.46)	51.5 (2.7)	34.5 (1.6)**	79.1 (6.5)	63.3 (7.4)
	5.0	1.4 (0.1)	3.6 (0.46)	60.3 (4.1)	37.5 (1.7)**	90.4 (3.9)	72.0 (3.0)**
	10.0	1.8 (0.2)*	6.1 (1.3)*	81.2 (10.0)**	63.0 (6.7)**	155.3 (5.2)**	129.3 (9.5)**
	20.0	2.9 (0.3)**	7.9 (1.0)**	108.0 (7.7)**	82.9 (5.4)**	182.3 (11.4)**	142.8 (8.7)**
Saline	—	1.0 (0.2)	2.5 (0.2)	50.2 (2.8)	25.4 (1.7)	74.6 (8.1)	53.6 (4.1)
Haloperidol	0.025	1.1 (0.1)	3.1 (0.4)	64.9 (5.2)*	34.0 (3.0)*	88.2 (7.8)	57.0 (4.6)
	0.05	1.1 (0.1)	3.2 (0.4)	67.0 (6.9)*	29.6 (7.4)	106.3 (9.3)**	57.2 (4.6)
	0.1	1.35 (0.1)*	3.9 (0.3)**	77.4 (3.2)**	38.5 (5.7)*	195.2 (8.2)**	79.6 (7.3)**
	0.2	2.31 (0.2)**	6.4 (0.3)**	147.2 (8.1)**	60.8 (7.8)**	232.6 (19.8)**	112.9 (7.1)**
	0.4	3.1 (0.2)	6.2 (0.9)**	137.6 (12.8)**	61.6 (4.5)**	212.2 (33.6)**	106.5 (17.2)**

Results are in pmol mg^{-1} protein; mean (s.e.mean).
Rats were killed by decapitation 60 min after treatment.
* $P < 0.05$, ** $P < 0.02$; comparisons with saline by unpaired t tests.
Five rats were included in each experiment.

PFC where both Hal and CLZ had no effect on 3-MT steady-state content.

The effects of haloperidol and clozapine on the levels of brain DOPAC and HVA

The effects of low doses of Hal and CLZ on brain concentrations of DOPAC and HVA are summarized in Table 3. As shown, DOPAC and HVA content were elevated by both drugs in all 3 brain regions analysed. The minimum i.p. doses of CLZ and Hal that consistently elevated both DOPAC and HVA in all three brain regions were respectively 10.0 and 0.2 mg kg^{-1}. These minimal doses are similar to those observed for 3-MT accumulation (see Table 1). Furthermore, the ACC appears to be more sensitive than the ST, especially in terms of changes in HVA levels.

Discussion

Dopamine release can be measured directly by a variety of approaches: *in vivo* microdialysis (Zetterstrom *et al.*, 1985; Imperato & Di Chiara, 1984; 1985), push-pull perfusion (Besson *et al.*, 1971; Mora & Myers, 1977), and *in vivo* voltammetry (Gonon *et al.*, 1984; Gonon & Buda, 1985; Lane & Blaha, 1986). However, it has been shown through pharmacological (Di Giulio *et al.*, 1978; Wood, 1982a,b; 1983; Altar *et al.*, 1987) and lesion studies (Groppetti *et al.*, 1978; Wood, 1982a,b; Wood *et al.*, 1987a,b) that the steady-state concentration and accumulation of 3-MT are reliable indices of dopamine release from neurones (Kehr, 1981; Waldmeier *et al.*, 1981; Wood & Altar, 1988). Further, although the concentrations of DOPAC and HVA or its rate of synthesis have been employed for the assessment of dopamine release and impulse flow (Demarest & Moore, 1979; Roth *et al.*, 1976), these metabolites are generally regarded as indices of dopamine synthesis and dopamine turnover (Westerink, 1985; Nissbrandt *et al.*, 1989). In the discussion that follows changes in 3-MT (both steady-state changes and changes in accumulation) are used to indicate relative changes in dopamine release, whereas changes in DOPAC and HVA are used as indices of dopamine rate of utilization and turnover.

The most important observation in the present study is the existence of selective regional differences in the effects of Hal and CLZ on brain dopamine release. Both drugs increased 3-MT accumulation in the PFC, but it was elevated only by Hal in the ACC and the ST. In contrast, dopamine metabolism was equally increased by Hal and CLZ in all three brain regions examined. Results similar to the acute effects of Hal and CLZ on 3-MT accumulation were recently also observed after chronic (28 days) CLZ and Hal treatment (Egan *et al.*, 1991), suggesting that the therapeutic action of typical and atypical neuroleptics may be associated with their persistent potentiation of dopamine release in the PFC and perhaps other cortical regions (Wood & Altar, 1988). This notion is partly in line with other data implicating the mesocortical and meso-accumbens dopamine systems in the antipsychotic effects of neuroleptics (Stevens, 1973; Snyder *et al.*, 1974; Chiodo & Bunney, 1983; White & Wang, 1983; Thierry *et al.*, 1984).

The effect of Hal on 3-MT steady-state and accumulation in the ST are consistent with those found by other workers in the ST (Saller & Salama 1986; Nissbrandt *et al.*, 1989) and several cortical areas (Wood *et al.*, 1987a). The effects of Hal on 3-MT accumulation in the ACC and of CLZ on 3-MT steady-state and accumulation in the ACC and PFC have not previously been described. Further, as demonstrated here for CLZ (compare the effects of CLZ in Tables 2 and 3) and previously reported by others (Groppetti *et al.*, 1978; Moleman *et al.*, 1978; Zetterstrom *et al.*, 1985; Nissbrandt *et al.*, 1989), dopamine release and metabolism are clearly dissociable and hence may well represent different neuronal activities.

Persistent blockade of dopamine receptors, as manifested biochemically by sustained elevations in brain dopamine release and metabolism (Hernandez & Hoebel, 1989; Invernizzi *et al.*, 1990) have been linked in various ways, to the therapeutic benefits of neuroleptics and to the development of tardive dyskinesia. As can be deduced from Table 3, dopamine metabolism does not differentiate between the acute effects of typical from atypical neuroleptics. Therefore tardive dyskinesia is more likely to be associated with persistent stimulation of dopamine release in the ST than is stimulation of dopamine metabolism (Egan *et al.*, 1991). Further, if the ACC is at all involved in mediating the antipsychotic effect of neuroleptics, this effect does not seem to involve stimulation of dopamine release.

Except for one study (Saller & Salama 1986), there appears to be a consensus that CLZ and other atypical neuroleptics produce no or little change in 3-MT production in the ST (Groppetti *et al.*, 1978; Ponzio *et al.*, 1981; Wood *et al.*, 1983; Altar *et al.*, 1986). For example, the putative atypical neuroleptic, CGS 10746B, was shown to reduce 3-MT concentration in the ST (Alter *et al.*, 1986). Similarly, the selective dopamine D_1-receptor antagonist, SCH 23390 (Setler *et al.*, 1978; Huff & Molinoff, 1982; Hytell, 1983), which shares many pharmacological features with CLZ (Lorio *et al.*, 1983; Coward *et al.*, 1989; Farde *et al.*, 1989), has no effect on ST 3-MT accumulation (Boyar & Altar, 1987; Saller & Salama, 1986). The discordance between our findings and those of Saller & Salama, 1986, may result from methodological differences, e.g. greater microwave fixation power (7 versus 1.3 kW) and a considerably shorter period of 3-MT accumulation in our study (10 versus 30 min). Longer accumulation time might increase shunting of dopamine metabolism through *O*-methylation, thus producing higher apparent rates of 3-MT accumulation. This latter may become even more prominent

after treatment with CLZ which mainly stimulates dopamine acidic metabolite production.

While the ST (followed by the PFC) appeared to be the area most sensitive to Hal in terms of 3-MT production (Table 3) the ACC appears to be most responsive to CLZ and Hal in terms of changes in HVA steady-state content. Differential effects of Hal and CLZ on DOPAC and HVA in the ACC and ST have previously been described (Anden & Stock, 1973; Wilk *et al.*, 1975; Westerink & Korf, 1976). However, since Hal is known to inhibit the efflux of DOPAC and HVA from the brain (Moleman *et al.*, 1978; Westerink *et al.*, 1984), the elevations produced by Hal and CLZ may overestimate dopamine turnover or rate of utilization.

Since CLZ and other atypical neuroleptics (Farde *et al.*, 1989) preferentially bind to D_1 dopamine receptors (Rupniak *et al.*, 1985; Fuxe *et al.*, 1989; Invernizzi *et al.*, 1990), the failure of CLZ to increase 3-MT accumulation (and by implication, dopamine release) in the ST and ACC may be related to its relatively low affinity for release-modulating D_2-dopamine autoreceptors (Coward *et al.*, 1989; Farde *et al.*, 1989). Recent microdialysis studies have shown that the infusion of a number of dopamine D_1-specific compounds into the nigra did not release dopamine from either the nigra or the ST (Santiago & Westerink, 1991). Hence, unlike Hal which blocks both D_1 and D_2 receptors, CLZ effects on dopamine release are apparently primarily linked to its ability to interact with postsynaptic dopamine D_1 receptors. If we assume blockade of the dopamine postsynaptic receptor to be a general property of CLZ, then stimulation of dopamine release and metabolism in the PFC is probably also linked to blockade of postsynaptic dopamine D_1 receptors. This view does not agree with the concept that dopamine release (Altar *et al.*, 1987) and synthesis (Demarest & Moore, 1979) in the PFC are regulated by autoreceptors.

Evidence supporting the presence of dopamine autoreceptors in the cortex were in most cases extrapolated from studies related to experiments that used the so-called presynaptic doses of dopamine agonists (Chiodo *et al.*, 1984; Talmaciu *et al.*, 1986; Altar *et al.*, 1987). Inherent in these studies is the assumption that the doses of dopamine receptor agonists that were employed do not effectively stimulate dopamine postsynaptic receptors in the PFC. It can be argued, however that the so called presynaptic doses of dopamine agonists may stimulate postsynaptically non-dopaminergic neurones in the PFC. This stimulation might be responsible for the reduction in dopamine release via a direct negative feed back mechanism. Data generated here on the effect of CLZ on dopamine release in the PFC are compatible with this hypothesis.

In conclusion, comparison of the effects of single administrations of Hal and CLZ on dopamine release (as reflected by 3-MT production) and metabolism (as reflected by DOPAC and HVA contents) in the PFC, ACC, and ST, revealed differences that are apparently not clearly detected by either microdialysis measurement of dopamine release or changes in the dopamine acidic metabolites. The difference between typical and atypical neuroleptics as reflected in 3-MT production may offer an important and a convenient approach to the search for new agents for the treatment of schizophrenia.

The authors wish to thank Dr R. Jed Wyatt and Dr J. Stevens for their valuable criticisms. The excellent technical work of Mr Randall Alexander is greatly appreciated.

References

ALTAR, C.A. & HAUSER, K. (1987). Topography of substantia nigra innervation by D_1 receptor-containing striatal neurons. *Brain Res.*, **410**, 1–11.

ALTAR, C.A., WASLEY, A.M., LIEBMAN, J., GERHARDT, S., KIM, H., WELCH, J.J. & WOOD, P.L. (1986). CGS 10746B: an atypical anti psychotic candidate that selectively decreases dopamine release at behavioral effective doses. *Life Sci.*, **39**, 699–705.

ALTAR, C.A., WILLIAMS, C.B., OEI, E. & WOOD, P.L. (1987). Dopamine autoreceptors modulate the *in vivo* release of dopamine in the frontal, cingulate and entorhinal cortices. *J. Pharmacol. Exp. Ther.*, **242**, 115–120.

ANDEN, N.E. & STOCK, G. (1973). Effect of clozapine on the turnover of dopamine in the corpus striatum and in the limbic system. *J. Pharm. Pharmacol.*, **25**, 346–348.

ANGST, J., BENTE, D., HEIMANN, H., HELMCHEN, H. & HIPPIUS, H. (1971). Das klinische Wirkungsblid von Clozapine (untersuchung mit dem AMP_system). *Pharmakopsychiatrie*, **4**, 200–211.

BARNES, J.M., BARNES, N.M., CHAMPANERIA, S., COSTALL, B. & NAYLOR, R.J. (1990). Characterisation and autoradiographic localisation of 5-HT_3 receptor recognition sites identified with [^{3}H]-(S) -zacopride in the forebrain of the rat. *Neuropharmacology*, **29**, 1037–1045.

BARTHOLINI, G., HAEFELY, W., JALFRE, M., KELLER, H.H. & PLETSCHER, A. (1972). Effects of clozapine on central catecholaminergic neurone systems. *Br. J. Pharmacol.*, **46**, 736–740.

BERZEWSKI, H., HELMCHEN, H., HIPPIUS, H. & KANOWSKI, S. (1969). Das klinische Wirkungspektrum eines neuen dibenzdiazepinderivates. *Arzneimittelforschung*, **19**, 496–498.

BESSON, M.J., CHERAMY, A., FELTZ, P. & GLOWINSKI, J. (1971). Dopamine: spontaneous and drug induced release from the caudate nucleus in the cat. *Brain Res.*, **32**, 407–424.

BOYAR, W.C. & ALTER, C.A. (1987). Modulation of dopamine release by D_2 but not D_1 receptor agonists and antagonists. *J. Neurochem.*, **48**, 824–831.

CHIODO, L.A., BANNON, M.J., GRACE, A.A., ROTH, R.H. & BUNNEY, B.S. (1984). Evidence for the absence of impulse-regulating somatodendritic and synthesis-modulating nerve terminal autoreceptors subpopulations of mesocortical dopamine neurons. *Neuroscience*, **12**, 1–16.

CHIODO, L.A. & BUNNEY, B.S. (1983). Typical and atypical neuroleptics. Differential effects of 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 differently affects the activity of two subpopulations of midbrain dopamine neurons. *J. Neurosci.*, **5**, 2539–2544.

COSTALL, B., NAYLOR, R.J. & TYERS, M.B. (1988). Recent advances in the neuropharmacology of 5-HT_3 agonists and antagonists. *Rev. Neurosci.*, **2**, 41–65.

COWARD, D.M., IMPERATO, A., URWYLER, S. & WHITE, T.G. (1989). Biochemical and behavioral properties of clozapine. *Psychopharmacol.*, **99**, S6–S12.

DEMAREST, K.T. & MOORE, M.J. (1979). Comparison of dopamine synthesis modulation in the terminals of nigrostriatal, mesolimbic, tuberoinfundibular and tuberohypophyseal neurons. *J. Neural Transm.*, **46**, 263–277.

DI GIULIO, A.M., GROPPETTI, A., CATTABENI, F., GALLI, G.L., MAGGI, A., ALGERI, S. & PONZIO, F. (1978). Significance of dopamine metabolites in the evaluation of drugs acting on dopaminergic neurons. *Eur. J. Pharmacol.*, **52**, 201–207.

EGAN, M.F., KAROUM, F. & WYATT, R.J. (1991). Effects of acute and chronic clozapine and haloperidol on 3-methoxytyramine accumulation in rat prefrontal cortex, nucleus accumbens, and striatum. *Eur. J. Pharmacol.*, **199**, 191–199.

FARDE, L., WIESEL, F.A., NORDSTROM, A.-L. & SEDVALL, G. (1989). D_1- and D_2-dopamine receptor occupancy during treatment with conventional and atypical neuroleptics. *Psychopharmacol.*, **99**, S28–S31.

FUXE, K., VON EULER, G., AGNATI, L.F., SALLER, C.F., SALAMA, A. & GOLDSEIN, M. (1989). Acute treatment with clozapine blocks D_1 dopamine receptor binding in discrete brain areas of the male rat. *Neurosci. Lett.*, **106**, 169–174.

GONON, F.G. & BUDA, M. (1985). Regulation of dopamine release by impulse flow and by autoreceptors as studied by *in vivo* voltammetry in the rat striatum. *Neuroscience*, **14**, 765–774.

GONON, F., NAVARRE, F. & BUDA, M. (1984). *In vivo* monitoring of dopamine release in the rat brain with differential pulse voltammetry. *Anal. Chem.*, **56**, 573–575.

GROPPETTI, A., PARENTI, M., GALLI, C.L., BUGATTI, A., CATTABENI, F., DI GIULIO, A.M. & RACAGNI, G. (1978). 3-methoxytyramine and different neuroleptics: dissociation from HVA and DOPAC. *Life Sci.*, **23**, 1763–1768.

HERNANDEZ, L. & HOEBEL, B.G. (1990). Selective effect of clozapine in DA turnover in the prefrontal cortex in rats. 20th Ann. Meet. *Neuroscience Abs.*, **244**, 9.

HONIGFELD, G., PATION, J. & SINGER, J. (1984). Clozapine: antipsychotic activity in treatment-resistant schizophrenics. *Adv. Ther.*, **1**, 77–97.

HUFF, R.M. & MOLINOFF, P.B. (1982). Quantitative determination of dopamine receptor subtypes not linked to activation of adenylate cyclase in the striatum. *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 7561–7565.

HYTELL, J. (1983). SCH 23390 the first selective dopamine D-1 antagonist. *Eur. J. Pharmacol.*, **91**, 153–154.

IMPERATO, A. & DI CHIARA, G. (1984). Trans-striatal dialysis coupled to reverse phase high performance liquid chromatography with electrochemical detection: a new method for the study of the *in vivo* release of endogenous dopamine and metabolites. *J. Neurosci.*, **4**, 966–977.

IMPERATO, A. & DI CHIARA, G. (1985). Dopamine release and metabolism in awake rats after neuroleptics as studied by trans-striatal dialysis. *J. Neurosci.*, **5**, 297–306.

INVERNIZZI, R., MORALI, F., POZZI, L. & SEMANIN, R. (1990). Effects of acute and chronic clozapine on dopamine release and metabolism in the striatum of conscious rats. *Br. J. Pharmacol.*, **100**, 774–778.

KANE, J., HONIGFELD, G., SINGER, J. & MELTZER, H. (1988). Clozapine for the treatment-resistant schizophrenic: a double-blind comparison versus chlorpromazine/benztropine. *Arch. Gen. Psychiat.*, **44**, 789–796.

KAROUM, F. (1983). Mass fragmentography in the analysis of biogenic amines: A clinical, physiological and pharmacological evaluation. In *Methods in Amine Research.* ed. Parves, S., Nagatsu, T., Nagatsu, I. & Parves, H. pp. 237–255. New York: Elsevier.

KAROUM, F., SUDDATH, R.L. & WYATT, R.J. (1990). Chronic cocaine and rat brain catecholamines: long-term reduction in hypothalamic and frontal cortex dopamine metabolism. *Eur. J. Pharmacol.*, **186**, 1–8.

KEHR, W. (1981). 3-methoxytyramine and normethanephrine as indicators of dopamine and noradrenaline release in mouse brain *in vivo*. *J. Neural. Trans.*, **50**, 165–178.

LANE, R.F. & BLAHA, C.D. (1986). Electrochemistry *in vivo*: application to CNS pharmacology. *Ann. N.Y. Acad. Sci.*, **473**, 47–63.

LORIO, L.C., BARNETT, A., LEITZ, F.H., HOUSER, V.P. & KORDUBA, C.A. (1983). SCH 23390, a potential benzazepine antipsychotic with unique interaction on dopaminergic systems. *J. Pharmacol. Exp. Ther.*, **226**, 462–468.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

McMILLAN, B.A. & SHORE, P.A. (1978). Comparative effects of clozapine and alpha-adrenoceptor blocking drugs on regional noradrenaline metabolism in the rat brain. *Eur. J. Pharmacol.*, **52**, 225–230.

MELTZER, H.Y. (1989). Clinical studies on the mechanism of action of clozapine: the dopamine-serotonin hypothesis of schizophrenia. *Psychopharmacology*, **99**, S18–S27.

MOLEMAN, P., BRUINVELS, J. & VAN VALKENBURG, C.F.M. (1978). On the relation between haloperidol-induced alterations in DA metabolism in rat striatum. *Life Sci.*, **23**, 611–616.

MORA, F. & MYERS, R.D. (1977). Brain self-stimulation: direct evidence for the involvement of dopamine in the prefrontal cortex. *Science*, **197**, 1387–1389.

NISSBRANDT, H., SUNDSTROM, E., JONSSON, G., HJORTH, S., CARLSSON, A. (1989). Synthesis and release of dopamine in rat brain: comparison between substantia nigra pars compacta, pars reticulata, and striatum. *J. Neurochem.*, **52**, 1170–1182.

PONZIO, F., ACHILLI, G., PEREGO, C. & ALGERI, S. (1981). Differential effects of certain dopaminergic drugs on the striatal concentration of dopamine metabolites, with special reference to 3-methoxytyramine. *Neurosci. Lett.*, **27**, 61–67.

ROTH, R.H., MURRIN, L.C. & WALTERS, J. (1976). Central dopaminergic neurons: effects of alterations in impulse flow on the accumulation of dihydroxyphenylacetic acid. *Eur. J. Pharmacol.*, **36**, 163–171.

RUPNIAK, N.M.J., MARTIN, D.H., MANN, S., FLEMINGER, S., GAVIN, K., JENNER, P. & MARSDEN, D. (1985). Chronic treatment with clozapine, unlike haloperidol, does not induce changes in striatal D-2 receptor function in the rat. *Biochem. Pharmacol.*, **34**, 2755–2763.

SALLER, C.F. & SALAMA, A.I. (1986). 3-methoxytyramine accumulation: effects of typical neuroleptics and various atypical compounds. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **334**, 125–132.

SANTIAGO, M. & WESTERINK, B.H.C. (1991). Characterization and pharmacological responsiveness of dopamine release recorded by microdialysis in the substantia nigra of conscious rats. *J. Neurochem.*, **57**, 738–747.

SETLER, P.E., SARAN, H.M., ZIRKLE, C.L. & SAUNDERS, H.L. (1978). The central effects of a novel dopamine agonist. *Eur. J. Pharmacol.*, **50**, 419–430.

SNYDER, S.H., BANERJEE, S.P., YAMAMURA, H.I. & GREENBERG, D. (1974). Drugs, neurotransmitters, and schizophrenia. *Science*, **184**, 1243–1253.

STEVENS, J.R. (1973). An anatomy of schizophrenia? *Arch. Gen. Psychiat.*, **29**, 179–189.

STILLE, G., LAUENER, H. & EICHENBERGER, H. (1971). The pharmacology of 8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[b,e][1, 4]diazepine (clozapine). *Pharmacology*, **26**, 603–625.

TALMACIU, R.K., HOFFMAN, I.S. & CUBEDDU, L.X. (1986). Dopamine autoreceptors modulate dopamine release from the prefrontal cortex. *J. Neurochem.*, **47**, 805–870.

THIERRY, A.M., TASSIN, J.P. & GLOWINSKI, J. (1984). Biochemical and electrophysiological studies of the mesocortical dopamine system. In *Monoamine Innervation of Cerebral Cortex*, ed., Descarries, L., Reader, T.A. & Jasper, H.H. pp. 242–244. New York: Alan R. Liss.

WALDMEIER, P.C., LAUBER, J., BLUM, W. & RICHTER, W.J. (1981). 3-methoxytyramine: its suitability as an indicator of synaptic dopamine release. *Naunyn-Schmeidebergs. Arch. Pharmacol.*, **315**, 219–225.

WESTERINK, B.H.C. (1985). Sequence and significance of dopamine metabolism in the rat brain. *Neurochem. Int.*, **7**, 221–227.

WESTERINK, B.H.C. & KORF, J. (1976). Regional rat brain levels of 3,4-dihydroxyphenylacetic acid and homovanillic acid: concurrent fluorometric measurements and influence of drugs. *Eur. J. Pharmacol.*, **38**, 281–291.

WESTERINK, B.H.C., BOSKER, F.J. & WIRIX, E. (1984). Formation and metabolism of dopamine in nine areas of the rat brain: modification by haloperidol. *J. Neurochem.*, **42**, 1321–1327.

WHITE, F.J. & WANG, R.Y. (1983). Different effects of classical atypical antipsychotic drugs on A9 and A10 dopamine neurons. *Science*, **221**, 1161–1170.

WILK, S., WATSON, E. & STANLEY, M.E. (1975). Differential sensitivity of two dopaminergic structures in rat brain to haloperidol and to clozapine. *J. Pharmacol. Exp. Ther.*, **195**, 265–270.

WOOD, P.L. (1982a). Action of GABAergic agents on dopamine metabolism in the nigrostriatal pathway of the rat. *J. Pharmacol. Exp. Ther.*, **222**, 674–679.

WOOD, P.L. (1982b). A selective ion monitoring assay for dopamine and its metabolites using negative chemical ionization. *Biochem. Mass. Spec.*, **9**, 302–306.

WOOD, P.L. (1983). Opioid regulation of CNS dopaminergic pathways: a review of methodology, receptor types, regional variations and species differences. *Peptides*, **4**, 595–601.

WOOD, P.L. & ALTAR, C.A. (1988). Dopamine release *in vivo* from nigrostriatal, mesolimbic, and mesocortical neurons: utility of 3-methoxytyramine measurements. *Pharmacol. Rev.*, **40**, 164–187.

WOOD, P.L., KIM, H.S. & ALTAR, C.A. (1987a). *In vivo* assessment of dopamine and norepinephrine release in rat neocortex: gas chromatography-mass spectrometry measurement of 3-methoxytyramine and normetanephrine. *J. Neurochem.*, **48**, 574–579.

WOOD, P.L., KIM, H.S. & MARIEN, M.R. (1987b). Intracerebral dialysis: direct evidence for the utility of 3-MT measurements as an index of dopamine release. *Life Sci.*, **41**, 1–5.

WOOD, P.L., McQUADE, P.S., ETIENNE, P., LAL, S. & NAIR, N.P.V. (1983). Differential actions of classical and atypical neuroleptics on mouse nigrostriatal neurons. *Prog. Neuro-psychopharmacol. Biol. Psychiat.*, **7**, 765–768.

ZETTERSTROM, T., SHARP, T. & UNGERSTEDT, U. (1985). The effects of neuroleptic drugs on striatal dopamine release and metabolism in the awake rat studied by intracerebral dialysis. *Eur. J. Pharmacol.*, **106**, 27–37.

(Received June 4, 1991
Revised October 3, 1991
Accepted November 6, 1991)