Psychopharmacology (1993) 112:S90–S94

Psychopharmacology

Haloperidol and clozapine treatment and their effect on *M*-chlorophenylpiperazine-mediated responses in schizophrenia: implications for the mechanism of action of clozapine

René S. Kahn, Larry Siever, Michael Davidson, Corey Greenwald, and Clare Moore

Department of Psychiatry, Mount Sinai School of Medicine/Bronx Veterans Administration Hospital, 130 W. Kingsbridge Road, New York, NY 10468, USA

Abstract. Since clozapine is, in contrast to conventional neuroleptics, effective in treatment refractory schizophrenic patients its mechanism of action may be different from that of typical neuroleptics. Clozapine has been shown to display the highest binding affinity of all neuroleptics to one of the serotonin (5-hydroxytryptamine, 5HT) receptor subtypes, i.e., the $5HT_{1c}$ receptor. Furthermore, clozapine, in contrast to conventional neuroleptics, blocks the effect of 5HT agonists on ACTH and corticosterone release in animals. This study hypothesized that clozapine, but not haloperidol would block ACTH and prolactin release induced by the 5HT agonist, *m*-chlorophenylpiperazine (MCPP). MCPP (0.35 mg/kg PO) was administered after a 3-week drug-free period, after 5 weeks of haloperidol treatment (20 mg/day) and finally after 5 weeks of clozapine treatment (> 400 mg/day) in ten male schizophrenic patients. Clozapine, but not haloperidol, blocked the effect of MCPP on ACTH and prolactin release. These results suggest that clozapine, in contrast to haloperidol, is a functional 5HT antagonist. Since MCPP-induced ACTH and prolactin release may be (partially) $5HT_{1c}$ mediated, these results suggest that clozapine is a potent antagonist at the $5HT_{1c}$ receptor.

Key words: Clozapine – Haloperidol – Schizophrenia – M-Chlorophenylpiperazine

The efficacy of clozapine in treatment refractory schizophrenia (Kane et al. 1988), and its general superiority when compared to conventional neuroleptics in non-refractory schizophrenics (Fischer and Ferner 1976; Van Praag et al. 1976; Leon 1979; Shopsin et al. 1979; Claghorn et al. 1987), raises the question whether its mode of action differs from that of conventional neuroleptics.

Correspondence to: R.S. Kahn

At first glance, clozapine's pharmacology does not seem to differ essentially from that of the typical neuroleptics. However, clozapine's $5HT_2$ receptor binding is 10–30 times more pronounced than its DA_2 binding affinity, while for chlorpromazine the ratio is approximately 1 and for haloperidol it is 0.1 (Peroutka and Snyder 1980; Altar et al. 1986). Clozapine has the highest $5HT_2/DA_2$ ratio of all currently available antipsychotic drugs and, in contrast to conventional neuroleptics, such as haloperidol, clozapine appears to be a weak DA antagonist. While chronic administration of conventional neuroleptics increases DA_2 receptor sensitivity, chronic administration of clozapine does not (Seeger et al. 1982; Lee and Tang 1984; Rupniak et al. 1984; Jenner et al. 1985; Cohen and Lipinski 1986), suggesting that DA_2 blockade by clozapine is minimal. Again in contrast to conventional neuroleptics such as haloperidol, clozapine may have potent $5HT_2$ antagonistic properties. Clozapine alters $5HT_2$ receptor sensitivity after chronic administration (Lee and Tang 1984), an effect also seen with other $5HT_2$ antagonists (Leysen et al. 1986), while haloperidol does not (Andree et al. 1986). Similarly, clozapine, but not haloperidol, blocks the temperature and cortisol rise induced by the selective $5HT_2$ agonist, MK-212, in rats [but not the cortisol rise induced by the selective $5HT_{1a}$ agonist 8-OHDPAT (Nash et al. 1988)]. Finally, clozapine has the highest binding affinity of all neuroleptics to $5HT_{1c}$ receptors (Canton et al. 1990). Thus, clozapine appears to differ from the conventional neuroleptic, haloperidol, in binding potently to $5HT_{1c}$ and $5HT_2$ receptors and being a potent 5HT antagonist in animals. However, there are no reports comparing the effect of clozapine and haloperidol on 5HT receptor function in human subjects.

To compare the effects of clozapine and haloperidol on 5HT receptor function in man, their effect on responses induced by the 5HT receptor agonist, *m*-chlorophenylpiperazine (MCPP) was studied in schizophrenic patients. MCPP is a functionally selective 5HT receptor agonist that readily crosses the blood-brain barrier. It binds to all 5HT receptors, but most potently to $5HT_{1c}$

receptors. MCPP reliably induces the release of ACTH and prolactin and increases body temperature in animal and man (for review see Kahn and Wetzler 1991). MCPP-induced ACTH release is most likely a $5HT_{1c}$ mediated effect. This conclusion is based on studies finding that MCPP-induced ACTH/cortisol release can be blocked by the mixed $5HT_{1/2}$ antagonist metergoline (Mueller et al. 1986; Kahn et al. 1990a) and by the $5HT_{1c/2}$ antagonist, ritanserin in rodents and man (Bagdy et al. 1989; Seibyl et al. 1991). Because ritanserin blocks both $5HT_{1c}$ and $5HT_2$ receptors, results of these studies could mean that MCPP-induced ACTH release is either $5HT_{1c}$ or $5HT_2$ mediated. However, since MCPP is probably a $5HT_2$ antagonist (Conn and Sanders-Bush 1987), the effect of MCPP on ACTH release is most likely to be $5HT_{1c}$ mediated. In contrast to its effect on ACTH, MCPP's effect on prolactin may only partially $5HT_{1c}$ related in man, since MCPP-induced prolactin release is only partially blocked by ritanserin in monkeys (Heninger et al. 1988) and man (Seibyl et al. 1991).

The present study used MCPP in a dose of 0.35 mg/kg. The choice of the dose was based on prior studies showing that 0.25 mg/kg PO produced minimal hormonal responses in healthy controls, while 0.5 mg/kg induced a robust release of ACTH and prolactin but induced physical discomfort as well (Kahn et al. 1990b). In a dose-response study (Kahn et al., unpublished data) using 0.35 and 0.5 mg/kg in schizophrenic patients, the former dose reliably, but not maximally, increased hormone release in schizophrenic patients and it was well tolerated. Thus, this dose was chosen to examine whether clozapine would be able to block these responses. This study examined the effect of haloperidol and clozapine treatment on MCPP-induced ACTH and prolactin responses in ten male schizophrenic inpatients. Since in a previous study (Kahn et al. 1992), MCPP failed to increase temperature in the schizophrenic patients (in contrast to the healthy controls studied), effects of clozapine and haloperidol on temperature responses were not examined in this study. It was hypothesized that clozapine, but not haloperidol, would block MCPP-induced ACTH and prolactin release.

Materials and methods

Subjects. Ten male inpatients completed this study (age 43.5 ± 11.2 years; age of onset of illness: 22.5 ± 3.7 years; mean number of hospitalizations: 6.3 ± 4.2) after giving written informed consent. All subjects had normal laboratory (including thyroid indices) and physical exams, and were free of drug and alcohol abuse for at least 6 months. Subjects were given the Schedule for Affective Disorders and Schizophrenia (SADS) interview and received diagnoses based on Research Diagnostic Criteria (RDC) and DSMIIIR. Nine patients were diagnosed as having schizophrenia; one patient was diagnosed as schizoaffective (DSMIIIR), mainly schizophrenic (RDC).

Study design. During the last week of a minimum 4-week drug-free period (except for occasional use of up to 1000 mg chloral hydrate/24 h) ACTH and prolactin responses to MCPP (0.35 mg/kg PO) and placebo were measured (MCPP #1) in 29 male schizophrenic patients. Following these tests, patients were treated with haloperidol 20 mg/day for 5 weeks. The active MCPP challenge test was repeated during week 5 of haloperidol treatment (MCPP #2). After this treatment episode, patients who clinically responded to haloperidol ceased to participate in this study ($n=15$). Haloperidol non-responders ($n=14$) were taken off haloperidol, remained drug-free for about 2 weeks and were subsequently treated with clozapine up to 600 mg/day for 5 weeks. Clozapine was started with 25 mg q.h.s. on the first day of treatment and increased by approximately 25 mg/day to a minimum of 400 mg/day. The active MCPP test was repeated during week 5 of clozapine treatment (MCPP #3) in 12 patients (2 patients refused to participate in the challenge test on clozapine). One of the 12 patients who completed all three phases of the study was excluded from analysis because MCPP blood levels were undetectable during the active (and placebo) challenge test during the drug-free period. Another patient was excluded because his clozapine dose was reduced to 150 mg/day due to orthostatic hypotension. Thus, ten patients were finally included in this study, completing all three MCPP tests. Nine of those patients were receiving 600 mg/day and one patient 450 mg/day at the time of the challenge test on clozapine (mean: 585.0 ± 47.4 mg/day). BPRS and CGI were rated weekly throughout the whole study period. Results of the active MCPP test during the drug-free period on eight of the ten patients in this study have also been reported in a study comparing the effect of MCPP in 22 schizophrenic patients and 17 healthy subjects (Kahn et al. 1992).

Challenge test. For the challenge tests subjects fasted (except for water intake) from 11 p.m. on the night preceding the challenge study and woke at 7 a.m. Between 8.30 and 9.00 a.m. an IV infusion was started, which was removed at 1.30 p.m. Capsules were administered orally at 10.00 a.m. All subjects remained semirecumbent during the procedure, and were not allowed to smoke, drink, eat, or sleep. Blood samples were taken at 30-min intervals from 9 a.m. to 1:30 p.m. and assessed for ACTH and prolactin. MCPP blood levels were measured at 11:00, 12:00 and 13:00 hours.

Biochemical analysis. ACTH was measured using an immunoradiometric assay in EDTA-treated plasma without extraction (Nichols Institute; Los Angeles, CA), using two monoclonal antibodies. The first antibody is directed against the carboxyl-terminus of the ACTH molecule and is conjugated to an avidin-biotin coated bead, while the second antibody is directed against the amino-terminus of the ACTH molecule and is ^{125}I-labeled. All samples were run in duplicate. Minimum detection level is 1 pg/ml. Intra-assay and interassay coefficients of variation were 7% and 5% respectively.

Prolactin was quantified using a room-temperature modification of the radioimmunoassay materials provided by ICN/Micromedic (Carson CA). Samples, standards, and controls were assayed in 100 µl aliquots, and run under equilibrium conditions with 100 µl each of primary antibody and radioiodinated tracer. Phase separation is achieved by the addition of 0.5 ml of a goat anti-rabbit gamma globulin / polyethylene glycon solution followed by centrifugation, decanting and gamma counting the precipitate. All samples were run in duplicate. The prolactin radioimmunoassay has a sensitivity of 1.5 ng/ml. The intra-assay and interassay coefficients of variation were 4% and 10%, respectively. MCPP was assayed as described by Suckow et al. (1990).

Data analysis. Since (prolactin) data were not normally distributed non-parametric tests were used. To compare the effects of neuroleptic treatment (haloperidol and clozapine) on MCPP-induced responses, Wilcoxon Signed Rank tests were used on area under the curve using the time when MCPP was administered (10:00 a.m.) as baseline. Variables examined were ACTH, prolactin and MCPP blood level. Each of the dependent variables was treated as a separate study tested at the $P<0.05$ level of significance. For each dependent variable two comparisons were made, i.e. 1) the effect of MCPP on haloperidol compared to the effect of MCPP during the drug-free period and 2) the effect of MCPP on clozapine compared to the effect of MCPP during the drug-free period. Analogous to

the Bonferroni inequality for multiple comparisons, a corrected P-value was obtained by doubling the P-value for each of the two tests. All data are presented as mean ± standard deviation (SD), unless stated differently.

Results

ACTH

Figure 1 shows the MCPP-induced ACTH response over time and as area under the curve (AUC) (insert) during the drug-free state, during week 5 of haloperidol treatment and during week 5 of clozapine treatment. Clozapine significantly decreased baseline ACTH levels as compared to the drug-free state ($Z=2.312$, $P=0.021$, corrected $P=0.042$). Haloperidol did not affect baseline ACTH levels as compared to during the drug-free state ($Z=0.102$, $P=0.92$, corrected $P=0.96$). Clozapine significantly diminished MCPP-induced ACTH release as compared to MCPP-induced ACTH release during the drug-free state ($Z=2.293$, $P=0.022$, corrected $P=0.044$). Although MCPP-induced ACTH release on haloperidol was smaller than during the drug-free state, this difference was not significant ($Z=1.784$, $P=0.074$, corrected $P=0.148$).

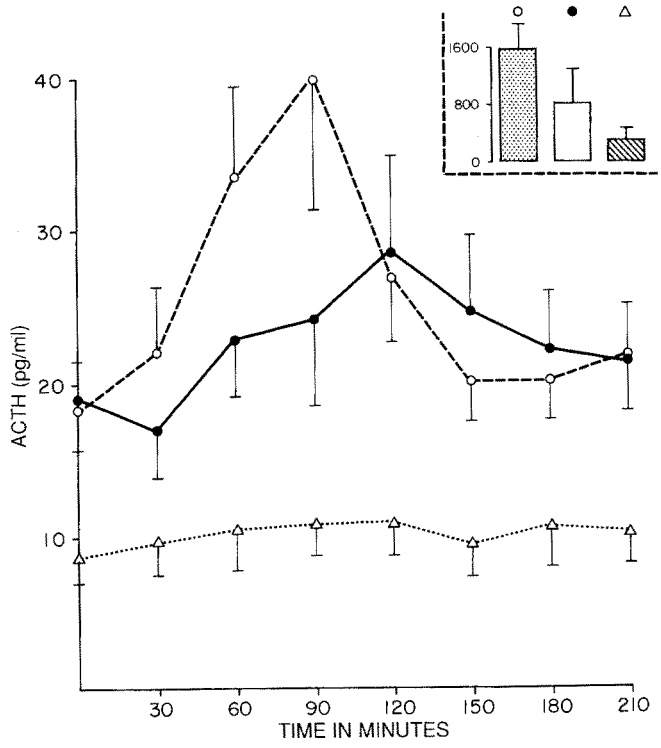

Fig. 1. Mean and standard error of the mean ACTH blood levels after administration of MCPP (0.35 mg/kg) during the drug-free state *(open circle)*, during haloperidol treatment *(closed circle)* and during clozapine treatment *(triangle)*. The *insert* shows the ACTH response to MCPP in the three conditions expressed as area under the curve

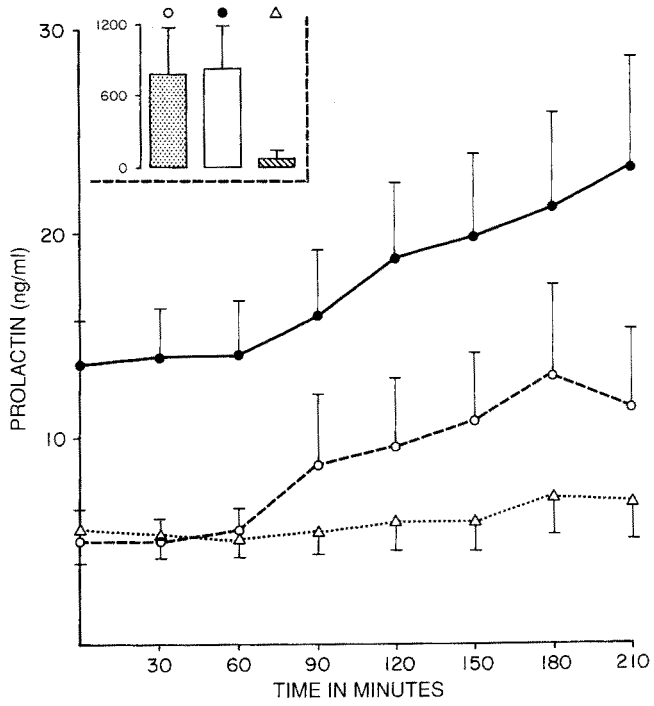

Fig. 2. Mean and standard error of the mean prolactin blood levels after administration of MCPP (0.35 mg/kg) during the drug-free state *(open circle)*, during haloperidol treatment *(closed circle)* and during clozapine treatment *(triangle)*. The insert shows the prolactin response to MCPP in the three conditions expressed as area under the curve

Prolactin

Figure 2 shows the MCPP-induced prolactin response over time and as area under the curve (AUC) (insert) during the drug-free state, during week 5 of haloperidol treatment and during week 5 of clozapine treatment. Haloperidol significantly increased baseline prolactin levels as compared to the drug-free state ($Z=2.803$, $P=0.005$, corrected $P=0.01$) but clozapine did not ($Z=1.274$, $P=0.20$, corrected $P=0.40$). Clozapine significantly diminished MCPP-induced prolactin release as compared to MCPP-induced ACTH release during the drug-free state ($Z=2.701$, $P=0.007$, corrected $P=0.014$) but MCPP-induced prolactin release on haloperidol was not significantly different from that during the drug-free state ($Z=0.153$, $P=0.88$, corrected $P=0.1$).

MCPP

Clozapine significantly raised MCPP levels as compared to MCPP levels during the drug-free state ($Z=2.524$, $P=0.012$, corrected $P=0.024$), but haloperidol treatment did not affect MCPP levels ($Z=1.54$, $P=0.12$, corrected $P=0.24$).

Discussion

This study found that clozapine treatment completely blocked MCPP-induced ACTH and prolactin responses,

despite its increasing MCPP blood levels. Haloperidol did not significantly alter the effect of MCPP on ACTH and prolactin release. The finding that clozapine but not haloperidol diminished the ACTH and prolactin response to MCPP suggests that clozapine, but not haloperidol, is a functional 5HT antagonist. As indicated earlier, animal studies found that clozapine, but not haloperidol, blocked temperature and cortisol responses induced by the $5HT_{(1c/2)}$ agonist, MK-212 (Nash et al. 1988).

The finding that clozapine blocked MCPP-induced ACTH and prolactin release is consistent with its high binding affinity to $5HT_{1c}$ receptors. Interestingly, while clozapine and haloperidol bind to $5HT_2$ receptors with about equal affinity (Canton et al. 1990; Leysen et al. 1993) (although the $5HT_2/DA_2$ ratio for clozapine is much higher), clozapine's binding affinity for the $5HT_{1c}$ receptor is more than 3 orders of magnitude greater than that of haloperidol (Canton et al. 1990). As indicated, MCPP-induced ACTH release appears mostly $5HT_{1c}$ mediated (Bagdy et al. 1989; Seibyl et al. 1991) and MCPP-induced prolactin release may be at least partially $5HT_{1c}$ mediated (Heninger et al. 1988; Bagdy et al. 1989a; Seibyl et al. 1991). Thus, the finding that clozapine blocked MCPP-induced ACTH and prolactin release is best explained by its blocking the $5HT_{1c}$ receptor. The fact that haloperidol failed to block MCPP-induced ACTH and prolactin release suggests that haloperidol does not display $5HT_{1c}$ antagonist properties. These conclusions are consistent with the 1000-fold higher affinity of clozapine to the $5HT_{1c}$ receptor than haloperidol. Thus, their effects at the $5HT_{1c}$ receptor may be one of the characteristics that separates clozapine from haloperidol.

Whether the ability of clozapine to block the $5HT_{1c}$ receptor may be related to its unique clinical efficacy needs to be further studied. However, preliminary findings suggest that MCPP-induced ACTH release predicts symptomatic improvement to clozapine responses. In 19 schizophrenic patients (of which the patients in the current study were a subset) placebo-corrected MCPP-induced ACTH release (obtained during the drug-free period prior to haloperidol and clozapine treatment) was significantly higher in the patients who eventually responded to clozapine than in those who failed to benefit from clozapine. Moreover, MCPP-induced ACTH release correlated significantly with reduction in global symptoms, particularly psychotic symptoms on clozapine (Kahn et al., in press). Thus, results from that study suggest that an index of $5HT_{1c}$ function (i.e., MCPP-induced ACTH release) may predict treatment response to clozapine, complementing the finding from this study that clozapine, but not haloperidol, is a functional $5HT_{1c}$ antagonist.

Another interesting difference between haloperidol and clozapine in this study is their effect on baseline ACTH levels. Clozapine, but not haloperidol, decreased baseline ACTH levels. This may suggest that blockade of $5HT_{1c}$ receptors, by decreasing 5HT function, decreases endogenous ACTH release. However, clozapine's effect on α-adrenergic receptors could also explain this effect. Finally, haloperidol, but not clozapine, raised baseline prolactin levels, as reported previously (see Meltzer 1989). This is consistent with the relative lack of DA antagonistic effects of clozapine in contrast to the potent DA antagonism of haloperidol (since DA inhibits prolactin release, DA antagonists are expected to increase prolactin levels).

Conclusions from this paper must remain tentative. The main limitation of this study is that it did not employ placebo tests during haloperidol and clozapine treatment. Since we were interested primarily in examining the effect of neuroleptic treatment on MCPP-induced responses, and administering six challenge tests in the same patient was found to be ethically unacceptable, it was felt that the study's goals could be met without administering placebo tests. A second problem is that since clozapine always followed haloperidol treatment, an order effect cannot be ruled out.

In summary, results from this study suggest that clozapine, but not haloperidol, is a functional 5HT antagonist. Indeed, several lines of evidence suggest that clozapine may be a $5HT_{(1c)}$ antagonist and that this property distinguishes it from haloperidol. Thus, studies examining the state of the $5HT_{1c}$ receptor in schizophrenia appear warranted. Such studies should be conducted in post-mortem brain samples and, once selective $5HT_{1c}$ are available, during in vivo challenge tests. Finally, development of $5HT_{1c}$ antagonists for the treatment of schizophrenia may also be useful.

References

Altar CA, Wasley AM, Neale RF, Stone GA (1986) Typical and atypical antipsychotic occupance of D2 and S2 receptors: an autoradiographic analysis in rat brain. Brain Res Bull 16:517–525

Andree TH, Mikuni M, Tong CY, Koenig JI, Meltzer HY (1986) Differencial effect of subchrnoic treatment with various neuroleptic agents on serotonin S2 receptors in rat cerebral cortex. J Neurochem 46:191–197

Bagdy G, Szemeredi K, Kanyicska B, Murphy DL (1989) Different serotonin receptors mediate blood-pressure, heart rate, plasma catecholamine and prolactin responses to *m*-chlorophenylpiperazine in conscious rats. J Pharmacol Exp Ther 250:72–78

Canton H, Verriele L, Colpaert FC (1990) Binding of typical and atypical antipsychotics to $5HT_{1c}$ and $5HT_2$ sites: clozapine potently interacts with $5HT_{1c}$ sites. Eur J Pharmacol 191:93–96

Claghorn J, Honigfeld G, Abuzzahab FS, Wang R, Steinbook R, Tuason V, Klerman G (1987) The risk and benefits of clozapine versus chlorpromazine. J Clin Psychopharmacol 7:377–384

Cohen BM, Lipinski JF (1986) In vivo potencies of antipsychotic drugs in blocking alpha-l noradrenergic and dopaminergic D2 receptors: implications for drug mechanisms of action. Life Sci 39:2571–2580

Conn PJ, Sanders-Bush E (1987) Relative efficacies of piperazines at the phosphoinositide hydrolysis-linked serotonergic $5HT_2$ and $5HT_{1c}$ receptors. J Pharmacol Exp Ther 242:552–557

Fischer-Cornelssen KA, Ferner UJ (1976) An example of European multicenter trials: mulispectral analysis of clozapine. Psychopharmacol Bull

Heninger GR, Krystal JH, Smith A (1988) Effects of D1 and D2 receptor antagonists and 5HT receptor agonists and antagonists on neuroendocrine function in rhesus monkeys. Soc Neurosci Abstr 324.7

Jenner P, Rupniak NM, Marsden CD (1985) Differential alteration of striatal D1 and D2 receptors induced by the long-term administration of haloperidol, sulpiride or clozapine to rats. Psychopharmacology (Suppl): 174–181

Kahn RS, Wetzler S (1991) *m*-Chlorophenylpiperazine as a probe of serotonin receptors: a review. Biol Psychiatry 30:1139–1166

Kahn RS, Kalus O, Cahn W, Wetzler S, Asnis GM, van Praag HM (1990a) The effect of 5HT antagonists of *m*-chlorophenylpiperazine-mediated responses. Psychiatry Res 33:189–198

Kahn RS, Wetzler S, Asnis GM, van Praag HM (1990b) The effects of *m*-chlorophenylpiperazine in normal subjects: a dose-response study. Psychopharmacology 100:339–344

Kahn RS, Siever L, Gabriel S, Amin F, Stern RG, DuMont K, Apter S, Davidson M (1992) Serotonin function in schizophrenia: effects of *m*-chlorophenylpiperazine in schizophrenic patients and healthy subjects. Psychiatry Res 43:1–12

Kahn RS, Davidson M, Siever L, Gabriel S, Apter S, Davis KL (1993) Serotonin function and treatment response to clozapine in schizophrenic patients. Am J Pychiatry (in press)

Kane J, Honigfeld G, Singer J, Meltzer H (1988) Clozapine for the treatment-resistant schizophrenic. Arch Gen Psychiatry 45:789–796

Lee T, Tang SW (1984) Lozapine and clozapine decrease serotonin (S2) but do not elevate dopamine (D2) receptor numbers in the rat brain. Psychiatry Res 12:277–285

Leon CA (1979) Therapeutic effects of clozapine: a 4-year follow-up of a controlled clinical trial. Acta Psychiatr Scand 59:471–480

Leysen JE, Van Gompel P, Gommeren W, Woestenborghs R, Janssen PAJ (1986) Down regulation of serotonin S2 receptor sites in rat brain by chronic treatment with the serotonin S2 antagonists: ritanserin and setoperone. Psychopharmacology 88:434–444

Meltzer HY (1989) Clinial studies on the mechanism of action of clozapine: the dopamine-serotonian hypothesis of schizophrenia. Psychopharmacology 99:S18–S27

Mueller EA, Murphy DL, Sunderland T (1986) Further studies of the putative serotonin agonist, *m*-chlorophenylpiperazine: evidence for a serotonin receptor mediated mechanism of action in humans. Psychopharmacology 89:388–391

Nash JF, Meltzer HY, Gudelsky GA (1988) Antagonism of serotonin receptor mediated neuroendocrine and temperature responses by atypical neuroleptics in the rat. Eur J Pharmacol 151:463–469

Peroutka SJ, Snyder SH (1980) Relationship of neuroleptic drug effects at brain dopamine, serotonin, alpha-adrenergic, and histamine receptors to clinical potence. Am J Psychiatry 137:1518–1522

Rupniak NM, Kilpatrick G, Hall MD, Jenner P, Marsden CD (1984) Differential alterations in striatal dopamine receptor sensitivity induced by repeated administration of clinically equivalent doses of haloperidol, sulpiride or clozapine in rats. Psychopharmacology 84:512–519

Seeger Th F, Thal L, Gardner EL (1982) Behavioral and biochemical aspects of neuroleptic induced dopaminergic supersensitivity: studies with chronic clozapine and haloperidol. Psychopharmacology 80 [Suppl]:182–187

Seibyl JP, Krystal JH, Price LH, Woods SW, D'Amico CD, Heninger GR, Charney DS (1991) Effects of ritanserin on the behavioral, neuroendocrine, and cardiovascular responses to metachlorophenylpiperazine in healthy subjects. Psychiatry Res 38:227–236

Suckow RF, Cooper ThB, Kahn RS (1990) High-performance liquid chromatographic method for analysis of plasma *m*-chlorophenylpiperazine. J Chromatogr 528:228–234

Van Praag HM, Korf J, Dols LCW (1976) Clozapine versus perphenazine: the value of the biochemical mode of action of neuroleptics in predicting their therapeutic efficacy. Br J Psychiatry 129:547–555