

Effects of Clozapine on Plasma Catecholamines and Relation to Treatment Response in Schizophrenia: A Within-Subject Comparison with Haloperidol

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We conducted a within-subject comparison of the effects of clozapine and haloperidol on plasma levels of neurotransmitters and metabolites, and related changes in specific plasma neurochemicals with clozapine response. The subjects were 14 inpatients with schizophrenia or schizoaffective disorder, who were refractory to haloperidol and at least one other typical antipsychotic medication. Subjects underwent, in the following order: a 6-week "fixed, flexible dose" haloperidol trial, followed by a 2–4 week medication-free phase, and a 6-week clozapine trial. Plasma levels of norepinephrine (NE), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylglycol (MHPG), and objective clinical ratings of total, positive, negative, and

depressive symptoms were obtained at the end of each phase. As expected, we found a substantial increase of plasma NE with clozapine but not with haloperidol. However, the increase in NE was not associated with improvement in total or positive symptomatology. There was some evidence for an association between improvement in negative symptoms and increased HVA on clozapine, as well as diminished HVA during the medication-free phase. The implications of these data for understanding the mechanisms of action of clozapine are discussed.

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Despite extensive evidence of clozapine's superior efficacy in the treatment of schizophrenia, its mechanism

of action remains unclear. Nonetheless, a series of clinical and preclinical investigations have yielded intriguing findings. Clozapine is the first effective antipsychotic with relatively weak binding to dopamine D2 receptors (see Brunello et al. 1995 for a review), compared with typical antipsychotics, a feature linked to its lack of extrapyramidal side effects and tardive dyskinesia (Nordstrom et al. 1995). Yet, clozapine clearly affects many neurotransmitter systems of potential relevance to the pathophysiology of schizophrenia. Preclinical studies have demonstrated relatively high affinity for the D4 receptor, an increased ratio of 5HT-2 to D2 receptor binding, and increased affinity for noradrenergic alpha-1 and alpha-2 adrenoceptors. It has also been suggested that clozapine's enhancement of dopamine

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activity in the prefrontal cortex could contribute to its partial efficacy in treating negative symptoms (Brunello et al. 1995; Lindenmayer 1995).

Several studies have attempted to relate clozapine's neurochemical effects with therapeutic response by comparing serum or cerebrospinal fluid (CSF) levels of neurotransmitters between different phases of treatment. The most striking finding to emerge from these studies is an increase in plasma NE (Lieberman et al. 1991; Davidson et al. 1993; Green et al. 1993; Breier et al. 1994). One of these studies (Breier et al. 1994) demonstrated a positive correlation between the NE increase on clozapine and improvement in both positive and total symptomatology, suggesting that one potential mechanism of action of clozapine is an enhancement of noradrenergic function. Changes in 3-methoxy-4-hydroxyphenylglycol (MHPG), a NE metabolite, with clozapine treatment have been less consistent (Lieberman et al. 1991; Pickar et al. 1992; Green et al. 1993). In general, plasma levels of the dopamine metabolite homovanillic acid (HVA) are reported to decrease during clozapine treatment (Pickar et al. 1992; Green et al. 1993) although not in all studies (Davidson et al. 1993).

In addition to these conflicting findings, prior studies have been hampered by a number of limitations. With one notable exception (Pickar et al. 1992), none of these studies conducted within-subject comparisons of neurotransmitter/metabolite levels between clozapine and typical antipsychotics. A major advantage of within-subject designs is that they permit the examination of intra-individual variation. Another limitation is the use of partially responsive patients, which could result in a truncated range on measures of clinical improvement, spuriously lowering correlations between neurochemical measures and clinical ratings. Moreover, in two studies, medication-free intervals were either not conducted (Breier et al. 1994), or were very brief, prohibiting the determination of absolute changes in neurotransmitter/metabolite levels. Finally, all of the previous studies utilized the Brief Psychiatric Rating Scale (BPRS) for clinical assessments; while the BPRS is effective at quantifying changes in total symptomatology, it is relatively imprecise in the separate quantitation of positive and of negative symptoms of schizophrenia. The use of the Positive and Negative Syndrome Scale (PANSS) (Kay et al. 1987) enabled us to more clearly assess positive and negative symptoms and their correlations with neurotransmitter changes over the course of treatment. This also permitted the examination of a potential relation between improvement in negative symptoms on clozapine and increased HVA, an indicator of enhanced dopamine activity, which may underlie the amelioration of these symptoms (Lindenmayer 1995).

In this investigation, we sought to test the following hypotheses in a within-subject design: 1) a marked in-

crease in plasma NE would occur with clozapine treatment, as compared to both a medication-free and haloperidol treatment interval; 2) improvement in positive symptomatology will be positively correlated with an increase in plasma NE; and 3) improvement in negative symptomatology will be positively correlated with an increase in plasma HVA. If clozapine has these effects, then one might expect a greater effect on positive/total and negative symptoms in the presence of diminished NE and HVA, respectively, at baseline. We therefore examined, in two related exploratory analyses, whether decreased medication-free levels of NE and HVA were predictive of improvement in these respective dimensions of symptomatology.

For this purpose, we obtained the above neurochemical measures within the same treatment-refractory schizophrenic subjects after six weeks of haloperidol treatment, a minimum two week medication-free interval, and at the end of six weeks of clozapine treatment. As a further assessment of NE function, plasma MHPG, a NE metabolite, was also measured. Two neuroendocrine measures, prolactin and cortisol, were also obtained during each phase. Comparisons between the medication-free and the two treatment phases were conducted for each neurochemical measure, and the relationship between symptomatic response and neurotransmitter/metabolite changes with clozapine was examined.

METHODS

Subjects

A total of 17 inpatients (9 M, 8 F; mean age = 36.2, SD = 11.9, range = 19–55) with a DSM-III-R diagnosis of chronic schizophrenia (N = 15) or schizoaffective disorder (N = 2) (American Psychiatric Association, 1987) were enrolled in this study. All subjects were inpatients on the Schizophrenia Research Unit (SRU) of the New York State Psychiatric Institute (NYSPI). All patients granted written informed consent to participate in this investigation of neurobiologic effects of clozapine in schizophrenia. Patients were diagnosed by the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al. 1994), a structured instrument which allows for systematic and comprehensive evaluation of psychiatric symptomatology, and detailed collection of medical and substance abuse history.

All patients met the following inclusion criteria: 1) treatment-refractory, as defined by a documented history of inadequate response to at least two different antipsychotic medications for at least six weeks at adequate doses (10 mg. of haloperidol or its equivalent); 2) no history of substance abuse for at least six months prior to the study, or any substance use within one

month of the study; and 3) no history of blood dyscrasias, seizures, or major medical problems.

Procedure

Description of Haloperidol, Medication-free, and Clozapine Phases. Prior to study entry (the "baseline" phase), fourteen subjects were on typical antipsychotics (loxitane, thiothixene, haloperidol, at doses ranging from 10–25 mg/day haloperidol equivalents), and anti-parkinsonian medications (benztropine 2–4 mg/day, trihexiphenidyl 2–8 mg/day)—two of these patients were also on divalproex sodium 1000–1500 mg/day; and three patients were on no medications. Any existing medications were tapered and discontinued over a 5–7 day period. Subjects were then entered into the six-week "fixed-flexible haloperidol" phase. The dosing schedule consisted of 5 mg/day for week one, 10 mg/day for week two, and 10–15 mg/day (at the discretion of the treating physician) for the remainder of this medication phase. All patients received benztropine mesylate 1–2 mg BID throughout the fixed-flexible haloperidol phase.

Response to haloperidol was defined based upon a minimal 20% improvement in the total PANSS score (see below) plus a total post-treatment PANSS score of less than 60; this criterion was analogous to the response criterion of less than 35 on the BPRS, used in a previous major clozapine efficacy study (Kane et al. 1988). All 17 subjects completed the haloperidol phase, and none were rated as haloperidol responders at the end of the trial (total PANSS: baseline: mean = 76.08, SD = 15.05; week 6 haloperidol: mean = 78.85, SD = 17.46 (3.6% increase in total PANSS). Following completion of the haloperidol phase, the patients were tapered and discontinued from haloperidol over a five-day period. After discontinuation of haloperidol, patients were maintained free of medication for a minimum of two weeks (mean = 20 days; range = 14–29 days) (the "medication-free" interval).

Clozapine treatment was initiated at 25 mg/day and increased gradually to 300 mg/day over two weeks. Over the remainder of the trial, the dosage was increased as clinically indicated, to a maximum dose of 900 mg/day throughout the remainder of the clozapine phase. Thus, all patients received at least 300 mg/day of clozapine for four weeks. Of the 17 patients originally enrolled in the study, 14 completed the six week clozapine trial (8 M, 6 F; mean age = 34.64, SD = 11.55, range = 19–55; mean age of onset = 19.8, SD = 4.8; mean duration of illness = 14.7, SD = 10.5). Two patients (1 M, 1 F) were removed from the clozapine trial by the investigators due to potentially serious medical complications (atrial fibrillation and markedly elevated liver function tests, respectively), which resolved fol-

lowing clozapine discontinuation, and one female patient elected to withdraw from the trial after 10 days for unclear reasons.

Rating Scales

All subjects received research assessments of symptom status during the final week of each phase of the study. Symptoms were assessed by the PANSS (Kay et al. 1987), a rating scale which comprehensively quantifies positive and negative symptoms of schizophrenia. Depression was assessed using the Hamilton Depression Rating Scale (HDRS) (Hamilton 1960). Our group has demonstrated excellent reliability with these instruments ($\kappa = 0.80$ or above). One of three trained clinicians (two with Master's degrees and one with a Ph.D. in clinical psychology) completed the diagnostic and symptom evaluations. Although raters were not blind to medication status, they were not aware of the purposes of the present study and were not informed of the subjects' neurotransmitter/metabolite levels.

At the end of the medication-free and clozapine phases, extrapyramidal side effects and tardive dyskinesia were assessed by trained raters using the Simpson-Angus Scale and the Abnormality Involuntary Movement Scale (AIMS), respectively. These ratings were available on 10 of the 14 patients completing the clozapine trial.

Neurotransmitter Studies

All blood studies were conducted during the last 3–5 days of the haloperidol, medication-free, and clozapine phases. Samples were drawn on two occasion, two days apart during each phase. Patients were on a low monoamine diet, which begun 12 hours prior to the first blood draw and ceased following the second blood draw. Prior to each venipuncture, subjects had no food intake from midnight of the previous night, received no morning medications, and were kept at bed rest. Blood was collected between 7 and 8 AM from an antecubital vein with the patient in a sitting position.

For the preparation of plasma to be used for the neurotransmitter and metabolite assays, blood was collected in oxylated tubes, inverted 15–20 times, and immediately placed on ice. Blood was centrifuged at 2000 r/min for 15 minutes. For the preparation of sera for the hormone assays, blood was drawn into barrier tubes, allowed to sit for 15 minutes, and centrifuged as for the plasma preparation. Both plasma and sera were stored at -20 degrees C.

Laboratory Assays. NE was quantified using high performance liquid chromatography with a triple cell amperometric detection system (Eisenhofer et al. 1986). HVA and MHPG were assayed by gas chromatogra-

Table 1. Neurotransmitter (HVA, NE, MHPG) and Hormone (Prolactin, Cortisol) Levels During Each Study Phase (Mean and Standard Deviation)^a

	Haloperidol Mean (sd)	Medication-Free Mean (sd)	Clozapine Mean (sd)	F	df	p
NE (pg/ml)	129.0 (88.0) (n = 9)	95.6 (74.9) (n = 13)	550.7 (411.1) (n = 13)	12.37	(2,16)	.001 ^b
HVA (ng/ml)	9.18 (3.80) (n = 10)	8.84 (2.10) (n = 12)	9.65 (3.14) (n = 11)	1.19	(2,16)	.33
MHPG (ng/ml)	5.58 (1.56) (n = 9)	5.53 (2.09) (n = 11)	7.41 (3.31) (n = 10)	1.50	(2,14)	.26
Prolactin (ng/ml)	40.49 (48.6) (n = 9)	7.07 (3.22) (n = 10)	8.27 (2.95) (n = 10)	4.37	(2,14)	.05 ^c
Cortisol (µg/dl)	12.62 (5.63) (n = 9)	14.30 (9.46) (n = 10)	12.82 (5.88) (n = 10)	1.32	(2,14)	.30

^aThe means and standard deviations reported here are derived from all neurotransmitter/metabolite and hormone results obtained. ANOVA was carried out only on subjects with measures at each time point; however, cases excluded from the ANOVA did not differ appreciably with respect to neurotransmitter/metabolite and hormone levels from included cases (results available on request).

^bMedication-free vs. clozapine: 95.6 vs. 550.7, $t(12) = 4.17$, $p < .001$; medication-free vs. haloperidol: 125.6 vs. 129.0, $t(8) = 0.13$, $p = .90$; clozapine vs. haloperidol: 641.8 vs. 129.0, $t(8) = 3.68$, $p < .01$.

^cMedication-free vs. clozapine: 7.36 vs. 8.36, $t(8) = 1.18$, $p = .27$; medication-free vs. haloperidol: 7.24 vs. 40.49, $t(8) = 2.12$, $p < .07$; clozapine vs. haloperidol: 8.41 vs. 44.01, $t(7) = 2.09$, $p < .08$.

phy/mass spectrometry with deuterated internal standards (Fri et al. 1974; Jimerson et al. 1981). Prolactin and cortisol were quantified using radioimmunoassay (Mircomedic RIA kit) (Sinha et al. 1973).

Values for each neurotransmitter, metabolite, and hormone were averaged for analysis.

Data Analysis

Analysis of data was carried out using SPSS for Windows (SPSS 1995). Repeated-measures analysis of variance ANOVA was used for assessing main effects, and comparisons of means were conducted by paired *t*-tests. Because the distributions of the neurotransmitter data demonstrated significant skewness and/or kurtosis, the non-parametric Spearman coefficient was used for correlations.

RESULTS

Neurotransmitter Data

The mean levels of each neurotransmitter (NE, HVA, MHPG) and hormones (prolactin, cortisol) at each phase (haloperidol, medication-free, clozapine) are presented in Table 1.

As expected, a significant main effect of medication phase was demonstrated for NE ($F(2,16) = 12.37$, $p < .001$). Post-hoc pairwise comparisons demonstrated a significant increase of plasma NE with clozapine treatment relative to the medication-free phase (95.6 (74.9) vs. 550.7 (411), $t(12) = 4.17$, $p < .001$) and to the haloperidol phase (641.8 (457.7) vs. 129.0 (88.0), $t(8) = 3.68$, $p < .01$). Individual levels of NE during each phase are illustrated in Figure 1. There was no NE increase with

haloperidol, compared to the medication-free phase ($p = 0.90$). No significant main effect of medication phase was observed for HVA ($p = .3$) or for MHPG ($p = .26$).

As expected, a significant main effect of medication phase was also demonstrated for prolactin ($F(2,14) = 4.37$, $p < .05$), and pairwise comparisons showed a nearly significant increase with haloperidol (7.24 (3.37) vs. 40.45 (48.55), $t(8) = 2.12$, $p < .07$), but not with clozapine (7.36 (3.28) vs. 8.36 (3.11), $t(8) = 1.18$, $p = .27$). No significant main effect of medication phase appeared for cortisol ($p = .30$).

Clinical Effects

Symptomatology. As expected, clozapine led to significant clinical improvement, as compared to the medication-free interval, for positive symptoms (mean PANSS positive symptom score (SD) medication-free vs. clozapine = 24.64 (7.31) vs. 15.0 (5.51), $t(13) = 5.03$, $p < .0001$) and total symptomatology (mean total PANSS (SD) medication-free vs. clozapine = 91.86 (20.96) vs. 67.64 (15.11), $t(13) = 4.67$, $p < .0001$). There was a strong trend for improvement in negative symptoms (mean PANSS negative symptom score (SD) medication-free vs. clozapine = 23.64 (7.53) vs. 20.50 (6.15), $t(13) = 2.11$, $p < .06$). Depression was also significantly improved by clozapine relative to the medication-free phase (mean HDRS medication-free vs. clozapine = 16.93 (5.15) vs. 9.07 (4.94), $t(13) = 5.0$, $p < .0001$). Because of the possibility that this latter finding was due to diminished negative or positive symptoms, we conducted a follow-up analysis of covariance, controlling for the change in negative and positive symptoms. The improvement in

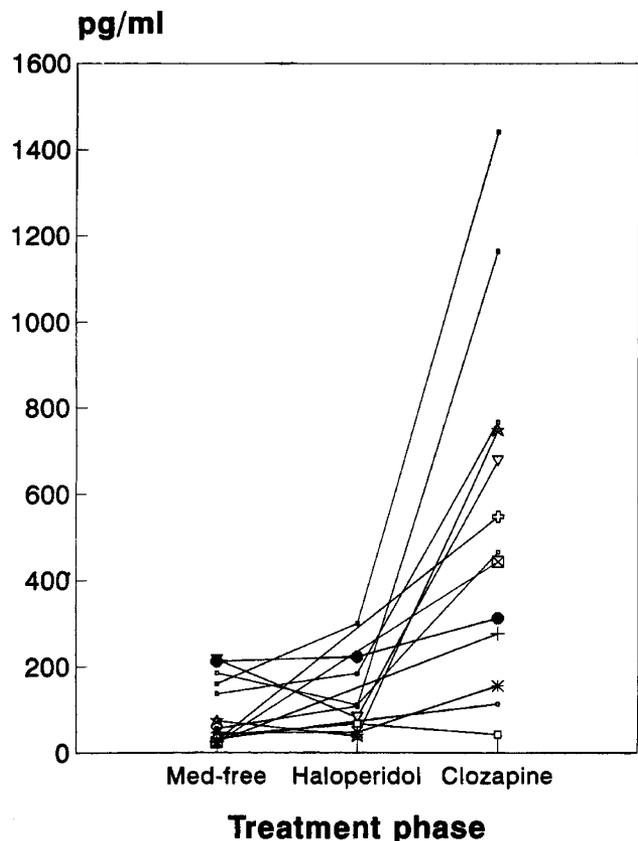


Figure 1. NE level (pg/ml) during each phase (medication-free, haloperidol, clozapine).

depression with clozapine treatment remained significant after controlling for change in both negative ($p < .002$) and positive symptoms ($p < .05$).

The proportion of clozapine treatment-responders, as defined by at least a 20% improvement in the total PANSS accompanied by a post-treatment score of less than 60, was 4/14 (29%).

Extrapyramidal Side Effects and Tardive Dyskinesia. Treatment with clozapine, as compared to the medication-free phase, was not associated with significant improvement in extrapyramidal side effects (mean Simpson-Angus total score (SD) medication-free vs. clozapine: 3.44 (2.56) vs. 3.20 (4.34), $t(7) = 0.41, p = .70$). There was no significant association between change in extrapyramidal side effects and improvement in negative symptoms ($r = .20, p = .64$). Tardive dyskinesia did improve significantly on clozapine (mean AIMS total score (SD) medication-free vs. clozapine: 8.11 (8.82) vs. 2.20 (4.13), $t(7) = 2.49, p = .04$).

Correlations between Neurotransmitter Level and Clinical Response

We next attempted to demonstrate relationships between change in neurotransmitter levels between the medication-free and clozapine phases and change in symptomatology.

Contrary to our hypothesis, there was no significant correlation between the increase in plasma NE and clin-

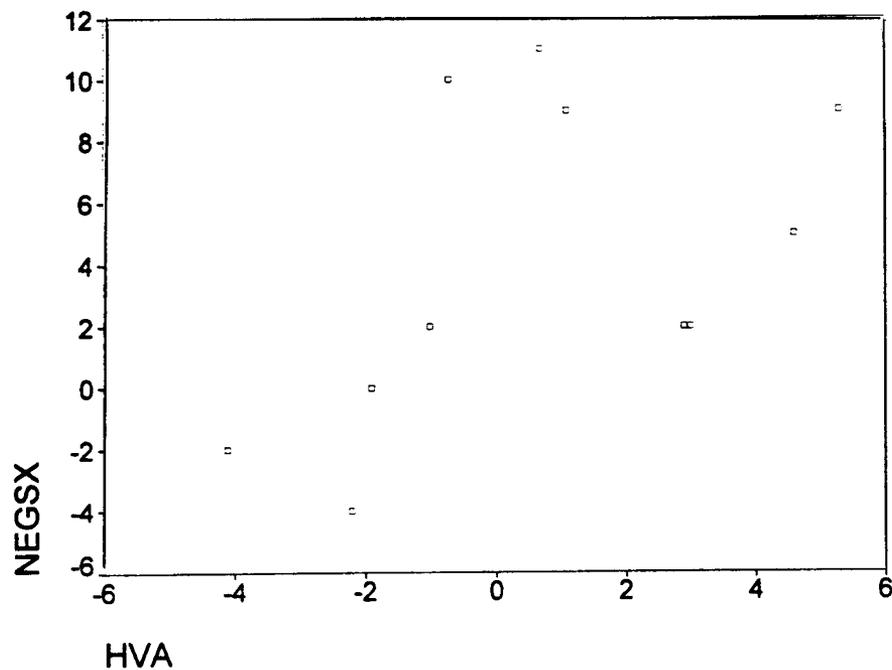


Figure 2. Correlation between change in negative symptom scores from the PANSS (Neg. sx; + = improvement) and change in homovanillic acid (HVA) level (ng/ml), during clozapine treatment.

$\rho = .56, p < .08$

ical response on the positive symptomatology scale ($\rho = -.10$). Visual inspection of the NE \times clinical response plot confirmed the statistical results; in particular, the finding could not be accounted for by the presence of outliers. In addition, the increase in NE was not correlated with improvement in total symptomatology. ($\rho = .11$). For the NE metabolite MHPG, exploratory analyses similarly did not reveal any significant correlation between change in MHPG and improvement in either positive ($\rho = -.33$) or total ($\rho = -.42$) symptomatology.

With respect to HVA, there was a trend for a positive correlation between increased HVA from the medication-free to clozapine phases and improvement in negative symptoms ($\rho = .56, p < .08$) (Figure 2), consistent with our hypothesis.

In two exploratory analyses, we examined whether medication-free levels of plasma NE and HVA predicted response to clozapine, with respect to positive/total symptoms and negative symptoms, respectively. The medication-free level of NE was not predictive of improvement in either total symptomatology ($\rho = .18$) or positive symptomatology ($\rho = .06$). Lower medication-free HVA was correlated with improvement in negative symptomatology ($\rho = -.61, p < .05$) (Figure 3).

DISCUSSION

We have demonstrated in this within-subjects study that clozapine treatment led to a significant, nearly six-

fold increase in plasma NE. This was in contrast to haloperidol, which did not alter plasma NE levels. To our knowledge, this is only the second study to contrast the effects of clozapine and haloperidol within the same subjects, replicating Pickar et al. (1992). Increases in plasma NE with clozapine as compared to haloperidol have also been demonstrated in studies utilizing separate clozapine- and haloperidol-treated groups (Lieberman et al. 1991; Davidson et al. 1993; Green et al. 1993; Breier et al. 1994).

However, we did not find a relation between increased NE and improvement in positive symptoms of schizophrenia. Indeed, previous studies also failed to report a relation between NE level and clinical response (Lieberman et al. 1991; Davidson et al. 1993; Green et al. 1993). In fact, to date, only one study has shown a significant correlation between increased NE and clinical improvement (Breier et al. 1994). In that study, improvement in the BPRS total score and positive symptom score were each positively associated with an increase in plasma NE. While our lack of an association might be attributed to a relatively small sample size, Breier et al.'s study also utilized a small sample ($N = 11$). Yet, the lack of an observed relation between NE and positive symptoms does not necessarily imply that the NE increase is unrelated to clozapine's mechanism of action. Indeed, studies have demonstrated that idazoxan, a selective alpha-2 adrenergic antagonist, which enhances noradrenergic neurotransmission, has significant efficacy in augmenting the antipsychotic re-

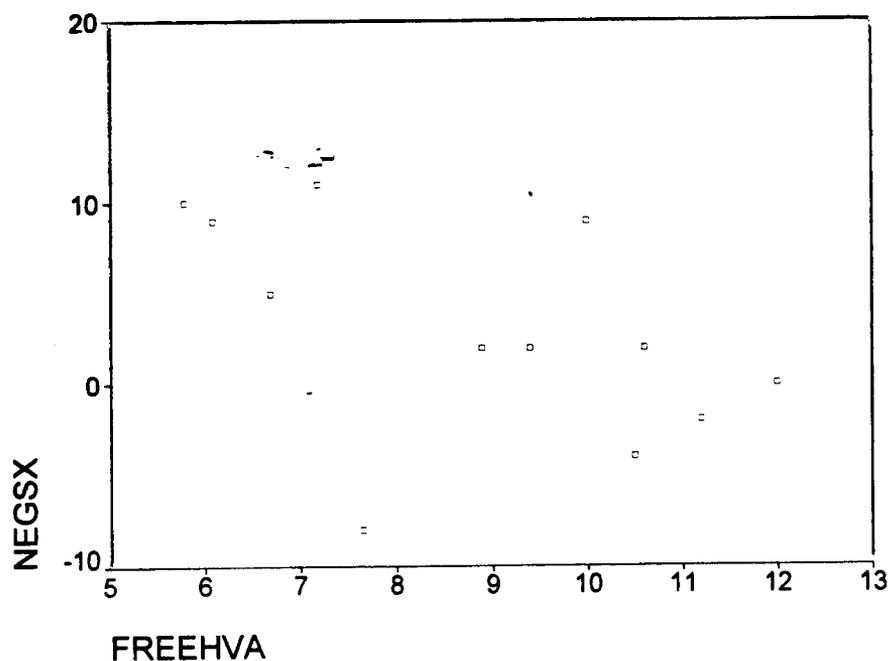


Figure 3. Correlation between change in negative symptom scores from the PANSS (Neg. sx; + = improvement) and medication-free homovanillic acid (free HVA) level (ng/ml), during clozapine treatment.

$$\rho = -.61, p < .05$$

sponse to typical antipsychotics in treatment-resistant patients with schizophrenia (Litman et al. 1993; Litman et al. 1996).

Several theories have been advanced to explain the increase in plasma NE by clozapine. These include α 1-adrenergic blockade, which would increase NE through reflexive sympathetic outflow mechanisms, α 2-adrenergic blockade, which might interfere with this receptor's presynaptic modulatory function on noradrenergic cells, leading to increased NE outflow, and NE reuptake blockade (Breier 1994), which produces increased synaptic NE concentrations. Each of these effects has been demonstrated in preclinical studies. While one would expect that increases in extra-neuronal NE concentrations would lead to increased MHPG, one of its principal metabolites (Kopin et al. 1984), we found only a small, marginally significant increase in plasma MHPG, consistent with a previous study (Pickar et al. 1992). Conceivably, another NE metabolic pathway may have been activated instead, or, as hypothesized by Breier et al. (1994), there may have been additional blockade of NE reuptake.

Our third hypothesis, that increased HVA is positively correlated with an improvement in negative symptoms, is consistent with our finding of a trend for an association. In line with this finding, we also showed a potential relation between diminished HVA during the medication-free interval and improvement in negative symptoms on clozapine—conceivably, those individuals whose negative symptoms were partially attributable to low HVA were most responsive to clozapine's enhancement of dopamine function. These findings are of interest in light of low prefrontal dopamine activity in schizophrenic patients (Davis et al. 1991), which might underlie negative symptoms (Deutch 1992), and findings that dopamine agonists appear to produce improvement in negative symptoms (Bodkin et al. 1996; Boyer et al. 1995; Ohmori et al. 1993; see Lindenmayer 1995, for a review). Furthermore, clozapine increases the basal output of dopamine neurons projecting to the prefrontal cortex (Moghaddam 1994). However, this finding must be regarded as tentative due to the small sample size, the fact that the association was only a trend, and the overall lack of change in plasma HVA.

The lack of change in plasma HVA with both clozapine and haloperidol is inconsistent with some previous studies. Clozapine has been shown to decrease HVA levels in some (Green et al. 1993; Pickar et al. 1992), but not in all studies (Davidson et al. 1993; Lieberman et al. 1991), and decreased HVA has been correlated with overall clinical improvement (Green et al. 1993; Pickar et al. 1992). However, we observed no significant correlation between change in HVA on clozapine and improvement in total or positive symptomatology. Yet, since a fraction of NE is metabolized into HVA, it is possible that the substantial increase in NE

by clozapine in our study may have obscured a decrease in HVA (see Davidson et al. 1993).

All of our findings occurred in the context of an expected rate of response to therapeutic dosages of clozapine with respect to total symptomatology, significant improvement in total and positive symptoms, and near-significant improvement in negative symptoms. The expected rise in prolactin on haloperidol but not clozapine served to validate clozapine's lack of effect on D2 receptors in this study.

An additional finding worthy of comment was significant improvement in depression with clozapine. This was not accounted for solely by improvement in positive or negative symptoms. Other recent studies have reported similar effects of clozapine on depression in patients with schizophrenia (Lindenmayer et al. 1994; Azorin 1995; Meltzer and Okayli 1995; Zarate et al. 1995). However, our exploratory analyses did not demonstrate any significant correlations between improvement in depression and any neurochemical measure during the clozapine phase.

Several factors limit the scope and interpretations to be derived from this study. First, our sample size was small for correlational analyses, resulting in low statistical power, which may have limited our ability to demonstrate a relationship between change in total/positive symptoms with the NE increase. A power analysis revealed that a medium effect size ($r = 0.50$) could be detected for 13 subjects with only 44% power.

Second, the design was not counterbalanced: all subjects underwent the three phases—fixed haloperidol, medication-free, and clozapine—in the same sequence. Thus, one cannot rule out the possibility of an order effect, with respect to treatment response and neurochemical changes. In addition, the fact that raters were not blind to medication status could have been a source of bias in measuring treatment response, although this would not explain the neurochemical findings, as these levels were objectively quantified by laboratory personnel unaware of medication phase.

Third, our sample was selected based upon stringent criteria for refractoriness to clinical treatment, and our subjects were inpatients on a schizophrenia research unit. Therefore, one must be cautious in generalizing our findings to less refractory patients and those from community samples. Of note, the only study to demonstrate a relation between NE increase and symptomatologic improvement was on an outpatient sample (Breier et al. 1994), in contrast to most other studies.

Fourth, given that our neurochemical measures were obtained from plasma, one must be cautious in interpreting these data as reflecting brain neurotransmitter activity. A large proportion of plasma HVA is generated by peripheral dopaminergic and noradrenergic neurons and by other peripheral tissues (Friedhoff and Silva 1995), and plasma NE arises mainly from periph-

eral sympathetic neurons (Lake et al. 1984). Additional problems are the difficulty in inferring regional brain neurotransmitter activity from plasma measures, and the fact that mixed venous blood may only partially represent systemic NE because of local tissue removal. However, it has been argued that *changes* brought about by antipsychotics should be correlated between peripheral and central neurotransmitters, irrespective of their different origins; a good example of this is the sequence of events occurring at dopaminergic synapses in the brain and parallel changes in plasma HVA levels (Friedhoff and Silva 1995; Davis et al. 1991). Similarly, medications that affect NE levels in the periphery generally produce the same effects in the brain following entry into the central nervous system (Goldstein et al. 1987), and CSF levels of NE and MHPG are significantly correlated with plasma levels of these neurotransmitters (Ziegler et al. 1977; Jimerson et al. 1981).

Fifth, although blockade of serotonin receptors is suspected in the mechanism of action of clozapine, we did not quantify levels of the serotonin metabolite 5-HIAA. However, clinical studies have not demonstrated significant changes in CSF 5-HIAA during clozapine treatment (Lieberman et al. 1991; Pickar et al. 1992). Moreover, we did not demonstrate a change in serum cortisol, which is believed to reflect serotonin activity to some degree (Meltzer and Maes 1995). This lack of change is consistent with a study by Breier et al. (1994), although Pickar et al. (1992) did report diminished cortisol following clozapine treatment, which was interpreted as possibly reflecting clozapine's antiserotonergic effect.

Sixth, neurotransmitter and metabolite levels were measured during only one week of each phase of study. If clozapine caused a change in NE or HVA levels prior to six weeks, followed by a return to baseline levels, a potential relationship between therapeutic response to clozapine and neurotransmitter/metabolite levels may have been missed. Such relationships have been observed most notably for alterations in HVA early in the course of haloperidol treatment (Duncan et al. 1993).

Finally, it is possible that greater than six weeks may be necessary for certain neurochemical effects and their relation to symptomatology to become manifest. Increasing evidence suggests that improvement continues in both positive and negative symptoms with an extended duration of treatment (Kane et al. 1988; Jalenques and Coudert 1992; Breier et al. 1993).

CONCLUSION

We have replicated, using a within-subjects design, previous findings of a marked increase in plasma NE following a six week trial of clozapine, in contrast to haloperidol. However, we did not demonstrate a relationship

between NE and clinical response to clozapine. In addition, our results were suggestive of a relation between increased plasma HVA and improvement in negative symptoms, although this finding must be regarded as tentative. Future studies using larger sample sizes, counterbalanced, randomized, placebo-controlled designs, more frequent measures of neurotransmitters/metabolites, and extended treatment intervals are warranted.

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REFERENCES

- American Psychiatric Association (1987): Diagnostic and Statistical Manual of Mental Disorders, 3rd ed., revised. Washington DC, APA
- Azarin JM (1995): Long-term treatment of mood disorders in schizophrenia. *Acta Psych Scand* 338 (suppl):20–23
- Bodkin JA, Cohen BM, Salomon MS, Cannon SE, Zornberg GL, Cole JO (1996): Treatment of negative symptoms in schizophrenia and schizoaffective disorders by selegiline augmentation of antipsychotic medication. A pilot study examining the role of dopamine. *J Nerv Mental Disease* 184:295–301
- Boyer P, Lecrubier Y, Puech AJ, Dewailly J, Aubin F (1995): Treatment of negative symptoms in schizophrenia with amisulpride. *Br J Psych* 166:68–72
- Breier A (1994): Clozapine and noradrenergic function: Support for a novel hypothesis for superior efficacy. *J Clin Psych* 55:122–125
- Breier A, Buchanan RW, Irish D, Carpenter WT (1993): Clozapine treatment of outpatients with schizophrenia: outcome and long-term response patterns. *Hosp Comm Psych* 44:1145–1149
- Breier A, Buchanan RW, Waltrip RW, Listwak S, Holmes C, Goldstein D (1994): The effect of clozapine on plasma norepinephrine: Relationship to clinical efficacy. *Neuropsychopharmacology* 10:1–7
- Brunello N, Masotto C, Steardo L, Markstein R, Racagni G (1995): New insights into the biology of schizophrenia through the mechanism of action of clozapine. *Neuropsychopharmacology* 13:177–213
- Davidson M, Kahn RS, Stern RG, Hirschowitz J, Apter S, Knott P, David KL (1993): Treatment with clozapine and its effect on plasma homovanillic acid and norepinephrine concentrations in schizophrenia. *Psych Res* 46:151–163
- Davis KL, Kahn RS, Ko G, Davidson M (1991): Dopamine in schizophrenia: A review and reconceptualization. *Am J Psych* 148:1474–1486

- Deutch AY (1992): The regulation of subcortical dopamine systems by the prefrontal cortex: interactions of central dopamine systems and the pathogenesis of schizophrenia. *J Neural Trans* 36 (Suppl):61–89
- Duncan E, Wolkin A, Angrist B, Sanfilippo M, Weiland S, Cooper TB, Rotrosen J (1993): Plasma homovanillic acid in neuroleptic responsive and nonresponsive schizophrenics. *Biol Psych* 34:523–528
- Eisenhofer G, Goldstein DS, Stull R, Keiser HR, Sunderland T, Murphy DL, Kopin IJ (1986): Simultaneous liquid chromatographic determination of 3,4-dihydroxyphenylglycol, catecholamines, and 3,4-dihydroxyphenylalanine in plasma and their responses to inhibition of monoamine oxidase. *Clin Chem* 32:2030–2033
- Fri CG, Wiesel FA, Sedvall G (1974): Simultaneous quantification of Homovanillic Acid and 5-hydroxyindoleacetic acid in cerebrospinal fluid by mass fragmentography. *Life Sci* 14:2469–2480
- Friedhoff AJ, Silva RR (1995): The effects of neuroleptics on plasma homovanillic acid. In Bloom FE, Kupfer DJ (eds), *Psychopharmacology: The Fourth Generation of Progress*. New York, Raven Press, pp. 1229–1233
- Goldstein DS, Zimlichman R, Kelly GD, Stull R, Bacher JD, Keiser HR (1987): Effect of ganglion blockade on cerebrospinal fluid norepinephrine. *J Neurochem* 49:1484–1490
- Green AI, Alam MY, Sobieraj JT, Pappalardo KM, Wateraux C, Salzman C, Schatzberg AF, Schildkraut JJ (1993): Clozapine response and plasma catecholamines and their metabolites. *Psych Res* 46:139–149
- Hamilton M (1960): A rating scale for depression. *J Neurol Neurosurg Psych* 23:56–62
- Jalenques I, Coudert AJ (1992): A new therapeutic approach to drug-resistant schizophrenia: clozapine. Long-term prospective study in 16 patients [French]. *Acta Psych Bel* 92:323–338
- Jimerson DC, Markey SP, Oliver JA, Kopin IJ (1981): Simultaneous measurement of plasma 4-hydroxy-3-methoxyphenylethylene glycol and 3,4-dihydroxyphenylethylene glycol by gas chromatography mass spectrometry. *Biomed Mass Spec* 8:256–258
- Kane J, Honigfeld G, Singer J, Meltzer H (1988): Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. *Arch General Psych* 45:789–796
- Kay SR, Fiszbein A, Opler LA (1987): The Positive and Negative Syndrome Scale (PANSS) for schizophrenia. *Schizophr Bull* 13:261–276
- Kopin IJ, Jimerson DC, Markey SP, Ebert MH, Polinsky RJ (1984): Disposition and metabolism of MHPG in humans: Application to studies in depression. *Pharmacopsychiatry* 17:3–8
- Lake CR, Chernow B, Feuerstein G, Goldstein DS, Ziegler MG (1984): The sympathetic nervous system in man: Its evaluation and the measurement of plasma norepinephrine. In Ziegler MG, Lake CR (eds), *Frontiers of Clinical Neuroscience: Norepinephrine*, Vol. 2. Baltimore, Williams & Wilkins Company, pp. 1–26
- Lieberman J, Johns C, Pollack S, Masiar S, Bookstein P, Cooper T, Iadorola M, Kane J (1991): Biochemical effects of clozapine in cerebrospinal fluid of patients with schizophrenia. *Advances in Neuropsychiatry and Psychopharmacology. Schizophr Res* 1:341–349
- Lindenmayer JP (1995): New pharmacotherapeutic modalities for negative symptoms in psychosis. *Acta Psych Scand* 388:15–19
- Lindenmayer JP, Grochowski S, Mabusat L (1994): Clozapine effects on positive and negative symptoms: a six-month trial in treatment-refractory schizophrenics. *J Clin Psychopharmacol* 14:201–204
- Litman RE, Hong WW, Weissman EM, Su TP, Potter WZ, Pickar (1993): Idazoxan, an alpha-2 antagonist, augments fluphenazine in schizophrenic patients: A pilot study. *J Clin Psychopharmacol* 13:264–267
- Litman RE, Su TP, Potter WZ, Hong WW, Pickar D (1996): Idazoxan and response to typical neuroleptics in treatment-resistant schizophrenia: Comparison with the atypical neuroleptic, clozapine. *Br J Psych* 168:571–579
- Meltzer HY, Maes M (1995): Effect of pindolol pretreatment on MK-212-induced plasma cortisol and prolactin responses in normal men. *Biol Psych* 38:310–318
- Meltzer HY, Okayli G (1995): Reduction of suicidality during clozapine treatment of neuroleptic-resistant schizophrenia: impact on risk-benefit assessment. *Am J Psych* 152:183–190
- Micromedic RIA kit: Micromedic Systems, Inc., Subsidiary of Rohm and Haas Co., Horsham, PA
- Moghaddam B (1994): Preferential activation of cortical dopamine neurotransmission by clozapine: functional significance. *J Clin Psych* 55 (suppl B):27–29
- Nordstrom AL, Farde L, Nyberg S, Karlsson P, Halldin C, Sedvall G (1995): D1, D2, and 5-HT2 receptor occupancy in relation to clozapine serum concentration: a PET study of schizophrenic patients. *Am J Psych* 152:1444–1449
- Nurnberger JL, Blehar MC, Kaufmann CA, York-Cooler C, Simpson SG, Harkavy-Friedman J, Severe JB, Malaszynski D, Reich T (1994): Diagnostic Interview for Genetic Studies. Rationale, unique features, and training. NIMH Genetics initiative. *Arch Gen Psych* 51:849–859
- Ohmori T, Koyama T, Inoue T, Matsubara S, Yamashita I (1993): B-HT 920, a dopamine D2 agonist, in the treatment of negative symptoms of chronic schizophrenia. *Biol Psych* 33:687–693
- Pickar D, Owen RR, Litman RE, Konicki PE, Gutierrez R, Rapaport MH (1992): Clinical and biologic response to clozapine in patients with schizophrenia. *Arch Gen Psych* 49:345–353
- Sinha YM, Selby FW, Lewis OJ, Vanderlaan WP (1973): A homologous RIA for human prolactin. *JCEM* 36:509–516
- SPSS 6.1. Windows & Base Package. January 1996. Chicago, Illinois.
- Zarate CA, Tohen M, Baldessarini RJ (1995): Clozapine in severe mood disorders. *J Clin Psych* 56:411–417
- Ziegler MG, Lake CR, Wood JH, Brooks BR, Ebert MH (1977): Relationship between norepinephrine in blood and cerebrospinal fluid in the presence of a blood-cerebrospinal fluid barrier for norepinephrine. *J Neurochem* 28:677–679