

Mechanism of Peripheral Noradrenergic Stimulation by Clozapine

Igor Elman, M.D., David S. Goldstein, M.D., Ph.D., Graeme Eisenhofer, Ph.D., Joan Folio, R.N., Anil K. Malhotra, M.D., Caleb M. Adler, M.D., David Pickar, M.D., and Alan Breier, M.D.

Elevated plasma norepinephrine (NE) levels is a relatively consistent clinical effect of clozapine. Plasma NE levels reflect an interplay of release, reuptake, metabolism, and excretion. To explore the mechanism of clozapine-induced plasma NE elevation, we measured arterial plasma levels of NE and other catechols during intravenous infusion of tritium-labeled NE (^3H -NE) in schizophrenic patients treated with clozapine, fluphenazine, or placebo. Clozapine-treated patients had markedly higher levels of NE than did the patients treated with fluphenazine or placebo. NE spillover averaged more than three times higher in clozapine-treated patients; whereas NE clearance did not differ among the groups. Production of

^3H -dihydroxyphenylglycol (^3H -DHPG), a purely intraneuronal metabolite of ^3H -NE in clozapine-treated patients was normal, indicating that clozapine did not affect neuronal uptake of NE. Because plasma levels of DHPG and dihydroxyphenylacetic acid (DOPAC), deaminated metabolites of catecholamines, in clozapine-treated patients were normal, clozapine also did not seem to inhibit intraneuronal monoamine oxidase (MAO). High plasma NE levels in clozapine-treated patients, therefore, resulted from increased NE spillover rather than decreased reuptake, metabolism, or clearance.

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Clozapine is an atypical neuroleptic with efficacy superior to that of conventional antipsychotic agents (Kane et al. 1988; Leiberma et al. 1989; Breier et al. 1994a; Breier et al. 1994b; Meltzer 1995; Meltzer et al. 1996). A relatively consistent clinical effect of clozapine is a robust increase in norepinephrine (NE) levels, both in cerebrospinal fluid (CSF) (Ackenheil 1989; Leiberma et al. 1989; Leiberma et al. 1991; Pickar et al. 1992) and in plasma (Pickar et al. 1992; Green et al. 1993; Davidson et al. 1993; Breier et al. 1994b; Breier 1994; Brown et al.

1997). Plasma NE increases have been related to clinical improvement (Breier et al. 1994b; Breier 1994), suggesting that this neurochemical effect may be involved in clozapine's therapeutic mechanism of action. In contrast, traditional antipsychotic agents studied to date have no or negligible influence on NE levels (Pickar et al. 1992; Breier et al. 1994b; Brown et al. 1997).

The mechanism of clozapine-induced NE elevation has not been established. Plasma levels of any endogenous biochemical represent the ratio of the rate of release of the substance into the bloodstream (spillover) and clearance of the substance from the bloodstream. Clozapine-induced elevations in plasma NE levels, therefore, could result from increased spillover, decreased clearance, or a combination of both.

Neuronal uptake by sympathetic nerves constitutes a determinant of plasma NE spillover (Eisenhofer et al. 1996) and clearance (Esler et al. 1981; Eisenhofer et al. 1991). After neuronal uptake of NE, some of the NE in the axoplasm undergoes oxidative deamination by mitochondrial monoamine oxidase (MAO), forming dihy-

From the Experimental Therapeutics Branch (IE, AKM, CMA, DP, AB), Clinical Neuroscience Branch (DSG, GE, JF), National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland.

Address correspondence to: Igor Elman, M.D., Massachusetts General Hospital, Harvard Medical School, West End House, 16 Blossom Street, Boston, Massachusetts 02114, USA.

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droxyphenylglycol (DHPG). Inhibition of neuronal uptake, therefore, could explain the finding of normal plasma DHPG levels, despite high plasma NE levels, in clozapine-treated patients (Breier et al. 1994b; Breier 1994).

The present study applied a comprehensive neurochemical approach to examine the mechanisms responsible for clozapine-induced elevations in plasma NE concentrations. This approach was based on intravenous infusion of tracer amounts of ^3H -NE in schizophrenic patients treated with clozapine, fluphenazine, or placebo. Because formation of ^3H -DHPG from ^3H -NE requires both neuronal uptake and intraneuronal deamination by MAO, measurement of plasma ^3H -DHPG responses provided the basis for assessing both of these processes. Plasma levels of the NE precursor dihydroxyphenylalanine (DOPA) and of the dopamine metabolite DOPAC were also examined to provide information about NE synthesis and further information about intraneuronal metabolism by MAO.

METHODS

Subjects

Ten clozapine-, 7 fluphenazine-, and 7 placebo-treated patients meeting *DSM-IV* criteria for schizophrenia, as determined by structured diagnostic interview, underwent this study at the National Institutes of Health Clinical Center. All subjects gave written informed consent to participate in the protocol, which was approved by the National Institute of Mental Health Review Board. All subjects were in good physical health, as evidenced by physical examination, ECG, and screening blood tests. The treatment groups did not differ in demographic or clinical characteristics (Table 1).

The patients were on a stable dose of medication or medications free for a minimum of 2 weeks. Doses were varied to achieve maximal clinical efficacy (clozapine mean dose \pm SD: 385.00 \pm 178.4 mg per day; fluphenazine mean dose \pm SD: 21.02 \pm 3.95 mg per day).

Procedure

The subjects were studied after having fasted and refrained from alcohol, tobacco, caffeine, or physical activity for at least 10 hours. With the patient supine, an arterial catheter was inserted percutaneously in a radial artery after local anesthesia of the overlying skin. An antecubital intravenous catheter was inserted into the contralateral arm. After 60 min, ^3H -NE ([1,7- ^3H]NE, 14-22 Ci/mmol; New England Nuclear, Boston, MA) in 50 cc normal saline was infused intravenously at 0.75 ml/min. Arterial blood samples (7–8 ml) were obtained at 15, 30, 45, and 60 min after the ^3H -NE infusion began.

Biochemical Variables

Blood for assays was collected in heparinized tubes and placed on wet ice. After separation by refrigerated centrifugation, the plasma was stored at -80°C . Plasma catechol levels were assayed using liquid chromatography with electrochemical detection (Eisenhofer et al. 1986). Concentrations of ^3H -NE and ^3H -DHPG were measured by scintillation spectrometry on effluent from the chromatographic column (Eisenhofer et al. 1991). Interassay coefficients of variation were 6.5% for NE, 3.9% for ^3H -NE, 8.4% for DHPG, 4.7% for ^3H -DHPG, 5.9% for DOPA, and 11.6% for DOPAC. Intra-assay coefficients of variations were 1.9% for NE, 3.2% for ^3H -NE, 3.7% for DHPG, 7.6% for ^3H -DHPG, 3.8% for DOPA, and 3.9% for DOPAC.

The rate of release of endogenous NE into arterial plasma and the rate of clearance from plasma was determined with the following equations: $CL = IR/[^3\text{H}]\text{-NE}$; $SP = CL \times \text{NE}_a$, where CL is the arterial plasma NE clearance (ml/min), IR is the infusion rate of [^3H]-NE, [^3H]-NE is the arterial plasma concentration of [^3H]-NE, SP is the NE spillover rate (pmol/min) into arterial plasma, and NE_a is the arterial plasma concentration of endogenous NE (Esler et al. 1979). Neuronal uptake (Eisenhofer et al. 1991) and NE stores (Eisenhofer et al. 1991; Eisenhofer et al. 1996) were assessed from the ^3H -DHPG and ^3H -NE concentrations.

Table 1. Demographic and Clinical Characteristics^a of Schizophrenic Patients Treated with Clozapine ($n = 10$), Fluphenazine ($n = 7$), and Placebo ($n = 7$)

Patient Characteristic	Clozapine	Fluphenazine	Placebo	F, df, p
Age (yr)	38.0 (8.7)	39.3 (7.7)	43.6 (4.4)	1.21, 2, 0.32
Sex ^b (M/F)	9/1	4/3	5/2	
Age of onset (yr)	20.1 (6.8)	26.9 (6.5)	27.1 (8.4)	2.7, 2, 0.09
Duration of illness (yr)	17.9 (12.9)	12.4 (6.1)	16.4 (9.1)	0.60, 2, 0.56
No. hospitalizations	9.5 (8.1)	9.3 (10.4)	4.4 (4.2)	0.97, 2, 0.40

^aData are presented as mean (standard deviation).

^b $\chi^2 = 2.44$, $df = 2$, $p = .30$.

Statistical Analyses

The data were analyzed using Statistica (StatSoft, Inc., Tulsa OK 74104). Results across the 4 sampling points were averaged and expressed as means \pm standard deviation (SD). Differences among the treatment groups were assessed by one-way repeated-measures analysis of variance (ANOVAs), with drug condition (clozapine, fluphenazine, placebo) as the grouping factor. When a group effect was significant, post hoc Newman-Keuls *t*-tests were performed. Time-related trends in ^3H -DHPG/ ^3H -NE ratios were examined by two-way ANOVAs, with drug condition as the grouping factor and time as the within-subjects factor. A *p* value less than .05 defined statistical significance. All tests were two-tailed.

RESULTS

Catechol Levels

Clozapine-treated patients had more than three times higher arterial plasma NE levels ($F = 9.39$; $df = 2$; $p = .001$, Figure 1) than patients treated with fluphenazine or placebo. Post hoc analyses revealed significantly higher NE levels in subjects treated with clozapine than with fluphenazine ($p = .002$) or placebo ($p = .005$). There were no significant NE differences between fluphenazine and placebo treatment groups. The groups did not differ in plasma levels of DOPA or DOPAC (Table 2).

NE Spillover

NE spillover was increased by more than threefold in clozapine-treated patients ($F = 9.41$; $df = 2$; $p = .001$) (Figure 1), as compared with both of the other groups (fluphenazine, $p = .004$; placebo, $p = .002$). Fluphenazine did not alter NE spillover.

NE Clearance

The three treatment groups did not differ in plasma NE clearance ($F = 0.42$; $df = 2$; $p = .66$) (Figure 1).

Time-Related Trends in ^3H -DHPG/ ^3H -NE Ratio

^3H -DHPG/ ^3H -NE ratios increased progressively with time ($F = 18.53$; $df = 3$; $p < .0001$). The increases did not differ among the groups, as indicated by a statistically nonsignificant interaction effect for group with time ($F = 0.91$; $df = 6,3$; $p = .49$, Figure 2).

DISCUSSION

The major finding of this study is that high plasma NE levels in clozapine-treated patients result entirely from

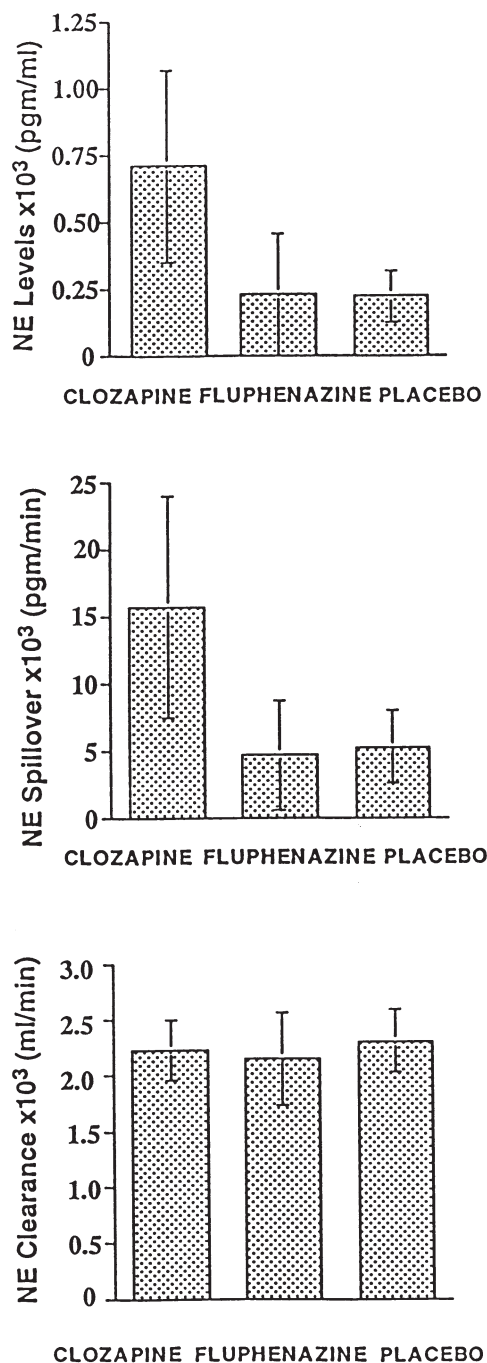


Figure 1. NE plasma level, NE spillover, and NE clearance in schizophrenic patients treated with clozapine ($n = 10$), fluphenazine ($n = 7$), and placebo ($n = 7$).

increased NE spillover and not from decreased clearance. Several factors determine NE spillover, other than exocytotic release in response to sympathetic nerve traffic. Probably the most important is reuptake. Estimates of the efficiency of reuptake of endogenously released NE range up to about 90% (Iversen 1973; Esler et al. 1990). Clearly, even a small amount of inhibition of re-

Table 2. Arterial Plasma Catechol Levels^a (pgm/ml) in Schizophrenic Patients Treated with Clozapine (*n* = 10), Fluphenazine (*n* = 7), and Placebo (*n* = 7)

Catechol	Clozapine	Fluphenazine	Placebo	F, df, <i>p</i>
DHPG	721.78 (137.99)	831.36 (361.31)	765.25 (177.48)	0.45, 2, 0.64
DOPA	1490.75 (271.54)	1394.21 (213.84)	1362.18 (271.54)	0.56, 2, 0.58
DOPAC	1303.86 (196.23)	1290.57 (447.94)	1043.08 (551.37)	1.17, 2, 0.33

^aData are presented as mean (standard deviation).

uptake would augment the amount of NE entering the plasma for a given rate of exocytotic release from the terminals. The finding of high plasma NE levels without a corresponding increase in plasma DHPG would support this sort of mechanism, because plasma DHPG reflects the metabolism of axoplasmic NE (Goldstein et al. 1988; Goldstein 1995). All other things being the same, decreased reuptake of NE would increase NE spillover but decrease plasma DHPG levels. Because we previously observed this pattern in clozapine-treated patients (Breier et al. 1994b), the present study focused especially on the issue of possible neuronal uptake blockade by clozapine.

Neuronal reuptake contributes to clearance of circulating NE in humans (Esler et al. 1981; Eisenhofer et al. 1991). The present finding of no change in NE clearance in clozapine-treated patients argues against inhibition of neuronal uptake as a basis for high plasma NE levels in these patients. To examine neuronal uptake more specifically, we measured the arterial plasma ³H-DHPG

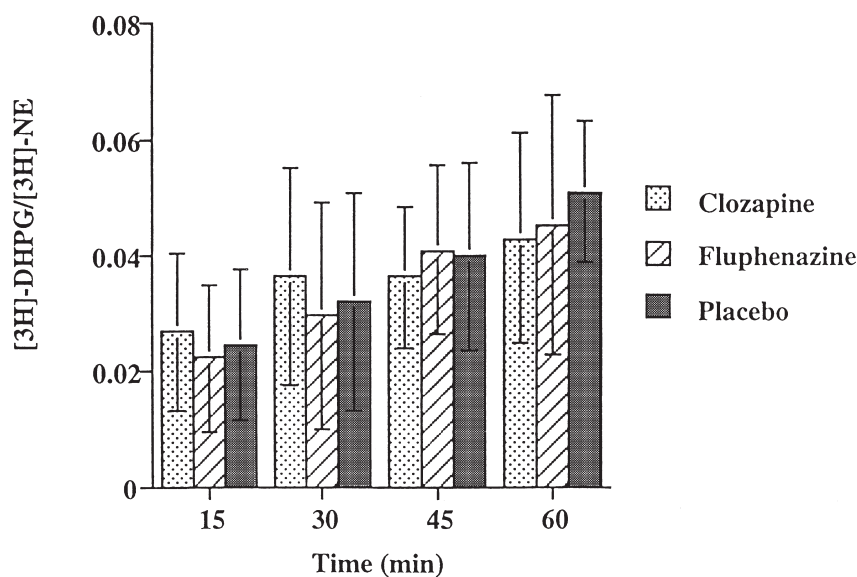
response during ³H-NE infusion. Production of ³H-DHPG did not differ among the treatment groups, with or without correction of ³H-DHPG levels for concurrent plasma ³H-NE levels. Because ³H-DHPG production in this setting results virtually exclusively from metabolism of ³H-NE in the sympathetic axoplasm (Goldstein et al. 1988; Goldstein 1995), the finding of normal ³H-DHPG and ³H-DHPG:³H-NE responses eliminates decreased neuronal reuptake as the mechanism of high plasma NE levels in clozapine-treated patients.

A buildup of NE in the axoplasm, such as by inhibition of MAO and of the vesicular monoamine transporter, could increase NE release by a nonexocytotic process, via exit through the membrane transporter (Eisenhofer et al. 1986; Hovevey-Sion et al. 1990). The present finding of normal plasma levels of DHPG, ³H-DHPG, and DOPAC in clozapine-treated subjects renders MAO inhibition a highly unlikely mechanism of action of clozapine. Moreover, blockade of the vesicular monoamine transporter accelerates the rate of attain-

Time Effect (F=18.53; df=3; p<0.00001)

Group effect-N.S.

Group x Time-N.S.

**Figure 2.** [³H]DHPG/[³H]-NE ratio in schizophrenic patients treated with clozapine (*n* = 10), fluphenazine (*n* = 7), and placebo (*n* = 7).

ment of plateau ^3H -DHPG concentrations during ^3H -NE infusion (Eisenhofer et al. 1991), and in the present study, clozapine treatment did not alter the slowly progressive increase in plasma ^3H -DHPG levels.

Clozapine produces blockade of $\alpha 1$ and $\alpha 2$ adrenoceptors (Arbilla et al. 1978; Souto et al. 1979; Richelson and Nelson 1984; Baldessarini and Frankenburg 1991). These actions could increase sympathoneural outflows reflexively in response to systemic vasodilation and also augment NE release for a given amount of nerve traffic, by blocking inhibitory modulatory $\alpha 2$ -adrenoceptors on sympathetic nerves (Breier et al. 1994b; Breier 1994). This synergy could then markedly increase exocytotic release of NE. This mechanism alone would not explain the clear finding of normal plasma DHPG levels in clozapine-treated subjects, replicating previous results (Breier et al. 1994b). Because plasma DHPG levels generally parallel plasma NE levels during sympathetic stimulation, by increased reuptake of the released NE (Goldstein et al. 1988; Goldstein 1995), adrenoceptor blockade alone would not account for the pattern of a large increase in plasma NE levels with no change in plasma DHPG levels. Because of the lack of DHPG changes, clozapine would not also be expected to increase NE spillover by only a central mechanism that would increase sympathoneural outflows.

A chronic, substantial increase in the rate of sympathetic nerve traffic might lead to a situation where the rate of exit of NE from the innervated tissues would exceed the rate of synthesis of NE. This would deplete the NE stores and, thereby, decrease production of DHPG. Decreasing the size of the storage pool of NE increases the rate of rise in the specific activity of ^3H -DHPG during ^3H -NE infusion (Eisenhofer et al. 1991; Eisenhofer et al. 1996). In the present study, however, clozapine treatment did not affect the rate of increase in arterial plasma ^3H -DHPG concentrations. A decreased storage pool of NE, therefore, does not explain the discrepancy between plasma NE and DHPG responses to clozapine.

If a chronic increase in the rate of sympathetic nerve traffic increased the relative proportion of vesicles fused with the membrane surface, then we might expect a relative increase in DHPG formation from reuptake of released NE and a relative decrease in DHPG formation from net leakage from the vesicles into the axoplasm. Thus, over-all DHPG production might remain unchanged. Patients with disorders presumably associated with chronically compensatory increased sympathetic nerve traffic, such as dihydropteridine reductase deficiency (Goldstein et al. 1995), Menkes disease (Kaler et al. 1993), familial dysautonomia (Axelrod et al. 1996), and partial deficiency of L-aromatic-amino-acid decarboxylase (unpublished observations), do have high plasma NE:DHPG ratios, consistent with a shift in the vesicles toward the membrane surface, where loss of NE via leakage and metabolism would be offset by loss of NE

via exocytosis. Therefore, a mechanism of clozapine's action may involve fusion of NE storage vesicles with the axonal membrane.

In conclusion, clozapine elevates arterial plasma NE levels via increases in NE spillover; whereas, NE reuptake, synthesis, or metabolism are not affected by this drug.

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