Pakistan Journal of *Pharmaceutical Sciences*
V0l.13(1), January 2000, pp.47-55

NEUROCHEMICAL AND EXTRAPYRAMIDAL EFFECTS OF ATYPICAL NEUROLEPTIC CLOZAPINE IN RATS

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ABSTRACT:

In view of a possible role of serotonin (5-hydroxytryptamine; 5-HT) and dopamine (DA) in neuroleptic-induced muscle rigidity and catalepsy, the present study is designed to investigate the neurochemical and extrapyramidal effects of atypical antipsychotic/ neuroleptic durg i.e., Clozapine (CZP) on the metabolism of serotonin and dopamine particularly in the caudate (a region of the brain involved in the control of movement), accumbens and rest of the rat brain. Interaperitoneal (i.p) injections of CZP at doses of 5.0 & 10mg/kg decreased significantly ($p<0.01$) locomotor activity in familiar (home cage) environment. CZP produced a significant (P<0.0I) cataleptic response only at doses of 10mg / kg used. Maximal cataleptic effects in rats occurred at high doses of CZP. Acute administration of CZP significantly ($p<0.01$) decreased levels of NA in accumbens at all the doses used. Significant increases ($p<0.01$) in the levels of NA observed in rest of the brain only at moderate dose (5mg/kg) of CZP. Results showed significant ($p<0.01$) increases in the levels of caudate DA following the administration of CZP at 10mg/kg. However administration of CZP at all the doses produced similar significant ($p<0.01$) increases in the levels of HVA in all the regions of the rat brain. Overall insignificant effects of CZP occurred on brain regional TRP. However, plasma TRP significantly ($p<0.01$) increased at 2.5mg/kg dose of CZP. Administration of CZP at doses of 2.5 and 10mg/kg significantly ($p<0.01$) decreased 5-HT levels in the rest of the brain. Administration of CZP produced insignificant ($p>0.05$) effects on 5-HIAA levels in the caudate and accumbens regions but CZP at doses of 2.5 and 5mg/kg significantly ($p<0.01$) decreased 5-HIAA levels in the rest of the brain.

Neurochemical and extrapyramidal effects of atypical antipsychotic *(clozapine)* are discussed in relation to a potential therapeutic profile in rats.

INTRODUCTION

Schizophrenia is a chronic, severe and disabling brain disease. The term schizophrenia was first used in 1911 by Eugen Bleuler, a Swiss psychiatrist, literally means split mind and many people still believe incorrectly that the condition causes a split personality (which is an uncommon problem involving dissociation) (Bones, 1993). Classically, the symptoms of schizophrenia have been classified as “positive” and “negative” The positive symptoms are those typical of psychosis and include delusions and illogicality (Baldessarini, 1990). The negative symptoms include

blunted affect, impaired emotional responsiveness, and apathy, loss of motivation and interest, and social withdrawal (Rifkin, 1993), Antipsychotic medications have been available since the mid-1950s. These medications reduce the psychotic symptoms of schizophrenia and usually allow the patient to function more effectively and appropriately (Meltzer, 1993).

A number of new antipsychotic durgs (the so-called "atypical antipsychotics") have been introduced since 1990 (Seeman, 1990). Antipsychotic treatment with the so-called "atypical" neuroleptics, as defined by the lack of extrapyramidal side effects in its strict sense, has made great advances in the last decades with the advent of newly developed antipsychotic agents (Casey, 1993). Atypical antipsychotic agents were developed in response to problem with typical agents, including lack of efficacy in some patients, lack of improvement in negative symptoms, and troublesome adverse effects, especially extrapyramidal side effects (EPSs) and tradive dyskinesia (TD) (Meltzer, 1993). Atypical antipsychotics differ from typical psychotics in their "limbic- specific" dopamine type-2 (D2)-receptors binding and high ratio of serotonin type-2 (5-HT-2)-receptor binding to D2 binding (Ichikawa et al., 2001). The first atypical neuroleptic drug was *clozapine,* also referred to as "dirty drug" or "rich drug" because of its broad receptor binding profile (Casey, 1993).

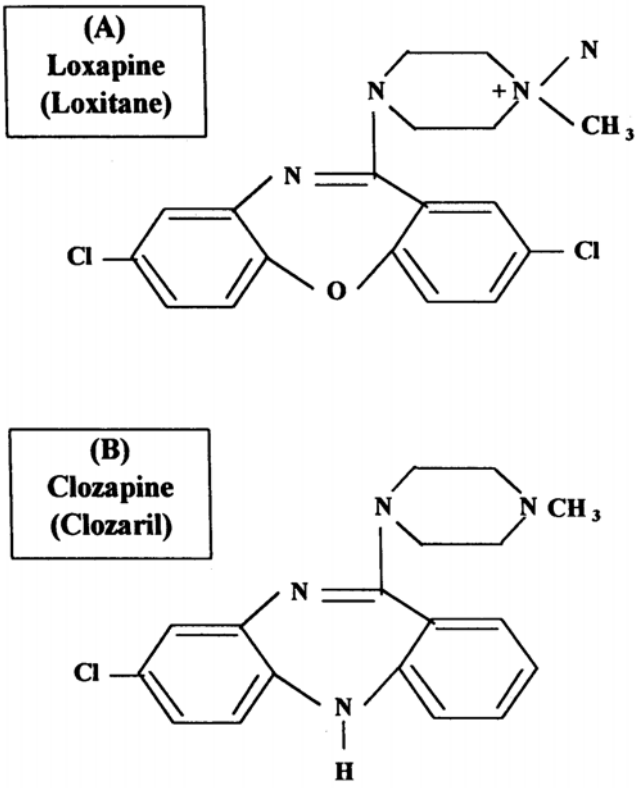

Fig. 1: Structural formulas of atypicl antipsychotic drugs: (A) Loxapine (Loxitane) and (B) Clozapine (Clozaril).

Clozapine has been the starting point for several different, newly developed, antipsychotics. Among these, the most prominent are risperidone, olanzapine, sertindol, ziprasidone and amisulpride (Tarazi et al., 2001). All of these newly developed; atypical antipsychotics show a high degree of efficacy in the treatment of positive symptoms of schizophrenia in combination with a lack of or a reduce degree of EPSs (Casey, 1993). Atypical antipsychotics are often considered fast-line agents for the treatment of schizophrenia and are promising treatment alternatives for other psychiatric and neurologic conditions (Meltzer, 1993), First synthesized in 1959, clozapine (clozaril) is a dibenzodiazepine derivative (Fig. 1) with unique preclinical and clinical characteristics (Coward, 1992). In preclinical studies, clozapine, like other antipsychotic drugs blocks conditioned avoidance behaviors, a measure that is considered predictive of anttipsychotic activity. However, unlike other antpsychotic drugs, clozapine does not cause catalepsy, block apomorphine- or amphetamine-induced stereotyped behaviors, elevate serum prolactin, or casue dopamine receptor hypersensitivity in laboratory animals (Bunney, 1992). Clozapine is further distinguished from other antipsychotic drugs by its relatively higher affinity for D-1 than for D-2 dopaminergic receptors, its higher affinity for 5-HT-2B serotonergic than for D-2dopaminergic receptors, and its strong affinity for the D-4 dopaminergic receptors (Casey, 1993; Meltzer, 1992). In clinical studies, clozapine has been shown to have differential clinical efficacy for treatment-resistant schizophrenia patients and to be associated with a low incidence of EPSs (Meltzer, 1997). The combination of these preclinical and clinical characteristics has led clozapine to be termed an "atypical antipsychotic" (Liberman, 1996; Buckman and Malan, 1996; Kapur and Seeman, 2001).

The present study is designed to investigate the extrapyramidal and monoaminergic effects of atypical neuroleptic namely *clozapine* in rats.

MATERIALS AND METHODS

Animals:

This investigation used experimentally naive, Male Albino Wistar rats (purchased from HEJ Research Institute of Chemistry, Pakistan), weighing 200-250gm. The rats were housed individually in cages with saw-dust covered floor where they had free access to dry food pellets and fresh tap water at room temperature with a regular 12 hours light dark schedule. Behavioral experiments were conducted between 09:00 a.m. to 11:00 a.m. in a separate testing room. All animals were handled with 2-3 days before starting the experiments.

Drug And Injections:

Clozapine (MW=326.83; RBI Research Biochemicals; USA purchased from their suppliers was used for the treatment. The drug was prepared in 0.1 N HCI with pH adjustment at 5.5 using 1 N NaOH and injected intraperitoneal (i.p.) at doses of 2.5, 5 and 10 mg/ml/kg of body weight. Control animals were injected with 0.9%NaCl (1 ml/kg body weight).

Experimental Protocol:

The animals were injected with saline or CZP at doses of 2.5, 5 and 10mg/kg. Activities were monitored in familiar (home cage) env[i]ronment 10min post-injections for 10min. Animals were decapitated lhr post-injections to collect blood in heparinized centrifuge tubes. Blood was centrifuge to obtain plasma. Brains *were* quickly removed and frozen at -70°C for the determination of plasma and brain tryptophan and brain catechol and indoleamines by high

performance liquid chromatography with electrochemical detection (HPLC-EC) (Haleem and Parveen, 1994, Batool et al., 2001).

Behavioral Studies:

A: Home Cage Activity:

Transparent Perspex cages (26x26x26cm) with sawdust covered floor were used to monitor activity in familiar environment. Rats were placed individually in these cages to get familiar with the environment. 10min later the animals were injected with various doses of CZP or vehicle. Numbers of cage crossings were counted 10min post-injection for 10minutes (Batool et al., 2001).

B; Cataleptogenic Effect:

Catalepsy was scored by the horizontal test bar. The forepaws were placed on a 9cm high horizontal bar while the hind-paws remained on the floor. The animals were injected i.p. with CZP (2.5, 5.0 & 10mg/kg). Testing was performed 30 min after the drug administration and the time to withdrawal of legs by the rats was measured. A cut-off time of 180sec was employed.

HPLC-EC Analysis of Biogenic Amines and their Metabolites and Tryptophan:

Brain samples were extracted as described elsewhere (Haleem et al., 1988). Tryptophan from plasma was also extracted similarly. A4um Novapak ODS, 4.6 mm i.d. xl5cm separation column was used. The solvent system was methanol (14%) octyl sodium sulfate (OSS; 0.23%) and EDTA (0.05%) in 0.1M phosphate buffer of pH 2.9. Electrochemical detection of brain catechol and indoleamines was done at an operating potential of 0.8V (glassy carbon electrode Vs an Ag/ AgCI reference electrode). Tryptophan was determined in a separate run at an operating potential of 1.0V (Haleem and Batool, 1996; Batool et al, 2001).

In the present investigation the result are presented as means ± S.D. Behavioral data on the effects of various doses of CZP on home cage activity and catalepsy and data on the effects of these drugs on plasma and brain tryptophan and brain catecholamines and indoleamines were analyzed by one way analysis of variance (ANOVA). Posthoc comparisons were done by Newman-Keuls test: Values $p<0.05$ were considered statistically significant.

RESULTS

A: Behavioral Effects of Clozapine:

Fig.2 show the effects of CZP injected at doses of 2.5, 5.0 & 10mg/kg on home cage activity (Fig 2A) and catalepsy (Fig 2B) in rats. Data analyzed by one way ANOVA showed that home cage locomotion (monitored as the number of cage crossings)/ 10min was significantly ($p<0.01$) decreased at all the doses of CZP (Fig 2A). Significant ($p<0.01$) cataleptic effect (immobile posture on inclined surface) was produced only at dose of 10mg/kg (Fig 2B).

B: Neurochemical Effects of Clozapine:

Fig. 3 and Fig 4 exhibit neurochemical data obtained form rest of the brain, caudate and accumbense following the acute administration of CZP at doses of 2.5, 5.0 & 10mg/kg.

Fig. 3 shows the effects of CZP at doses of 2.5, 5.0 & 10mg kg on brian, regional catecholamines in rats. ANOVA (df=3,8) revealed a significant effect on brain regional NA (Fig

3A) (Rest of the brain; F=7.000; p<0.05: Caudate; F=1.874; p>0.05; Accumbens; F= 97.1; P<0.01), DOPAC (Fig 3B): (Rest of the brain; F=38.86; p<0.01: Caudate; F=6.296; p<0.05: Accumbens; F=1.192; p>0.05), DA (Fig 3C): (Rest of the brain; F=22.31; p<0.01: Caudate; F=129.71; p<0.01) and HVA (Fig 3D): (Rest of the brain; F=9.350; p<0.01 Caudate; F=30.715; p<0.01. Accumbens; F--41.42; p<0.01).

Fig. 4 shows the effects of CZP at doses of 2.5 5 & 10mg/kg on brain regional indolcamines in rats. ANOVA (df-3,8) revealed a significant effect on brain regional TRP (Fig 4A) (Rest of the brain; F=0.2332; p>O.05: Caudate; F=2.192; p>0.05:Accumbens; F=0.495; p>0.05), plasma) TRP (Fig 4B): (F=9.24; p<0.01), 5-HT (Fig 4C) (Rest of the brain; F=8.698) and 5-HIAA (Fig 4D): (Rest of the brain; F=5.827; p<0.05: Caudate; F=4.688; p>0.05: Accumbens; F=6.140; p<0.05).

Acute administration of CZP significantly (p<0.01) increased levels of NA in accumbens at all the doses used. Significant increase (p<0.01) in the levels of NA observed in rest of the brain only at moderate dose (5mg/kg) of CZP. Results showed significant (p<0.01) increase in the levels of caudate DA following the administration of CZP at 10mg/kg. However, administration of CZP at all the doses produced similar significant (p<0.01) increases in the levels of HVA in all the regions of the rat brain. Overall insignificant effects of CZP occurred on brain regional TRP. However, plasma TRP significantly (p<0.01) increased at 2.5mg/kg dose of CZP. Administration of CZP at doses of 2.5 and 10mg/kg significantly (p<0.01) decreased 5-HT levels. Administration of CZP produced insignificant (p>0.05) effects on 5-HIAA levels in the caudate and accumbens regions but CZP at doses of 2.5 and 5mg/kg significantly (p<0.01) decreased 5-HIAA levels in the rest of the brain.

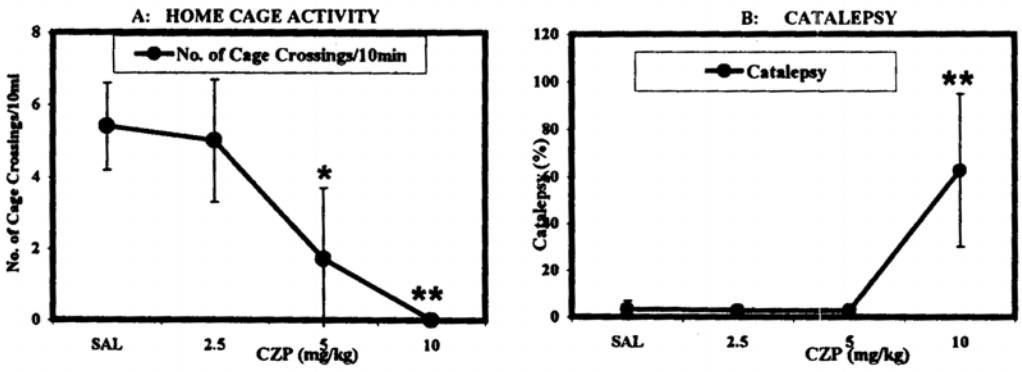

Fig. 2: Effects of clozapine (2.5, 5.0 & 10mg/kg) on Home Cage Activity (No. of Cage Crossings /10min; 2A) and Catalepsy (Immobile posture on inclined surface; cut off time 3 min; 2B). Value are means ± S. D. (n=6) 15min after the drug or saline administration scored for 10min and 3min respectively. Significant differences by Nawman-Keuls test:
*p<0.05, **p<0.01 from saline injected controls following one way ANOVA.

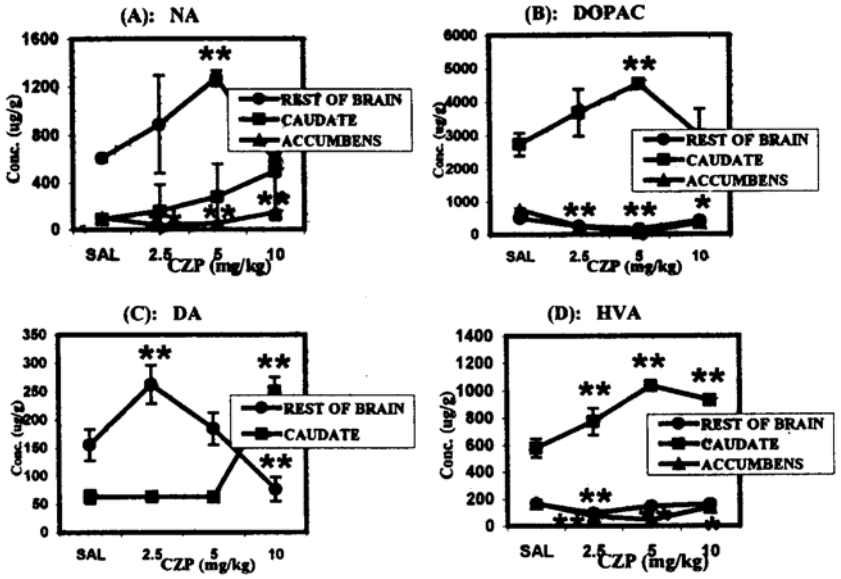

Fig 3: Effects of CZP (2.5, 5.0 & 10mg/kg) on brain regional NA (3A), DOPAC (3B), DA (3C) and HVA (3D). Values are means ± S.D. (n=6) lhr after the saline or drug administration. Significant differences by Newman-Keuls test:
*p<0.05, **<0.01 from saline injected controls following one-way ANOVA.\

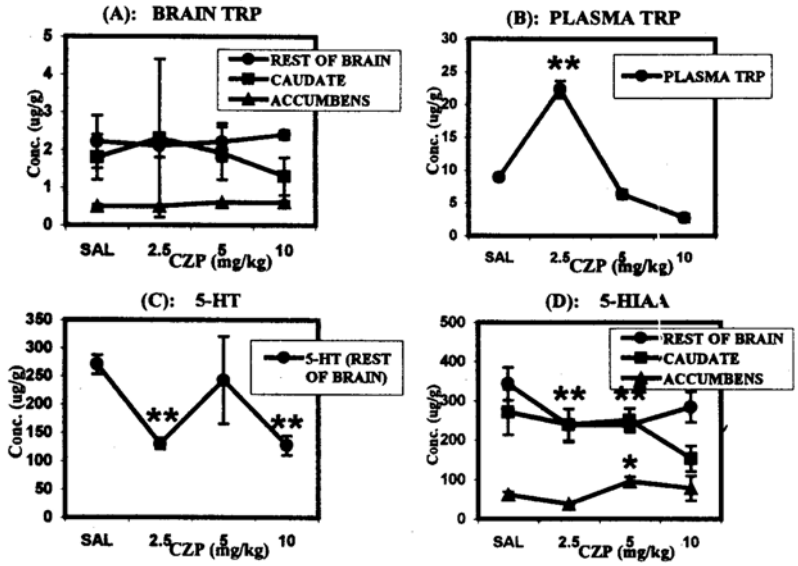

Fig 4: Effects of CZP (2.5, 5.0 & l0mg/kg) on brain regional TRP (4A), plasma TRP (4B) and 5-HT (4C) and 5-HIAA (4D). Values are means ± S.D. (nom) lhr after the saline or drug administration. Significant differences by Newman-Keuls test: *p<0.05, **<0.01 from saline injected controls following one-way ANOVA.

DISCUSSION

Neuropleptic drug-induced acute etrapyramidal syndromes (EPSs) are one of the major limitations to effective neuroleptic treatment (Casey, 1994; Casey, 1996). These disorders have both motor (objective) and mental (subjective) aspects, which must be considered in any evaluation and differential diagnosis of treatment-related side effects (Casey, 1997). Acute EPSs are commonly explained on the basis of dopamine D2 receptor antagonism, whereas, the atypical (clozapine) neuroleptic produces only minimal motor system side effects (Casey, 1995). Serotonin) (5-HT-2) antagonists often reduce or prevent catalepsy in rodents (Ichikawa and Meltzer, 2000).

Typical antpsychotic drugs, chlorpromazine (10mg/kg) and haloperidol (1mg/kg) occupied predominantly D2 receptors in the striatum and the nucleus accumbens (Hollerman et al., 1992), on the other hand, atypical antipsychotic drugs, clozapine (10mg/kg), occupied mainly 5-HT-2 receptors and low or minimum occupancy of D2 receptors (Knable et al., 1997). These characteristics may be relevant to their weak potency in producing EPSs in man or catalepsy in rodents (Casey, 1997). However, further studies are needed to elucidate regional differences in receptor occupancies by these antipsychotic drugs. The properties of atypical antipsychotics are often explained on the basis of their activity at different sites of action. The atypical antipsychotic clozapine is reported to preferentially enhance cortical, as opposed to striatal, DA concentrations (Ichikawa and Meltzer, 1991).

In the present study dose-dependent administration of clozapine showed that EPSs of clozapine are very small (Fig 2B). It is interesting to note that CZP-induced increases of HVA in the caudate (Fig 3D) and also in the rest of the brain are much smaller. Neuroleptic-induced HVA increases are often taken as a measure of D2 antagonist activity. Differential responses of the drug for 5-HT receptors may also contribute in the minimal cataleptic effect of clozapine. Thus administration of clozapine decreased 5-HT and 5-HIAA concentration in the rest of the brain (Fig 4C & 4D).

Accumbens is a region of the brain known to be involved in emotional control (Marcus et al., 2001). Thus neuroleptics would be expected to bind to D2 receptors in this brain region to produce antipsychotic effects. Inability of CZP to increase HVA concentration in this brain region suggests that dopaminergic terminals of this brain region behave differently. Important finding of the present study is that CZP decreased HVA concentration in this brain region. CZP, the first novel antipsychotic, has relatively low activity at D2 receptors, a high affinity for D4 receptors and a greater 5-HT-2 than D2 antagonism. Hence, CZP and other novel antipsychotics can be classified as such by this later characteristic. These agents should also be considered for refractory schizophrenia. Patients whose schizophrenia does not respond to one of these agents may respond to another. Further research should involve clinical trials, given the long periods needed to establish efficacy, and should address many remaining questions about the novel agents.

CONCLUSION AND SIGNIFICANCE

The present results as a whole show atypical neuroleptics CZP (binding studies show that CZP's highest affinities, for binding to receptors in the brain, to be for dopamine D-4, serotonin$_{1C}$ sertonin$_2$ alpha-1 (an adrenergic receptor), muscarinic (an acetylcholine receptors, and histamine-1 receptors, but moderate affinity is also seen for many other receptor subtypes) produce lasser EPSs

than typical neuroleptics. In this regared CZP is a superior antipsychotic than conventional neuroleptics

ACKNOWLEDGEMENTS

We authors would like to thanks for Pakistan Science Foundation (PSF) for financial support.

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