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PREFERENTIAL BLOCKADE BY CLOZAPINE OF HYPERLOCOMOTION INDUCED BY NON-COMPETITIVE NMDA ANTAGONIST MK–801

I. NINAN AND S. K. KULKARNI*

Pharmacology Division,
University Institute of Pharmaceutical Sciences,
Panjab University, Chandigarh – 160 014

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Abstract : Effect of clozapine on MK–801–induced hyperlocomotion and stereotypy as well as open field behavior was studied. Clozapine (0.1 – 7.5 mg/kg) dose-dependently blocked MK-801(0.5 mg/kg)-induced stereotypy. Both total and ambulatory responses were blocked by even the lower doses (0.1 – 0.5 mg/kg) of clozapine. In open field test, clozapine selectively blocked hyperambulation induced by MK–801 (0.1 mg/kg) whereas it potentiated MK–801 (0.1 mg/kg)-induced stereotypy at all the doses used. Haloperidol (0.25 and 0.5 mg/kg) and SCH 23390 (0.5 and 1 mg/kg) showed a dose-dependent effect on MK–801–induced behaviors while sulpiride (25 and 50 mg/kg) failed to modify MK–801–induced open field behavior. This study supports the preferential effect of clozapine on dopamine receptors located in mesolimbic area and further suggests the possibility of using open field behavior induced by MK–801 as a model for studying atypical antipsychotics.

Key words : clozapine, hyperlocomotion, MK–801, open field test, stereotypy

INTRODUCTION

Unlike the classical antipsychotics, atypical antipsychotics (clozapine being the prototype) are reported to produce very low incidence of extrapyramidal side effects and are more effective in treating "deficit" psychotic symptoms, such as alogia, avolition, affective flattening, social withdrawal, anhedonia and more severe cognitive deficits (1). Although the molecular basis for this diverse type of action is far from clear, it is postulated that at physiologic concentrations effective in schizophrenia, typical antipsychotic agents preferentially bind to the dopamine D_2 receptor subtype whereas clozapine would bind to the dopamine D_4 receptor subtype (2). It has been further suggested that the D_4 receptor selectivity together with the localization of D_4 receptors in mesolimbic area may be responsible for the novel actions of clozapine.

MK–801, a non-competitive NMDA antagonist induces a complex behavioral syndrome in the rodents which included hyperlocomotion, stereotypy and ataxia

*Corresponding Author

(3, 4). Similar behavioral effects have been shown by the NMDA receptor antagonists namely, DL-2amino-5-phosphonovleric acid (AP-5) and 3-(2-carboxypoperazin-4-yl)-propyl-1-phosphonoic acid (CPP), or following systemic administration of PCP, ketamine and SKF 10,047 (5–7). These behavioral alterations, atleast in part, are mediated by activation of the mesolimbic dopaminergic system as a result of an interaction between glutamatergic and dopaminergic transmission (8). The glutamate hypothesis of schizophrenia suggests a relative deficiency of glutamate transmission that may result in a dysregulated dopamine system. However, there is also evidence that MK–801 stimulated locomotion can be produced by dopamine-independent mechanisms (9, 10). Diana and Sagaratella (1994) showed that MK–801 increases locomotor/exploratory activity in open field test (11). It has been previously demonstrated that antipsychotic agents including haloperidol, chlorpromazine, and clozapine antagonized PCP-induced locomotion and stereotypy (12, 13). More recently, it has been shown that haloperidol nonselectively antagonized the MK–801-induced sniffing as well as spontaneous sniffing, whereas clozapine was shown to selectively antagonize the MK–801-induced sniffing (14). Although clozapine does not bind directly to the MK–801 binding site on the NMDA-glycine receptor complex, it is reported to block MK–801 discriminative cue (15). Selective antagonism of these behaviours has been proposed as an animal model of neuroleptic-resistant schizophrenia (8).

The present study aims to explore the differential effects of clozapine on locomotion and stereotypy induced by MK–801 and to further evaluate the validity of this model for studying atypical antipsychotics.

METHODS

Animals: The Balb/C mice and Wistar rats (Central Animal House, Punjab University, Chandigarh) weighing 20–30 g and 150–250 g, respectively were used. The animals were given free access to standard pellet food and water and were habituated to laboratory conditions before the test. All experiments were undertaken between 09.00 and 17.00 h.

Measurement of stereotypy: Measurement of cumulative stereotypy was done by placing mice individually in glass containers. Sniffing, rearing, licking, biting, gnawing and grooming were observed as stereotypic behaviors for 2 h after drug administration. The intensity of stereotypy was recorded as described by Costall and Naylor (16). The cumulative stereotypy score was calculated by adding all the scores for the purpose of comparison (17).

Measurement of locomotor activity: Measurement of locomotor activity (ambulation) and total activity was done using computerized animal activity meter (Opto Varimex mini, Columbus Instruments, Ohio, USA). Briefly, after 30 min of drug treatment mice were individually placed in a transparent plastic cage (30 × 23 × 22 cm) and the activity was recorded for 5 min after allowing the mice to adapt to the new environment for 2 min. An array of 11 infrared emitter/detector pairs (spaced at

2.65 cm intervals; beam wave lengh=875nm; distance between the sensors=50 cm) measured the animal activity along single axis of motion, the digital data being displayed on the front panel meters as ambulatory and total activity. The ambulatory option in the instrument automatically differentiates between actual ambulatory movements and stereotypic movements such as scratching, grooming and digging. The locomotion was expressed in terms of total photobeam counts per 5 min per animal.

Open field test : The apparatus consisted of a circular arena of diameter 80 cm, surrounded by a 30 cm high wooden wall. The arena painted white, was divided in to 25 small sections, bright light (five 60 W bulbs) and high sound (buzzer attached to amplifier) served as stimuli (18).

After 30 min of drug administration, each rat was carefully placed in a particular section next to the wall and allowed to explore the open field for 2 min. During this period, the ambulation and stereotypic behaviours such as rearing and preening were recorded.

Drugs : Clozapine (Sandoz, Switzerland), MK-801 [5-methyl-10, 11-dihydro-5H-dibenzo (a, d) cyclohepten-5, 10-imine] (dizocilpine; Merck, Sharp and Dohme, Rahway, NJ, USA), haloperidol (Searle, Skokie, IL, USA), (-)sulpiride hydrochloride (Research Biochemicals Inc., Natick, MA, USA), SCH23390 [R(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1-H-3-benzazepine-7-o1 hydrochloride] (Schering Plough Co., Bloomfield, NJ, USA). The drug solutions were made in distilled water, except clozapine and haloperidol. Clozapine was dissolved in a few drops of dilute hydrochloric acid while haloperidol was dissolved in a drop of glacial acetic acid, volume was made up with distilled water and pH adequately adjusted. All drugs were administered intraperitoneally in a constant volume of 1 or 0.5 ml per 100 g of body weight of mice or rats respectively. The selection of doses was based on previous reports from our laboratory. In combination studies antagonists were administered 30 min prior to agonist treatment.

Statistical analysis : The data expressed as mean ± SEM were subjected to analysis of variance (ANOVA) followed by Student's-test. $P<0.05$ was considered statistically significant.

RESULTS

Effect of clozapine on MK-801-induced stereotypy and hyperlocomotion : MK-801 (0.1, 0.25 and 0.5 mg/kg) showed a dose-dependent increase in locomotion and stereotypic behaviors such as severe rearing, sniffing and grooming. The peak effect was observed at 30 min and the effect lasted for 2 h. Clozapine *per se* (0.1, 0.25 and 0.5 mg/kg) showed a dose-dependent increase in ambulatory and total activity scores but at higher dose (1, 2, 5 and 7.5 mg/kg) it decreased the ambulatory and total activity score in a dose-dependent manner (Table I). Clozapine (0.1 to 2 mg/kg) dose-dependently blocked MK-801 (0.5 mg/kg)-induced stereotypy, whereas higher doses (5 and 7.5 mg/kg) completely blocked it. The ambulation and total activity were sensitive even to lower doses of clozapine (Fig. 1A, B)

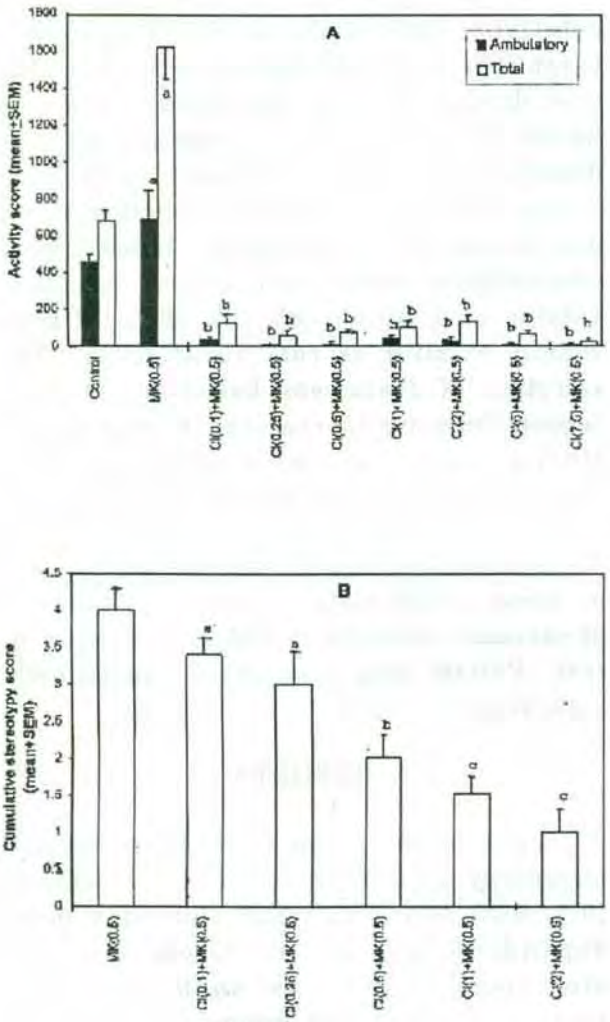

Fig.1 (A): Effect of clozapine (0.1–7.5 mg/kg) on MK-801 (0.5 mg/kg)-induced locomotor activity (both ambulatory and total). $^{a}P<0.001$ as compared to control group, $^{b}P<0.001$ as compared to MK-801 group. (n=5–7).

(B): Effect of clozapine (0.1–2 mg/kg) on MK-801 (0.5 mg/kg)-induced stereotypy. $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$ as compared to MK-801 group (n=5–7).

Effect of haloperidol, sulpiride and SCH 23390 on MK-801-induced stereotypy and hyperlocomotion : Haloperidol (0.25 and 0.5 mg/kg) blocked MK-801(0.5 mg/kg)- induced hyperlocomotion and stereopy dose-dependently. (Fig. 2A, B). Similarly, both sulpiride (25 and 50 mg/kg) and SCH 23390 (0.5 and 1 mg/kg) dose-dependently blocked

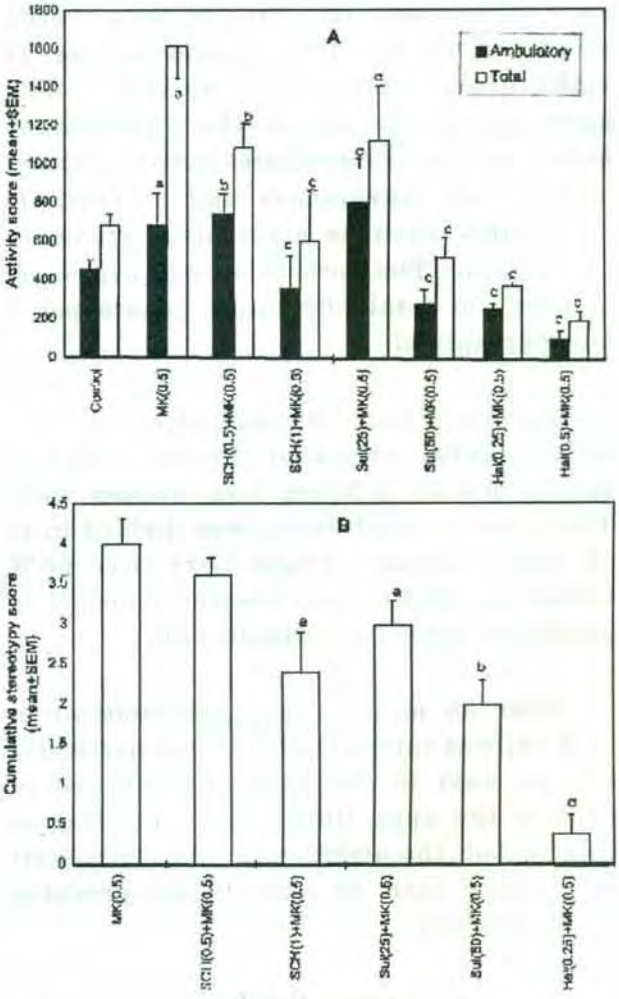

Fig.2 (A): Effect of SCH 23390 (0.5 and 1 mg/kg), sulpiride (25 and 50 mg/kg) and haloperidol (0.25 and 0.5 mg/kg) on MK-801 (0.5 mg/kg)-induced locomotor activity (both ambulatory and total). $^{a}P<0.001$ as compared to control, $^{b}P<0.001$ as compared to MK-801 group (n=5–7).

(B): Effect of SCH 23390 (0.5 and 1 mg/kg), sulpiride (25 and 50 mg/kg) and haloperidol (0.25 mg/kg) on MK-801 (0.5 mg/kg)-induced stereotypy. $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$ as compared to MK-801 group (n=5–7).

MK-801 (0.5 mg/kg)-induced hyperlocomotion and stereotypy.

Effect of clozapine on MK-801-induced ambulation and stereotypy in open field: MK-801 (0.1 and 0.25 mg/kg) produced increase in ambulation but stereotypic behaviors such as rearing and preening did not change significantly as compared to control group. A higher dose of MK-801 (0.5 mg/kg) produced total ataxia. Clozapine *per se* (0.1 to 2 mg/kg) did not change ambulation but increased the stereotypic score. At doses 5 and 7.5 mg/kg, clozapine decreased ambulation. Clozapine (5 mg/kg) did not show any change in stereotypic score but at 7.5 mg/kg dose it almost completely blocked the stereotypy (TableI).

In interactive study clozapine (0.1, 0.25, 0.5, 5 and 7.5 mg/kg) inhibited the hyperambulation produced by MK-801 (0.1 mg/kg) whereas clozapine (1 and 2 mg/kg) did not affect ambulation. Clozapine (0.1, 0.25, 0.5, 1, 2, 5 and 7.5 mg/kg) potentiated stereotypy (Table II).

Effect of haloperidol, sulpiride and SCH23390 on MK-801-induced ambulation

TABLE I : Effect of clozapine *per se* on locomotion (mice) and open field behaviour (rats) respectively.- $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$ as compared to control.

No.	Treatment (mg/kg)	n	Locomotor activity (mean ± SEM)		Open field behaviour (mean ± SEM)	
			Ambulatory	Total	Ambulation	Stereotypy
1.	Saline	5	453.8±47.3	681.5±60.6	47.8 ± 4.55	12.8±0.85
2.	Clozapine (0.1)	5	477±26.2	720.8±26.2	50.4 ± 7.8	32.6±2.25^{c}
3.	Clozapine (0.25)	5	579±117.2	822±142.4	53.4 ± 2.93	32.2±2.47^{c}
4.	Clozapine (0.5)	5	625.6±53.3^{a}	902.2±62.7^{a}	49.6 ± 10.8	28.8±5.7^{a}
5.	Clozapine (1)	5	143.6±28.5^{b}	349±79.9^{b}	57 ± 5.7	23.8±2.05^{b}
6.	Clozapine (2)	5	295±55^{b}	426.8±64.3^{b}	57.4 ± 18.5	18.4±5.35
7.	Clozapine (5)	5	29.4±19.7^{c}	62.2±29^{c}	29 ± 7.38^{a}	24±3.39^{a}
8.	Clozapine (7.5)	5	8.2±6.2^{c}	27.8±13.5^{c}	5 ± 2.36^{c}	3.8±1.9^{b}

TABLE II : Effect of clozapine (0.1–7.5 mg/kg) on hypermbulation and stereotypy induced by MK-801 (0.1 mg/kg) in open field. $^{a}P<0.001$ as compared to control group, $^{b}P<0.05$, $^{c}P<0.0$, $^{d}P<0.001$ as compared to MK-801 group (n=5–7).

No.	Treatment (mg/kg)	Open field behaviour (mean ± SEM)	
		Ambulation	Stereotypy
1.	Saline	47.81±4.55	12.83±0.85
2.	MK-801 (0.1)	126.82±12.6^{a}	18.81±4.65^{a}
3.	Clozapine (0.1) + MK-801 (0.1)	85.83±16.5	27.22±4.86^{d}
4.	Clozapine (0.25) + MK-801 (0.1)	69.81±15.9^{b}	26.17±4.28^{d}
5.	Clozapine (0.5) + MK-801 (0.1)	84.83±15.4	22.21±4.26^{b}
6.	Clozapine (1) + MK-801 (0.1)	111.24±17.4	27.11±5.4^{c}
7.	Clozapine (2) + MK-801 (0.1)	116.23±19.9	36.86±13.15
8.	Clozapine (5) + MK-801 (0.1)	48.62±11.41^{c}	39.67±31.21^{d}
9.	Clozapine (7.5) + MK-801 (0.1)	56.63±7.78^{c}	38.21±5.48d

TABLE III : Effect of SCH 23390 (0.5 mg/kg), sulpiride (25 and 50 mg/kg) and haloperidol (0.25 and 0.5 mg/ kg) on MK-801 (0.1 mg/kg)-induced hyperambulation and stereotypy in openfield. [a]p<0.05 as compared to control, [b]p<0.05, [c]p<0.01, [d]p<0.001 as compared to MK-801 group. (n=5 – 7).

No.	*Treatment (mg/kg)*	*Open field behaviour (mean ± SEM)*	
		Ambulation	*Stereotypy*
1.	Saline	47.81 ± 4.55	12.83 ± 0.85
2.	MK-801 (0.1)	126.82 ± 12.6[a]	18.81 ± 4.65[a]
3.	SCH 23390 (0.5) + MK-801 (0.1)	54.61 ± 16.12[c]	16.21 ± 5.51
4.	Sulpiride (25) + MK-801 (0.1)	82.21 ± 9.95[b]	17.14 ± 4.39
5.	Sulpiride (50) + MK-801 (0.1)	129.1 ± 23.6	16.42 ± 8.14
6.	Haloperidol (0.25) + MK-801 (0.1)	119.84 ± 15.23	10.42 ± 3.76
7.	Haloperidol (0.5) + MK-801 (0.1)	38.44 ± 10.82[c]	1.41 ± 0.92[d]

and stereotypy in open field : Haloperidol (0.25 mg/kg) did not block MK-801 (0.1 mg/ kg)-induced hyperambulation and stereotypy but in higher dose (0.5 mg/kg) it blocked both hyperambulation and stereotypy. Sulpiride (25 and 50 mg/kg) did not affect both hyperambulation and stereotypy induced by MK-801 (0.1 mg/kg), SCH 23390 (0.5 and 1 mg/kg) dose-dependently blocked hyperambulation but it completely blocked MK-801-induced stereotypy at 1 mg/kg dose (Table III).

DISCUSSION

Clozapine has got unique preclinical as well as clinical profile. Clozapine does not block the classical apomorphine-induced stereotypy and does not produce catalepsy. Other atypical antipsychotics like risperidone exhibit catalepsy at higher doses. Similarly, clozapine neither induce extrapyramidal symptoms nor alter prolactin secretion in human beings. One of the mechanisms suggested for this atypical nature of action is its selective blockade of dopamine D_4 receptors located predominantly in mesolimbic areas which is mainly responsible for the behaviors associated with schizophrenia (19). Seeman et al, (20) reported that the density of D_4 receptor in the human striatum has been shown to be approximately 10% of the total population of the D_2-like receptors. It was originally shown that dopaminergic mechanisms in the striatum were involved in stereotyped behavior, whereas the mesolimbic area (the nucleus accumbens and the olfactory tubercle) were involved primarily in locomotor activity. The major findings of the present study i.e., (a) clozapine showing different action from haloperidol against MK-801-induced hyperlocomotion and stereotypy and a similar atypical behavior in open field test and (b) clozapine showing a selective inhibition of hyperlocomotion as compared to stereotypy, demonstrated a predominant effect of clozapine on dopamine receptors in mesolimbic area.

Clozapine increased the locomotion when studied in activity meter but without any change in ambulation when studied in open field. Clozapine showed an increase in stereotypy in open field. This locomotor stimulatory effect may be due to the anticholinergic action of clozapine (21). Muscarinic cholinergic antagonists such as scopolamine are well known to produce hyperactivity in mice (22, 23). Several lines of evidence suggest that anticholinergics may produce hyperactivity through direct or indirect interactions with dopaminergic systems (24, 25). Lower doses of clozapine almost completely blocked MK-801-induced hyperlocomotion but the effect on stereotypy was dose-dependent and less severe. Interestingly in open field, clozapine potentiated MK-801-induced stereotypy whereas ambulation was inhibited by lower and higher doses with median doses having no effect. One explanation for these findings could possibly be offered by the earlier report of high binding affinity of clozapine at dopamine D_1 receptors compared to D_2 receptors and its site specific action at the dopamine receptors in mesolimbic area (26). Haloperidol, the classical antipsychotic dose-dependently blocked both hyperambulation and stereotypy induced by MK-801. In open field test, lower dose of haloperidol did not block hyperambulation or stereotypy whereas the higher dose significantly blocked both. These observations support the predominant dopamine D_2 blockade in both mesolimbic area and nigrostriatal area by haloperidol. The dopamine D_1 antagonist, SCH 23390 dose-dependently blocked both hyperlocomotion and stereotypy. Similar results were observed in open field also suggesting greater role of dopamine D_1 receptor in these behaviours. Sulpiride, yet another atypical antipsychotic and selective dopamine D_2 blocker dose-dependently blocked both hyperlocomotion and stereotypy induced by MK-801. In open field. The stereotypic behaviour remain unaffected by pretreatment with sulpiride whereas lower dose of sulpiride blocked hyperambulation induced by MK-801.

Clozapine did not modify MK-801-induced stereotypy or even potentiated it in open field. The potentiation of stereotypy could be due to the 5-HT_{2A} receptor blockade by clozapine. Many of the 5-HT_{2A} antagonists have been reported to increase nigrostriatal dopaminergic activity (27, 28). Similarly sulpiride did not affect stereotypy in open field test. Compared with classical antipsychotics, atypical antipsychotics reported to have relatively lower affinity for dopamine D_2 receptors, whereas the substituted benzamides like sulpiride are postulated to block dopamine D_2 receptors selectively within mesolimbic brain areas (29).

In conclusion, our results support the preferential effect of clozapine on dopamine receptors located in mesolimbic areas at lower doses being responsible for its atypical profile. It further suggests the possibility of using open field behaviour induced by MK-801 as a model for studying atypical antipsychotics.

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