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Anti-schizophrenic activities of histamine H_3 receptor antagonists in rats treated with MK-801

Danish Mahmood[1], Mohd Akhtar[1]

[1] *Department of Pharmacology, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi*

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Abstract

Animal models based on N-methyl-d-aspartate (NMDA) receptor blockade have been widely reported. Ketamine and MK-801, the two noncompetitive antagonists of NMDA receptors, produce behaviors related to schizophrenia and exacerbated symptoms in patients with schizophrenia.

The study presented here investigated the effect of subchronic dosing (once-daily, 7 day) of histamine H_3 receptor (H_3R) antagonists, ciproxifan (CPX) (3 mg/kg, i.p.) and clobenpropit (CBP) (15 mg/kg, i.p) including clozapine (CLZ) (3.0 mg/kg, i.p.) and chlorpromazine (CPZ) (3.0 mg/kg, i.p.), the atypical and typical antipsychotic, respectively, on MK-801(0.2 mg/kg, i.p.)-induced locomotor activity, and dopamine and histamine levels in rats. Atypical and typical antipsychotic was used to serve as clinically relevant reference agents to compare the effects of the H_3R antagonists. MK-801 significantly increased horizontal activity which was reduced with CPX and CBP.

The attenuation of MK-801-induced locomotor hyperactivity produced CPX and CBP were comparable to CLZ and CPZ. Dopamine and histamine levels were measured in striatum and hypothalamus, respectively, of rat brain.

The MK-801 induced increase of the striatal dopamine level was reduced in rats pretreated with CPX and CBP including CLZ. CPZ also significantly lowered striatal dopamine levels, though the decrease was less robust compared to CLZ, CPX and CBP. MK-801 increased histamine content although to a lesser degree. Subchronic treatment with CPX and CBP exhibited further increased histamine levels in the hypothalamus compared to MK-801 treatment alone. Histamine H_3 receptor agonist, R-α methylhistamine (10 mg/kg, i.p.) counteracted the effect of CPX and CBP.

The findings of the present study support our previous work showing positive effects of CPX and CBP on MK-801-induced schizophrenia like behaviors in rodents. However, clinical studies have reported no antipsychotic effects with histamine H3 receptor antagonists.

Key words

MK-801, histamine H_3 receptor, ciproxifan, clobenpropit, locomotor hyperactivity

INTRODUCTION

Animal models of schizophrenia-like behavior based on the blockade of N-methyl-d-aspartate (NMDA) receptors have been extensively used and is a well established method of mimicking psychoses in laboratory conditions [1]. Although, major explanatory hypothesis for the pathophysiology of schizophrenia has been the dopamine hypothesis, which has stated that a dysfunction of dopamine neurotransmitter system underlies the behavioral abnormalities accompanying schizophrenia. However, in the brains of schizophrenic patients, altered levels of dopamine or dopamine receptors were not generally observed upon postmortem examination, indicating an alternate explanation for the etiology of schizophrenia. The alternate explanation suggested was the glutamate dysfunction hypothesis. This hypothesis was based on the observation that phencyclidine (PCP), an NMDA receptor antagonist, administration closely mimicked behaviors related to schizophrenia [2]. Two other noncompetitive antagonists of the NMDA receptors, ketamine and MK-801, also produced schizophrenia-like behaviors and exacerbated symptoms in patients with schizophrenia [3]. NMDA is a subtype of glutamate receptors [4], and glutamate transmission is known to play key role in several behavioral systems including motor activity, learning, and memory. Further, reduced NMDA receptor expression in mice was reported to display schizophrenia like behaviors [5]. These findings led to an extensive use of PCP- and MK-801-treated animals as models for schizophrenia to study putative chemical agents with antischizophrenic activities.

In rodents, systemic MK-801 administration results in a variety of schizophrenia-like behaviors including hyperlocomotion, disruption of prepulse inhibition, impaired performance in learning and memory tasks, and decreased social behaviors [4]. In addition, MK-801 induces ataxia, head weaving, body rolling, and stereotyped motor patterns, and a single application of MK801 is well-known to induce psychosis-like behaviors [6, 7, 8, 9, 10]. Such behavioral syndromes have been used to model schizophrenia-like effects of NMDA antagonism. In particular, NMDA antagonist-induced hyperlocomotion has been used to compare the effects of typical and atypical antipsychotics as well as many other antipsychotic drugs with a variety of mechanism of action (e.g. mGluR2, GlyT1, etc.) [10]. These behavioral changes are reversible by antipsychotic medication applied to the animals and considered analogues of positive symptoms of psychoses in humans, further suggesting that MK-801 induced behaviors in rodents may serve as an animal model of schizophrenia.

Address for correspondence: Danish Mahmood, Dept of Pharmacology, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi-110062
E-mail: danishmahmood2000@gmail.com

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Histamine H_3 receptors is a presynaptic autoreceptor regulating synthesis and release of histamine from histaminergic neurons [11]. As heteroreceptors, H_3 receptors modulate the release of various neurotransmitters including dopamine, acetylcholine, norepinephrine, 5-hydroxytryptamine, gamma-aminobutyric acid (GABA), glutamate, substance P and tachykinins [12]. Histamine H_3 receptors are present in cerebral cortex, hippocampus, amygdala, nucleus accumbens, globus pallidus, striatum and hypothalamus, however, the highest density of H_3 receptors were found in basal ganglia, an important seat in the brain involved coordination of information from sensorimotor, motivational and cognitive brain areas to control behaviors such as movement and reward learning [13]. H_3 receptor antagonists have been reported to potentiate neurochemical and behavioral effects of haloperidol and enhance prepulse inhibition of startle in rats and modulate neurochemical and behavioral effects of methamphetamine [14, 15].

In our previous study, we reported that acute administration of CPX (3.0 mg/kg, i.p.) and clobenpropit (CBP) (15 mg/kg, i.p.) attenuate MK-801-induced schizophrenia-like behaviors[16]. However, there are reports that H_3 receptor inverse agonists do not possess antipsychotic-like properties and they increase motor effects of MK-801 in rats[17–19]. Therefore, the aim of the present study was to extend the findings of our previous study by looking at subchronic dosing and to substantiate our previous findings indicating antischizophrenic-like activities of histamine H_3 receptor antagonists. We intended to explore the effects of subchronic dosing of CPX and CBP on MK-801 induced schizophrenia-like behaviors in an open field. Further, several preclinical and clinical studies in the past have shown brain histamine neurons involvement in the pathogenesis of schizophrenia and have indicted histaminergic systems in psychotic disorders[13], and hyperactivity of dopamine is an established theory in schizophrenia. Therefore, we also investigated the effects of subchronic CPX and CBP doses on MK-801 induced effects on histamine and dopamine levels in the hypothalamus and striatum.

MATERIALS AND METHODS

Animals. The study was carried on Wistar albino 12-week old rats weighing 150–200 gm procured from Central Animal House Facility, Hamdard University. Rats were kept in a group of 6 animals per cage and maintained under standard conditions at 20 °C and 50%–55% humidity in a natural light and dark cycle, with free access to food and water, and housed in a CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) approved animal house facility of Jamia Hamdard. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC Protocol No.- 456). Utmost care was taken to ensure that animals were treated in the most humane and ethically acceptable manner. Animals were brought to experimental room and allowed one week acclimation period to adjust to the new housing, food, water, noises, smells, and light cycle before being used in experiments.

Study design. The experimental groups were divided into 12 groups; each consisting of 6 rats. I: vehicle; Group II: CPX; III: CBP; IV: RAMH; V: MK-801; VI: CPX+MK-801; VII: CBP+MK-801; VIII: RAMH+MK-801; IX: CLZ + MK-801; X: CPZ + MK-801; XI: CPX+RAMH+MK-801; and XII: CBP+RAMH+MK-801

Drugs and treatment. All the drugs used in the experimental study were procured from Sigma Chemicals Co. St. Louis, Missouri, USA). Drug solutions were made in normal saline (0.9% NaCl). All the drugs were administered intraperitoneally, and drug solutions were freshly made on the same day of experiment. The drug solutions were administered in volume of 1 ml/kg. In this study, we induced locomotor activity with MK-801 dose of 0.2 mg/kg, intraperitoneally similar to what was used in our prior study[13]. Higher doses (greater than 0.5 mg/kg) were reported to produce a typical motor syndrome characterized by head weaving, body rolling, ataxia and salivation[20]. Hence, we restricted the dose of MK-801 to 0.2 mg/kg, which did not produce these motor effects which could confound the interpretation of test results.

Open field activity. It was performed in open field consisting of an acrylic box ($40.6 \times 40.6 \times 40.6$ cm) fitted with two photobeam frames (16 beams/dimensions; 2.5 cm between beams; Coulbourn Instruments; Allentown, PA). The lower frame (2.5 cm above the arena floor) recorded horizontal locomotor activity while the upper frame (15 cm above the floor) records rearing. Open field chamber was connected to software (TruScan 2.0 version, Coulbourn Instruments) which recorded the beam breaks (100 milliseconds sampling rate). The locomotor activity was monitored for a period of 20 min beginning 10 min after the injection of MK-801. CPX (3 mg/kg, i.p.), CBP (15 mg/kg, i.p.), CLZ (3 mg/kg, i.p.) and CPZ (3 mg/kg, i.p.) were administered once-daily for 7 days. The dose of drugs chosen in this study is based on reported studies [16].

On the last day of dosing, rats were brought to the test room from the animal housing facility and kept for 30 min in the home cage for habituation. Following 24 h of the last day of the test drug dosing, locomotor testing was done. RAMH (10 mg/kg, i.p.), and MK-801 were administered 15 min and 10 min, respectively, before locomotor testing. All the test recordings were done between 9.00 am and 5.00 pm.

Liquid chromatography mass tandem spectroscopy (LC-MS/MS) estimation of brain striatal dopamine and hypothalamic histamine content in rat. The estimation of dopamine and histamine was done using LC-MS-MS technique with modification of the previous method by Fen Li Su et al. [21] in 2009. Following behavioral testing, rats were sacrificed by cervical dislocation using ether anesthesia. Brains were quickly harvested and placed on ice. The right and left striata, including hypothalamus were dissected and quickly frozen on dry ice, and then stored at -80 °C freezer until analysis. The limits of the hypothalamus for dissection were the optic chiasma at the anterior border, the mammillary bodies at the posterior border, and, on both lateral sides, the hypothalamic sulci. The tissue was finally cut dorsally at 2 mm from the ventral face.

Striatum/hypothalamus was homogenized in ice-cold methanol (1 g brain tissue in 4 ml of methanol) after weighed precisely. 1 ml of homogenate was pipetted out into 1.5 ml conical plastic centrifuge tube and centrifuged at 14,000 rpm

for 20 min. Then the supernatant was evaporated to dryness by vacuum freeze-drying. The dry residue was then reconstituted with 300 µl deionizer water and vortex-mixed for 10 s and added 300 µl chloroform – isopropanol (100:30, v/v). After vortex mixed for 2 min, the mixture was centrifuged at 3,000 rpm for another 5 min. The upper aqueous layer was injected into LC–MS/MS. The sample volume injected was 10 µl for both dopamine and histamine.

Calibration standards and control samples. Calibration samples were prepared in deionized water by adding standard solutions corresponding to a blank and various calibration concentrations ranging 0.3–1250 ng/ml for dopamine and 0.5–100 ng/ml for histamine were prepared. Control samples were prepared from a mixed brain tissue homogenate. LC–MS/MS analyses were carried out on a 4000 QTRAP LC-MS/MS System (AB sciex, Foster city, California, USA) with a hybrid triple quadruple/linear ion trap mass spectrometer (AB sciex, Foster city, California, USA) equipped with pressure ion. The analytes were separated on a thermo C-18 column (4.6 mm, 5 ml, SN: 1245575T, thermo electron corporation, USA) with column temperature at ambient temperature. The mobile phase were 0.05 % formic acid acetonitrile (92: 8, v/v) for the determination of dopamine. The flow rate was 0.8 ml/ min and the post-column splitting ratio was 4:1. The dopamine was obtained by pressure ion in the positive ion mode.

Statistical significance

The data were expressed as mean (±standard error of mean [SEM]). Results were analyzed by Analysis of Variance followed by Dunnett's t-test for multiple comparison tests. $p< 0.05$ were considered as significant. The comparison were predefined and made only against the vehicle and MK-801 treatment groups.

RESULTS

The effect of subchronic dosing of CPX and CBP on MK 801-induced locomotor activity in rats

Rats treated subchronically with CPX or CBP exhibited a significant reduction of hyperlocomotor activities induced by a single dose administration of MK-801 (0.2 mg/kg, i.p.). The horizontal activity increased from 2086.5±249.69 cm to 10577.10±249.69 cm [(p<0.01) F=131.91(11,60)] following MK-801 administration (Figure 1). Rats treated subchronically with CPX (3.0 mg/kg, i.p.) or CBP (15 mg/kg, i.p.) showed a significant (CPX: p<0.01; CBP: p<0.01) reduction in hyperlocomotor activity induced by MK-801 (3103.18±255.02 cm and 3260.03±147.96 cm, respectively; Figure 1a). The increased hyperlocomotor activity induced by MK-801 was, also, reduced (3342.72±213.30 cm and 4462.42±219.60, respectively) significantly (CLZ: p<0.01; CPZ: p<0.01) following administration of CLZ (3.0 mg/kg, i.p.) or CPZ (3.0 mg/kg, i.p.). The protective effects of the administration of both the H_3 antagonists were decreased (p<0.05) in RAMH (10 mg/kg, i.p.) pretreated groups. The MK-801-induced increase in horizontal activity was aggravated (p<0.05) following concurrent administration of RAMH and MK-801.

The *per se* effects of subchronic dosing of CPX and CBP of used in this study showed no influence on locomotor activity.

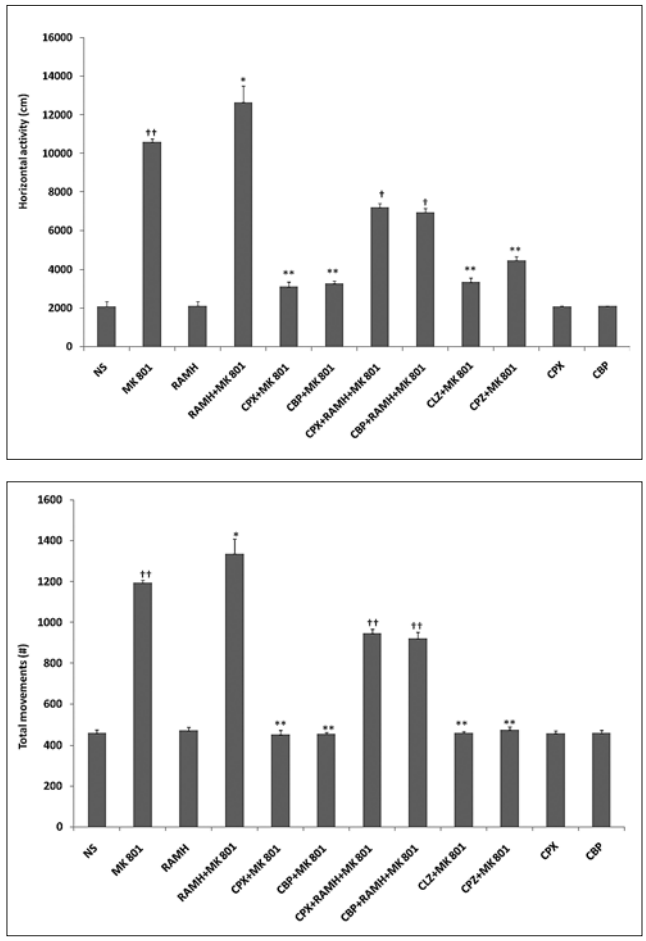

Figure 1. The subchronic dosing of histamine H3 receptor antagonists on MK 801-induced locomotor hyperactivity in rat.
Data represented as Mean± SEM. ANOVA followed by Dunnett's multiple comparison tests, $^{\dagger\dagger}p$<0.01 versus NS (normal saline), $^{**}p$<0.01 versus MK 801 (dizocilpine). CPX: ciproxifan; CBP: clobenpropit; CPZ: chlorpromazine; CLZ: clozapine; MK-801: dizocilpine; NS: normal saline; RAMH: R-α-methylhistamine. CPX, CBP and CLZ were administered 1h before locomotor testing; MK 801 was administered 10 min locomotor testing; CPZ was administered 30 min and RAMH 15 min before locomotor testing

The effect of subchronic dosing of CPX and CBP on dopamine and histamine levels in rats

The administration of MK-801 (0.2 mg/kg, i.p.) caused significant (p<0.01) elevation of the striatal dopamine level compared to control group rats. In CPX (3.0 mg/kg, i.p.) and CBP (15 mg/kg, i.p.) pretreated groups, there was a significant reduction (p<0.01; [F=16.84(11,60)]) on the MK-801-induced increase in the striatal dopamine levels which was recorded as 2580.31±219.80 ng/g-tissue and 2593.54±283.44 ng/g-tissue, respectively. The administration of CLZ or CPZ (3.0 mg/kg, i.p.) also, significantly reduced (p<0.01) the increased striatal dopamine level induced by Mk-801. Reduction in the striatal dopamine level mediated by CLZ or CPZ, were found to be comparable to the reduction produced by CPX or CBP administrations. CPZ (3.0 mg/kg, i.p.) administration also reduced dopamine level in striatum. The decrease of striatal dopamine level mediated by the administration of CPX and CBP further tended to elevate (p<0.05) in RAMH (10 mg/kg, i.p.) pretreated group when compared with CPX+MK-801 and CBP+MK-801 groups, respectively (Tab. 1).

The administration of MK-801 (0.2 mg/kg, i.p.) increased the hypothalamic histamine level. Subchronic dosing of

Table 1. The subchronic dosing of histamine H_3 receptor antagonists on dopamine and histamine levels in rat

Treatments (n=6)	Striatum	Hypothalamus
	Dopamine (ng/g-tissue)	Histamine (ng/g-tissue)
NS	2551.92 (±295.90)	245.39 (±17.04)
MK-801	3150.04 (±311.30)†	287.94 (±30.98)†
RAMH	2532.13 (±213.82)	245.16 (±32.01)
RAMH+ MK801	3530.99 (±127.08)*	265.48 (±8.54)*
CPX+ MK-801	2580.31 (±219.80)*	361.11 (±21.00)†
CBP+ MK-801	2593.54 (±283.44)*	344.13 (±0.21)†
CPX+RAMH + MK-801	2905.33 (±283.44)#	261.27 (±23.18)#
CBP+RAMH + MK-801	2867.54 (±130.48)$	259.68 (±13.67)$
CLZ+ MK-801	2514.17 (±237.44)*	296.11 (±20.31)†
CPZ+ MK-801	2639.83 (±277.71)*	253.01 (±24..47)*
CPX	2442.36 (±70.45)	241.36 (±12.73)
CBP	2490.84 (±125.0)	243.95 (±11.75)
F-value	F=16.84(11,60)	F=8.162 (11,60)

Data represented as mean (±SEM), ANOVA followed by Dunnett's multiple comparison t-test, †$p<0.05$ versus NS, *$p<0.05$ versus MK-801(dizocilpine), #$p<0.05$ versus CPX+MK 801; and $$p<0.05$ versus CBP+MK 801. CPX: ciproxifan; CBP: clobenpropit; CPZ: chlorpromazine; CLZ: clozapine; MK-801: dizocilpine; NS: normal saline; RAMH: R-α-methylhistamine. Following 24 h of the last day of the 7-day dosing with test drugs, MK 801 was administered 10 min and RAMH 15 min before locomotor testing

CPX and CBP exhibited further increased of histamine level in the hypothalamus compared to MK-801 alone. The administration of CLZ significantly [$p<0.05$; F=8.162 (11,60)] increased the hypothalamic histamine level compared to vehicle treatment group while CPZ (3.0 mg/kg, i.p.) decreased the histamine levels in comparison to MK-801 treatment group to 253.01±24.47 ng/g-tissue.

Treatment with RAMH (10 mg/kg, i.p.), in CPX+MK-801+RAMH or CBP+RAMH+MK-801 treatment group, tended to significantly ($p<0.05$) reverse the increase of histamine levels mediated by CPX or CBP administrations in CPX+MK-801 or CBP+MK-801 treatment group. The administration of CPX or CBP per se had no influence on either the dopamine levels in striatum or the histamine levels in hypothalamus. A description of the striatal dopamine level and hypothalamic histamine level is provided in Table 1.

DISCUSSION

In the past, several publications have reported the therapeutic potential of histamine H_3 receptor antagonists/inverse agonists in animal models of CNS disorders, including schizophrenia [13, 16, 22, 23, 24, 25, 26, 27]. H_3 antagonists have been investigated involving multiple facets of animal behaviors for their antipsychotic-like activities, and several preclinical studies have demonstrated antipsychotic like effects with H_3 antagonists and showed huge potential of histamine H_3 receptor antagonists as novel antipsychotic agents [16, 23, 24]. However, unfortunately clinical studies were mostly disappointing and failed to demonstrate appreciable therapeutic efficacy in psychosis [28, 29]. Further, animal studies reporting procognitive and antipsychotic effects of histamine H_3 receptor antagonists agonists [15, 16, 24, 30, 31, 32], has remained mostly inconclusive and indirect as animal models has their own limitations with respect to mimicking all of the characteristics of schizophrenic symptoms. However, research to investigate antipsychotic-like activities of H_3 receptor antagonists has continued despite contradictory reports [17, 18] as well as the recently reported clinical failures with ABT-288 and MK-0249 for cognitive disabilities associated with schizophrenia.

In the present study, we administered subchronic doses (once daily, 7day) of CPX and CBP to investigate their effect on MK-801-induced hyperlocomotion and, also, their effect on the modulation of histamine and dopamine levels in the brain following the behavioral testing. In the present study, we also utilised CLZ and CPZ as they serve as clinically relevant reference atypical and typical reference antipsychotic agents to compare the effect of histamine H_3R antagonists. RAMH is potent and selective H_3-agonist and is reported to antagonize most of the pharmacological effects observed with H_3-antagonists. In the present study, MK-801 administration in rats produced schizophrenia like behavior as observed from increase of locomotor activity in rat. A functional interaction between glutamate and dopamine systems has been established [33], and linked to schizophrenia suggesting that the behavioral activation associated with MK-801 may represent a valid model for detecting potential therapeutic agents in the treatment of schizophrenia [34].

The reproduction of whole of human psychotic behaviors in animal models of schizophrenia continues to pose limitations because the pathophysiology of negative symptoms are less well understood compared to positive symptoms, and have weak face validity and predictive validity. Further, most psychiatric disorders are very heterogeneous in nature and have diverse causality leading to a similar disease symptomatology allowing a syndromic diagnosis. Psychiatric symptoms such as hallucinations, obsessions, delusions, guilt, etc. are uniquely human and can only be inferred with significant limitations in animal models. Therefore, we cannot, necessarily, and realistically postulate that an abnormal behavior in rodent should or could be analogous to an abnormal human behavior[35]. Hence, there continues to be lack of novel antipsychotic agents offering complete remission in schizophrenia.

In our previous study, we reported pharmacological induced models with relevance to schizophrenia with acute administration of H_3 receptor antagonists CPX and CBP[16]. The finding of the present study with subchronic administration of CPX and CBP did not yield much difference suggesting that the sensitivity of MK-801 induced hyperlocomotion to detect antipsychotic-like activity is comparable regardless of the acute or subchronic administration of histamine H_3 receptor antagonists.

In the present study, we noted decrease in MK-801 induced hyperlocomotor response in open field in rats pre-treated with subchronic doses of CPX (3.0 mg/kg) and CBP (15 mg/kg). It has been reported that NMDA antagonists, including PCP, ketamine and MK-801, increase metabolism and release of dopamine in rodent brain [36, 37]. In addition, in the present study, we also observed an increase in dopamine level in striatum of mouse treated with MK-801 leading to hyperlocomotor responses [34]. In contradiction with the finding of our study, in 1991, Liljequist et al. [20] have reported that 0.2 mg/kg intraperitoneal dose of MK-801 increased the rate of dopamine depletion in the striatum and in the limbic forebrain of mice, whereas the rate of depletion of noradrenaline remained unaltered in the limbic forebrain and in the hippocampus. Hence, MK-801 was suggested

to facilitate the activation of dopaminergic mechanisms indirectly (perhaps by reducing glutamatergic activity) instead influencing dopamine neurons, directly. However, later studies supported the finding of our study showing increase dopamine release in the striatum. In 1996, in an *in vivo* microdialysis study, Miller and Abercrombie [38] reported that MK-801 (0.2 or 0.5 mg/kg, i.p.) significantly increased spontaneous dopamine release in the striatum, whereas treatment with vehicle elicited no change. The increase in striatal dopamine by MK-801 was suggested to result from the disinhibition of a tonic inhibitory influence of NMDA receptor producing increased neuronal firing of dopamine in substantianigra pars compacta, and also MK-801 increased neuronal firing of dopamine in the midbrain [38]. In the same year, Mathe et al. [39] reported that administration of MK-801 at 0.1 and 0.3 mg/kg, subcutaneously, significantly increased dopamine levels and its metabolites in the nucleus accumbens of freely moving rats. Histamine H_3 receptors are reported to colocalize in different neuronal populations and controls striatal dopaminergic and glutamatergic transmission [40]. In light of these studies, we could presume that the decrease of MK-801-induced increased dopamine level in the striatum by CPX and CBP could have been due to modulation of the synthesis and release dopamine and glutamate in the striatum from cortico-striatal afferents [40]. It was, further, reported that MK-801-induced modulation of locomotor activity and stereotyped behavior could be abolished or diminished by the depletion of dopamine from neuronal stores or by the pretreatment with reserpine, α-methyl-*p*-tyrosine and the administration of dopamine receptor antagonists, indicating primarily a dopamine-dependent behavioral effects of MK-801[39]. In a study, increased extracellular concentrations of dopamine including norepinephrine, and serotonin were reported in the nucleus accumbens following systemic treatment of MK-801 [41]. In another study, increased extracellular level of dopamine, and other neurotransmitter such as GABA, glutamate, serotonin and acetylcholine have been reported in the frontal area of rats and monkeys following systemic treatment with PCP [1, 41, 42] Large numbers of H_3 receptors have been demonstrated on nonhistaminergic neurons and their activation inhibits synthesis and release of several neurotransmitters, including dopamine, norepinephrine, GABA, and acetylcholine[22].

Striatum contains a high density of postsynaptic H_3 receptors, which are co-localized with both D_1 and D_2 receptors in the GABAergic dynorphinergic and enkephalinergic neurons, respectively [15, 43]. Activation of H_3 receptor has been reported to inhibit dopamine D_1 receptor dependent release of GABA from in rat striatum[44]. Further, an existence of a direct functional H3/D2- receptor interactions in striatopallidal neurons of the indirect pathway [15] and antagonistic intramembrane interaction between H_3 and D_2 receptors in striatal tissue has been reported [45]. Faucard et al., in a study involving double immunostaining for histidine decarboxylase and NMDA receptor subunits, have shown the presence of NMDA receptor subunits at histamine neurons of the rat TMN. They further observed immunoreactivity for the neuronal glutamate transporter EAAC1 near most histaminergic perikarya, indicating presence of strong histamine/glutamate functional interactions in the brain [46]. In line with these studies, it may, also, be proposed that attenuation of locomotor hyperactivity in rats, pretreated with subchronic doses of CPX and CBP, not only resulted from the blockade of dopamine receptor but involves complex interplay of several neurotransmitters including histamine, GABA, and glutamate in the brain leading to inhibition of locomotor hyperactivity.

Prefrontal cortex (PFC) is known to be involved in pathology of schizophrenia[47]. Recently, Molina et al. reported that NMDA receptor blockade acutely disrupts a synchronized action potential firing in the PFC of rat [48]. We may presume that CPX and CBP might have a facilitatory activity in synchronized firing of action potential in rat prefrontal cortex. The present study showed an increase in the hypothalamic histamine level in rats treated with MK-801. Rats treated with subchronic doses of CPX and CBP tended to further raise the level of histamine in the hypothalamus. Administration of MK-801 increased the turnover of histamine as indicated by increased histamine metabolite, tele-methylhistamine (*t*-MeHA), a biomarker of histamine turnover, in the cerebral cortex, striatum, hippocampus and hypothalamus. The further increase of histamine by CPX and CBP, as noted in this study, may be due to blockade of presynaptic H_3 autoreceptors, and is consistent with the recent finding showing that perfusion of the tuberomamillary nucleus with H_3 receptor antagonist, ABT-239, increased histamine release from tuberomamillary nucleus, nucleus bacillus magnocellularis, and cortex, but not from the striatum or nucleus accumbens [49]. Also, systemic administration of ABT-239 increased histamine release from nucleus bacillus magnocellularis, but not from the nucleus accumbens[49]. It was hypothesized that endogenous histamine modulate the activity of striatal neurons, and cause psychotic symptoms by activating H_3 receptors present at a high density on striatal neurons [15]. This would lead to hyperactivity of histaminergic neurons induced by drug produced psychotic symptoms, and cause antipsychotic-like properties by the blockade of H_3 receptors. CPX increase the histaminergic-neuron activity and lower schizophrenic symptoms by acing as an inverse agonist at presynaptic H_3 autoreceptors [50]. This may be implied that histaminergic neurons are not directly involved in mechanisms that produce psychotic symptoms but inhibit these mechanisms in a compensatory manner. Such a compensatory mechanism is most likely to cause histaminergic neuronal hyperactivity induced by psychotogenic drugs and NMDA- antagonists. At the same time, these compensatory mechanisms are facilitated by drugs that enhance histaminergic neuronal activity and show schizophrenic-like behaviors such as H_3 antagonists/inverse agonists and atypical neuroleptics[51]. In 1990, Tiedtke et al. [52]. reported a study comparing typical and atypical antipsychotic drugs on MK-801 induced stereotypy in rats. They showed that both typical and atypical antipsychotic drug decreased stereotypy but the typical one exhibited greater reduction in stereotypy. The study indicated that some of the effectiveness of antipsychotic drugs was mediated by indirect effects at the NMDA receptor [53]. In this study, CPX and CBP mediated reversal of MK-801 induced behavioral and neurochemical changes were nearly similar to CLZ and better than CPZ. Further, the finding of our study was concordant with the report that typical antipsychotic agents decrease histamine neuron activity, whereas atypical antipsychotic agents enhance histamine neurons [51], and also CLZ has been reported to possess H_3 receptor antagonistic activity [53], indicating that H_3

receptor antagonists may possess some antipsychotic-like activities though Burban et al reported complete lack of antipsychotic effect with H_3 receptor antagonist [17], and the other study reported that CPX alleviated the impact of NMDA receptor hypofunction on some forms of memory but exacerbated motor effect of MK-801 in rats [18]. Therefore, the antipsychotic potential of histamine H_3 receptor antagonists have yet to be convincingly demonstrated given conflicting preclinical and clinical findings.

CONCLUSION

In conclusion, the finding of the present study supports that CPX and CBP have preventive activities in some of the psychotic behaviors in experimental animal models. Histamine H_3 antagonists protect psychotic-like behaviors induced MK-801 by modulating dopamine and histamine levels in rat. Thus, the present study supports our previous study reporting antipsychotic-like activities with H_3 antagonists, CPX and CBP. However, further studies involving newer improved animal models of schizophrenia are warranted.

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