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In vivo microdialysis studies of striatal level of neurotransmitters after haloperidol and chlorpromazine administration

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Therapeutic success of atypical antipsychotics has focused the attention on the role of receptor systems other than dopaminergic system in the pathophysiology of neuroleptics-associated acute (Parkinson's like syndrome) and chronic (tardive dyskinesia) extrapyramidal side effects. This study was planned to investigate changes in striatal levels of norepinephrine, dopamine and serotonin after acute and chronic administration of classical neuroleptics (haloperidol and chlorpromazine). These changes were correlated with behavioural alterations in rats. *In vivo* microdialysis with HPLC/ECD system revealed that there was a marked decrease in striatal neurotransmitter contents (NE, DA and 5-HT), which was also correlated with severe cataleptic response in rats after acute administration of haloperidol (2 mg/kg) and chlorpromazine (20 mg/kg). Chronic administration of haloperidol (1 mg/kg for 21 days) and chlorpromazine (5 mg/kg for 21 days) resulted in time dependent increase in orofacial hyperkinetic movements. The microdialysis studies also showed a significant decrease in the striatal levels of all the neurotransmitters. The results provide evidence for the involvement of striatal adrenergic and serotonergic systems, besides dopaminergic system in neuroleptic-induced acute and chronic extrapyramidal symptoms.

Keywords: Atypical antipsychotics, *In vivo* microdialysis, Neurotransmitters, Tardive dyskinesia

Serotonergic, dopaminergic and nor-adrenergic pathways constitute major monoaminergic afferents to striatum, along with massive corticostriatal and thalamostriatal glutamatergic inputs. Interaction between these neuronal systems are of great interest for understanding the normal functioning of basal ganglia and the pathophysiology of neurological and psychiatric disorders associated with these structures[1-3].

Administration of typical neuroleptics like haloperidol induces catalepsy in rats, a phenomenon generally defined as the long-term maintenance of the animal in an abnormal posture[4]. This behavioural response has long been used as a model for the extrapyramidal side effects such as Parkinsonian-like bradykinesia associated with anti-psychotic use in humans[5]. Although anti-psychotic-induced catalepsy appears primarily to be due to the blockade of dopamine neurotransmission in the striatum, a number of other neurotransmitter systems have been reported to modulate this response[6].

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Chronic administration of typical neuroleptic drugs is associated with acute and delayed motor side effects including Parkinsonism, akathisia and tardive dyskinesia[7]. Tardive dyskinesia is a potentially irreversible and involuntary hyperkinetic disorder[7]. Although relationship between the tardive dyskinesia and long term haloperidol treatment has been established, pathophysiology of this motor disturbance is still unknown. Various hypotheses have been proposed including development of dopamine receptor supersensitivity, disturbed balance between dopaminergic and cholinergic system, dysfunction of GABA neurons, excitotoxicity via glutamate receptors and oxidative stress[8,9] Neither of them individually explains the pathogenesis of tardive dyskinesia. Possible involvement of different neurotransmitters such as dopamine, norepinephrine, serotonin and their interaction with glutamatergic excitatory pathways has been speculated[10,11].

In the present study, extracellular levels of norepinephrine, dopamine and serotonin have been determined in rat striatum after acute and chronic administration of typical neuroleptics by using *in vivo* microdialysis techniques, and attempt has also been made to correlate these alterations with associated behavioural dysfunction.

Materials and Methods

Animals—Male Wistar rats (150-180 g) bred in the Central Animal House facility of Panjab University were used. The animals were housed under standard laboratory conditions (25º ± 2ºC, 60-70% RH), maintained on a natural 12:12 L:D cycle and free access of food and water. Animals were acclimatized to laboratory conditions before the test. All the experiments were carried out between 0900 and 1500 hrs. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to the guidelines of Indian National Science Academy (INSA) for the use and care of experimental animals.

Drugs and treatment schedule—Animals were divided in 5 groups. Doses were selected on the basis of previous studies conducted in the laboratory.

Group	n	Treatment
1	6	Vehicle
2	8	Haloperidol (Serenace, Searle India; 2 mg/kg, ip, once)
3	8	Chlorpromazine (May & Baker, India; 20 mg/kg, ip, once)
4	6	Haloperidol (1 mg/kg, ip, once daily for 21 days)
5	6	Chlorpromazine (5 mg/kg, ip, once daily for 21 days)

All drugs were administered intraperitoneally in a constant volume of 0.5 ml/ 100 g of body weight of rat. Catalepsy score was measured for over 4 hr at various time points after haloperidol and chlorpromazine administration in group 2 and 3. In group 4 and 5, drugs were administered once daily at 0900 hrs for 21 days. Behavioural assessments were done 24 hr after the last dose (on day 22).

Catalepsy measurements—Measurement of catalepsy was done to assess acute side effects associated with neuroleptics. The development of severity of the four stages of catalepsy was scored[5] as follows:

Stage 1: rat moves freely when placed on the table (score=0)

Stage 2: rat moves only when touched or moved (score=0.5)

Stage 3: rat fails to correct posture in 10 sec when front paw placed alternatively, on a 3 cm high block (score=0.5 for each paw with a total score of 1 for this stage)

Stage 4: rat fails to correct posture in 10 sec when front paw placed alternatively, on a 9 cm high block (score=1 for each paw with a total score of 2 for this stage)

Thus the complete cataleptic response was described when the score was 3.5. A lower score meant an apparently lesser degree of catatonia. The scoring of catalepsy response was done at 1, 2, 3, 4 hr after the administration of different typical and atypical antipsychotics. Score at different time points were added for comparing cumulative catalepsy score[12].

Measurement of vacuous chewing movements and related behaviours—Measurement of vacuous chewing movements and related behaviours was done to assess chronic side effects associated with neuroleptics. On the test day, rats were placed individually in a small (30×20×30 cm) plexiglass cage for the assessment of oral dyskinesia. Animals were allowed for 10 min to get used to the observation cage before behavioural assessments. The behavioural parameters of oral dyskinesia were measured continuously for 5 min. Hand operated counters were employed to score vacuous chewing movements and related behaviours. Counting was stopped whenever the rat began grooming, and restarted when grooming stopped. In all the experiments the scorer was unaware of the treatment given to the animals[13,14].

In vivo microdialysis—Animals were anaesthetized with thiopental sodium (45 mg/kg, ip) diluted in distilled water. They were then mounted in a stereotaxic frame, and implanted with dialysis probe guide cannulae in the striatum (using Paxinos and Watson rat atlas, 1998) relative to bregma. Guide cannulas were attached to skull with dental acrylic and machine screw. Dialysis experiments were conducted 48-72 hr after the surgery in anesthetized animals. On the day of experiment, a semi-permeable polycarbonate dialysis probe [2 mm membrane with 0.5 mm diameter, molecular weight cutoff: 20,000 Da; (CMA-12 from CMA/Microdialysis, SE-171, 18-Solna, Sweden)] was inserted in the guide cannula. The dialysis probe was perfused with a physiological perfusion solution (Artificial cerebrospinal fluid; 147 m*M* NaCl, 3.0 m*M* KCl, 1.3 m*M* $CaCl_2$, 1.0 m*M* $MgCl_2$, and 1.0 Na_2PO_4 (*p*H 7.4), CMA/Microdialysis, Solna, Sweden) at a rate of 2.0 µl/min set by a micro infusion pump (Harvard Apparatus, Holliston, MA, USA). In acute experiments, drug was administered on the day of

experiment. It was administered after the achievement of stable monoamine release levels. This occurred after 3-4 hr of probe insertion. The sampling period was 1 hr upto 6 hr and then after 24 hr of drug administration. In chronic administration drugs were administered for 21 days. On day 18, after the administration of drug, surgery was performed. Online quantification of dopamine (DA), nor epinephrine (NE) and 5-Hydroxytryptamine (5-HT) in the dialysate was accomplished using HPLC/ECD (Waters, USA)[15,16]. (Fig. 1)

Analytical procedure—Biogenic amines (dopamine, norepineprine and serotonin) were estimated by HPLC (Waters system) with electrochemical detector as per Church[17]. Waters standard system consisting of a high pressure isocratic pump, a 20 µl sample injector valve, C18 reverse phase column and electrochemical detector were used. Data were recorded and analyzed with the help of EMPOWER SOFTWARE (Waters Pvt Ltd). Mobile phase was consisting of 2% citric acid, 2% $KHPO_4$, 1 m*M* EDTA, 1.2% MeOH, and 70 mg/ml of sodium octyl sulphate. *p*H of the mobile phase was adjusted to 3 with the help of HCl (6*N*). Electrochemical conditions for the experiment were +0.800 V, sensitivity ranges from 5-50 nA. Separation was carried out at a flow rate of 1 ml/min. Dialysate (20 µl) was injected manually through HPLC injection pump. Data were recorded and analyzed with the help of empower software[17,18].

Data analysis—Changes in the monoamines contents due to various treatments were expressed as percentage of baseline in each individual rat. The average levels of all the neurotransmitters in three samples preceding the drug application was defined as base line (100%) in case of acute experiments. In chronic neuroleptic treatment the experimental data of treated animal were compared with those of control animals.

Statistical analysis—One specific group of rats was assigned to one specific drug treatment condition. All the values are expressed as mean±S.E. The data were analyzed by using analysis of variance (ANOVA) followed by Dunnett's test. In all tests, $P<0.05$ was considered as the criterion for statistical significance.

Results

Behavioural studies—Acute administration of haloperidol (2 mg/kg) or chlorpromazine (20 mg/kg)

Fig. 1—*In vivo* microdialysis setup for the collection of extracellular fluid from rat striatum. (A) - Streotaxic apparatus for probe implantation; (B) - In vivo microdialysis set up; and (C) – HPLc/ECS detection system.

induced a severe cataleptic state appearing at the first hour after its injection, reaching a maximum plateau after the second hour. Total catalepsy score for haloperidol and chlorpromazine was 14±1.3 and 12±2.2 respectively as compared to control group (0). Chronic (21 days) administration of haloperidol (1 mg/kg, ip) or chlorpromazine (5 mg/kg, ip) resulted in a significant increase in vacuous chewing movements and related behaviours (tongue protrusions and facial jerkings) as compared to vehicle treated group (Table 1).

Microdialysis studies—Figure 2 shows the effect of single ip injection of haloperidol (2 mg/kg) or chlorpromazine (20 mg/kg) on the extracellular levels of DA, NE and 5-HT in the striatum. There was a tendency toward a decrease in extracellular concentrations of all the three neurotransmitters. In haloperidol (2 mg/kg) treatment extracellular levels of DA, NE and 5-HT reached a minimum level of 43% (3-4 hr), 22 % (4-5 hr) and 26% (2-3 hr) of base line respectively. Similarly, chlorpromazine (20 mg/kg) treatment showed a decrease in DA, NE and 5-HT levels to reach a minimum of 3% (3-4 hr), 20 % (3-4 hr) and 45% (5-6 hr) of base line. However, the levels of all three neurotransmitters returned towards base line levels after 24 hr.

Figure 3 shows the effect of chronic (21 days) administration of haloperidol (1 mg/kg, ip) or chlorpromazine (5 mg/kg, ip) on the extracellular striatal levels of DA, NE and 5-HT. There was a significant decrease in extracellular concentrations of all the neurotransmitters as compared to control. Haloperidol resulted in 62, 72 and 56% decrease in the levels of DA, 5-HT and NE, respectively. Chlorpromazine resulted in 55, 58 and 71% decrease in the levels of DA, 5-HT and NE, respectively.

Discussion

Acute and chronic administration of typical neuroleptics is associated with extrapyramidal side effects. The *in vivo* microdialysis analysis revealed that there was a decrease in the levels of DA, NE and 5-HT in extracellular striatal space which may be correlated with extrapyramidal effects seen in the animals.

The cataleptic immobility observed in rodents following typical neuroleptics (e.g., haloperidol or chlorpromazine) is an indicator of nigrostriatal dysfunction due to decreased levels of various neurotransmitters[19,20]. Time dependent correlation between catalepsy induced by typical antipsychotics and change in striatal neurotransmitters were noted in

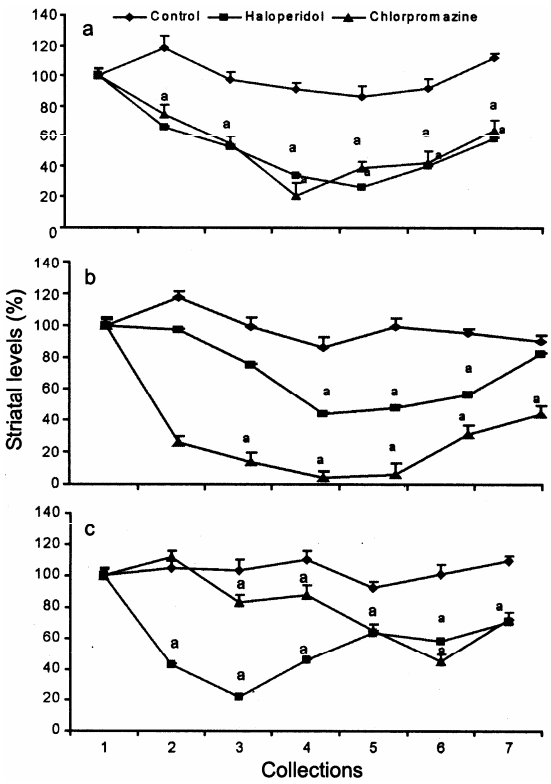

Fig. 2—Effect of acute administration of haloperidol (2 mg/kg) or chlorpromazine (20 mg/kg) on striatal (a) DA (b) NE (c) 5-HT levels in living animals by using *in vivo* microdialysis. [Values expressed as mean ± SE. $^{*}P \leq 0.05$ as compared to control group. Dialysate were collected at 7 different time points:1=0-1 hr, 2=1-2 hr, 3=2-3 hr, 4=3-4 hr, 5=4-5 hr, 6=5-6 hr and 7= 24 hr]

Table 1—Effect of chronic administration of haloperidol (1 mg/kg once daily for 21 days) and chlorpromazine (5 mg/kg once daily for 21 days) on orofacial dyskinetic movements in rat
[Values expressed in mean ± SE].

Treatment	Dose mg/kg	No. of VCM/5 min Day 0	Day 22	No. of Tongue protrusions/5 min Day 0	Day 22	No. of Facial jerkings/5min Day 0	Day 22
Control		2.3±0.5	3.2±0.9	0.4±0.01	1.2±0.1	0.6±0.02	1.1±0.09
HAL	1	1.6±0.4	62.0±5.7*	1.3±0.08	15.0±3.8*	1.2±0.08	18.0±5.3*
CPZ	5	2.0±0.6	55.0±7.0*	0.4±0.04	12.0±2.6*	0.8±0.04	15.0±3.3*

HAL=Haloperidol; CPZ=Chlorpromazine
*$P \leq 0.05$ as compared to control group

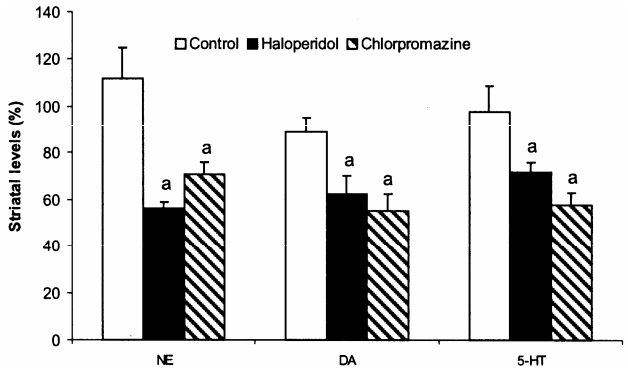

Fig. 3—Effect of chronic administration of haloperidol (1 mg/kg for 21 days) or chlorpromazine (5 mg/kg for 21 days) on striatal levels of (a) DA (b) NE (c) 5-HT levels in living animals by using *in vivo* microdialysis (% levels of day 0 were taken as 100%). [Values expressed in mean ± SE. $^{*}P \leq 0.05$ as compared to control group]

the present study. Microdialysis study showed that neuroleptic-induced catalepsy was well correlated with a decrease in the striatal levels of dopamine[21]. Acute treatment with haloperidol resulted in elevated level of 3, 4 dihydroxy phenyl acetic acid (DOPAC), homo vanillic acid (HVA) and increased metabolite/dopamine ratios in limbic and striatal brain areas[21]. Changing dopamine function relative to serotonin function, rather than changing dopamine *per se*, is associated with the therapeutic effect of haloperidol[22]. Exploration of serotonin-dopamine relationship may be more informative than examining each system in isolation. There is a decrease in the levels of dopamine and subsequent increase in its metabolite levels[21]. Increase in HVA is relative to 5-hydroxy indole acetic acid (5-HIAA), metabolite of serotonin[22]. In the present study, acute administration of typical neuroleptics resulted in significant decrease in striatal serotonin levels with the concomitant increase in catalepsy. It is either due to its direct effect or resultant effect on dopamine receptors. Further, in neuroleptic-induced catalepsy, acute behavioral effects of serotonin modulators in rodents include both an increase[23] and a reduction of locomotor activity, and the reasons for this discrepancy are not well understood. Previous studies have shown that clomipramine, a non-selective antidepressant, enhances the neuroleptic-induced catalepsy[24], while fluoxetine (at 5 mg/kg ip, in male mice) attenuates the phenomenon[25]. Selective serotonin reuptake inhibitors (SSRIs) also consistently attenuate the neuroleptic-induced catalepsy. The central serotonergic system modulates nigrostriatal dopaminergic transmission[19]. In short, it can be said that typical antipsychotics decrease serotonin levels in striatum which can be related to anti-cataleptic effects of SSRIs.

In addition to impaired dopaminergic neurotransmission, dysfunctional noradrenergic system has also been demonstrated in cataleptic behaviour. Intravenous infusion of NE (1.5 and 15 μg/kg) or L-threo-3, 4-dihydroxyphenylserine (DOPS), a synthetic precursor of NE, (2 and 4 mg/kg) in male wistar rats (240–290 g) significantly decreased haloperidol-induced catalepsy[25]. Pretreatment with Ro 40-7592, a catechol-o-methyltransferase inhibitor (COMT), potentiated and prolonged the anti-cataleptic effect of NA and DOPS. These findings suggest a peripheral site of NA mediated anti-cataleptic action which can be further potentiated by COMT inhibition[26]. This suggests the involvement of catecholamine metabolism in haloperidol-induced catalepsy. There was a significant decrease in striatal nor-epinephrine levels with an increase in cataleptic effect. This decrease could also be attributed to increased metabolism of nor-adrenaline.

Chronic administration of haloperidol and chlorpromazine is associated with increase in vacuous chewing movements and related effects. Besides this, Bishnoi *et al*[10]. explained about the change in the levels of monoamines and serotonin after chronic administration of typical neuroleptics[10]. There was significant decrease in the levels of all the three neurotransmitters in cortical and subcortical regions after chronic administration of haloperidol and chlorpromazine whereas the decrease was not significant in atypical neuroleptics such as clozapine and risperidone[18]. Present study further substantiates the point that there is significant decrease in the levels of monoamines which can be correlated to increased vacuous chewing movements and other related behaviours. Chronic administration of neuroleptics is associated with proliferation of D_2 receptors in caudate putamen and nucleus accumbens[11], selective tissue and neuronal increase in dopaminergic receptor sites and receptor bindings (increase in Bmax)[11], increase in striatal D_2 mRNA expression, augmentation of downstream signal transduction pathways involving CAMKII and nNOS up regulation[27]. This results in dopaminergic supersensitivity and concomitant decrease in dopamine levels in striatum.

The serotonergic input has been shown to make direct synaptic contact with dopaminergic neurons in both substantia nigra and ventral tagmental area, major areas involved in the movement disorders[28].

Serotonin modulates striatal dopamine release and could influence dyskinetic movement. Stimulation of dorsal raphe serotonergic fibres releases serotonin in the substantia nigra and this is associated with the decrease in dopamine related behaviours. Hence in accordance with literature reports the present results also suggest that there is a significant decrease in the levels of dopamine and serotonin after chronic haloperidol administration. 5-HT may help in neuronal preservation and indeed 5-HT can have a neuroprotective action in the striatum and other brain areas[3]. Apart from its neuromodulatory action, 5-HT may have a major role in preservation of the structural organization of CNS especially in neuroleptic-induced vacuous chewing movements and related behaviours in rodents[29-31].

Besides serotonin and dopamine, typical neuroleptics such as haloperidol and chlorpromazine on chronic administration also affect the density of adrenergic receptors in different regions of brain especially in the thalamic regions[10,32]. Recent receptor binding studies have confirmed that chronic administration of haloperidol increases the receptor binding sites and the B_{max} of adrenergic receptors which was not seen in chronic administration of clozapine suggesting the possible involvement of this aspect in the pathophysiology of tardive dyskinesia[32]. In our study, extracellular concentration of norepinephrine was decreased with the chronic administration of haloperidol and chlorpromazine. The decrease was simultaneous to the increase in orofacial hyperkinetic movements. It is gradually accepted that in case of tardive dyskinesia, glutamatergic transmission has been increased and high concentration of glutamate leads to oxidative damage as well as neuronal degeneration[9,33]. Glutamatergic transmission has been modulated by the concentration of dopamine, serotonin and norepinephrine in extracellular regions of brain. Hence, with change in the concentration of these neurotransmitters, neuroleptics either directly on their own or indirectly via glutamate transmission result in increased orofacial hyperkinetic movements.

In conclusion, the microdialysis studies also showed a significant decrease in the striatal levels of all the neurotransmitters. Present study provides evidence for the involvement of striatal adrenergic and serotonergic systems, besides dopaminergic system in neuroleptic-induced acute and chronic extrapyramidal symptoms.

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