Comparative neurochemical changes associated with chronic administration of typical and atypical neuroleptics: Implications in tardive dyskinesia

Mahendra Bishnoi*, Anil Kumarª, Kanwaljit Chopraª & Shrinivas K Kulkarni*ª†

*Centre with Potential for Excellence in Biomedical Sciences (CPEBS), Panjab University, Chandigarh 160 014, India
ªPharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160 014, India

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An important goal of current neuroleptic research is to develop antipsychotic compounds with the low incidence of extrapyramidal side effects. The therapeutic success and less side-effect of atypical anti-psychotics such as clozapine and risperidone has focused the attention on the role of receptor systems other than dopaminergic system in the pathophysiology of neuroleptics-associated extrapyramidal side effects. The present study compares the effect of chronic administration of typical and atypical antipsychotics on neurochemical profile in rat forebrain. The study was planned to study changes in extracellular levels of norepinephrine, dopamine and serotonin in forebrain region of brain and tried to correlate them with hyperkinetic motor activities (vacuous chewing movements (VCM’s), tongue protrusions and facial jerking) in rats, hallmark of chronic extrapyramidal side-effect of neuroleptic therapy tardive dyskinesia. Chronic administration of haloperidol (1 mg/kg) and chlorpromazine (5 mg/kg) resulted in significant increase in oro-facial hyperkinetic movements whereas clozapine and risperidone showed less significant increase in oro-facial hyperkinetic movements as compared to control. There were also significant decrease in the extracellular levels of neurotransmitters dopamine, norepinephrine and serotonin in fore-brain as measured by HPLC/ED after chronic administration of haloperidol and chlorpromazine. Chronic administration of atypical neuroleptics clozapine and risperidone resulted in the decrease in extracellular concentration of dopamine and norepinephrine but the effect was less significant as compared to typical drugs. However, treatment with atypical neuroleptics resulted in 3 fold increase in serotonin levels as compared to forebrain of control rats. Typical and atypical neuroleptics showed varying effects on neurotransmitters, especially serotonin which may account for the difference in their profile of side effects (Tardive dyskinesia).

Keywords: Atypical anti-psychotics, Neurotransmitters, Tardive dyskinesia,

Schizophrenia is a chronic severe mental illness affecting approximately 1% of the population¹. The first generation anti-psychotic medications such as chlorpromazine and haloperidol often described as typical ones produce numerous adverse effects of which most troublesome are extrapyramidal side effects such as Parkinsonism and tardive dyskinesia². The therapeutic success of clozapine and other newer atypical anti-psychotics has focused the attention on the role of receptor systems other than dopaminergic system in the pathophysiology of schizophrenia and associated extrapyramidal side effects particularly that of tardive dyskinesia³.

Tardive dyskinesia is a potentially irreversible and involuntary hyperkinetic disorder⁴. 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 a disturbed balance between dopaminergic and cholinergic system, dysfunction of GABA neurons, excitotoxicity via glutamate receptors and oxidative stress. 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 speculated⁵,⁶. High density of serotonin receptors especially 5HT1, 5HT2 and 5HT3 receptor subtypes in the basal ganglia region and their interaction with the dopaminergic system has led to the hypothesis that serotonin may play a role in movement disorders associated with basal ganglia⁷,⁸.

The serotonergic, dopaminergic and nor-adrenergic pathways constitute the major monoaminergic afferents to the striatum, along with the massive corticostriatal and thalamo-striatal glutamatergic

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*Correspondent author
Phone no: 0172-2534197, 2534114
Fax: 01722534197
E-mail: skpu@yahoo.com
inputs. Interaction between these neuronal systems are of great interest for understanding the normal functioning of basal ganglia as well as the pathophysiology of neurological and psychiatric disorders associated with these structures including Parkinson’s disorder, Huntington’s disease, Schizophrenia and Tardive dyskinesia. A number of studies have shown that dopaminergic and glutamatergic and/or serotonergic and glutamatergic systems are anatomically and functionally linked in the basal ganglia region.

In the present study neurochemical changes in extracellular levels of norepinephrine, dopamine and serotonin in forebrain of rats have been determined after chronic administration of different typical (haloperidol and chlorpromazine) and atypical (clozapine and risperidone) anti-psychotics and tried to correlate them with hyperkinetic motor activities (vacuous chewing movements (VCM's), tongue protrusions and facial jerking), characteristic of debilitating motor dysfunction tardive dyskinesia.

Materials and Methods

Animals—Male Wistar rats (180-220 g) bred in the Central Animal House facility of Panjab University were used. The animals were housed under standard laboratory conditions, maintained on a natural 12:12 hr light-dark cycle and free access to food and water. Animals were acclimatized to laboratory conditions before the test. Each animal was used only once in the experiment. All the experiments were carried out between 0900 and 1500 hrs. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of Indian National Science Academy for the use and care of experimental animals.

Drugs and treatment schedule—The following drugs were used in the present study. Haloperidol (Serenace, Searle, India), chlorpromazine, clozapine and risperidone were diluted with distilled water. All drugs were administered intraperitoneally in a constant volume of 0.5 ml/100 g of bodyweight of rat. Animals were divided in 5 groups. First group received vehicle, second group received haloperidol (1mg/kg) plus vehicle, third group received chlorpromazine (5 mg/kg) plus vehicle, fourth group received clozapine (5 mg/kg) plus vehicle, and fifth group received risperidone (2 mg/kg) plus vehicle. Drugs were administered once daily (0900) in the morning for 21 days and behavioural assessments were done 24 h after the last dose. Drug doses were selected on the basis of previous studies conducted in our laboratory and those reported in literature.

Behavioural assessment of orofacial dyskinesia—On the test day rats were placed individually in a small (30×20×30 cm) Plexiglas cage for the assessment of oral dyskinesia. Animals were allowed 10 min to get used to the observation cage before behavioural assessments. To quantify the occurrence of oral dyskinesia, hand operated counters were employed to score tongue protrusion and vacuous chewing frequencies (VCMs). In the present study VCM are referred to as single mouth openings in the vertical plane not directed toward physical material. If tongue protrusion, VCM occurred during a period of grooming, they were not taken into account. Counting was stopped whenever the rat began grooming, and restarted when grooming stopped. Mirrors were placed under the floor and behind the back wall of the cage to permit observation of oral dyskinesia when the animal was faced away from the observer. The behavioural parameters of oral dyskinesia were measured continuously for a period of 5 min. In all the experiments the scorer was unaware of the treatment given to the animals.

Neurotransmitters estimation—Biogenic amines (dopamine, norepinephrine and serotonin) were estimated by HPLC (Waters system) with electrochemical detector by the method of Church. 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. Mobile phase consisting of 2% citric acid, 2% K2HPO4, 1 mM EDTA, 1.2% MeOH, and 70 mg/ml of sodium octyl sulphate was used. pH of the mobile phase was adjusted to 3 with the help of HCl (6N). 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. Samples (20 μl) were injected manually.

On the day of experiment forebrain frozen samples were thawed. They were homogenized in homogenizing solution containing 0.1 M perchloric acid. After that samples were centrifuged at 12000 g for 15 min. The supernatant was further filtered through 0.25 μ nylon filters before injecting in the HPLC injection pump. Data were recorded and analyzed with the help of empower software.
Statistical analysis—One specific group of rats was assigned to one specific drug treatment condition and each group comprised six rats (n=6). 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

Body weight change—Body weight was significantly decreased in haloperidol (1 mg/kg, ip), chlorpromazine (5 mg/kg, ip) and clozapine (5 mg/kg, ip) treated groups as compared to control animals where as risperidone (2 mg/kg) showed no significant change as compared to control.

Behavioural assessment

Assessment of orofacial dyskinesia—Haloperidol (1mg/kg, ip) and chlorpromazine (5 mg/kg, ip) treatment resulted in significant increase in VCMs, tongue protrusion and facial jerking as compared to control. Although there was significant increase in clozapine (5 mg/kg, ip) treated group, it was significantly less as compared to haloperidol (1mg/kg, ip) and chlorpromazine (5 mg/kg, ip) treatment. Where as risperidone (2 mg/kg, ip) treatment resulted in insignificant increase in VCMs, tongue protrusion and facial jerking as compared to controls (Fig. 1).

Neurochemical assessment

Neurotransmitter level estimation in the forebrain—Chronic administration of haloperidol (34, 43, and 23%) and chlorpromazine (29, 35, and 64%) significantly decreased the dopamine, norepinephrine and serotonin levels in rat forebrain as compared to control animals. Although clozapine (19.5, 20.5%) and risperidone (15, 27.8%) resulted into significant decrease in dopamine and norepinephrine levels but was much less than the haloperidol and chlorpromazine treated groups. Where as clozapine (265%) and risperidone (228%) resulted into significant and many fold increase in serotonin levels as compared to control animals (Table 1).

![Fig. 1](image)

Table 1—Extracellular levels of dopamine, norepinephrine and serotonin in forebrain of the animals treated with vehicle, haloperidol (1 mg/kg, ip 21 days), chlorpromazine (5 mg/kg, ip 21 days), clozapine (5 mg/kg, ip 21 days), risperidone (2 mg/kg, ip 21 days).

<table>
<thead>
<tr>
<th>Treatment (mg/kg ip.)</th>
<th>Dopamine (pg/mg tissue)</th>
<th>Norepinephrine (pg/mg tissue)</th>
<th>Serotonin (pg/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>514± 45.2</td>
<td>1668± 56.2</td>
<td>1153± 45.2</td>
</tr>
<tr>
<td>Haloperidol (1)</td>
<td>339.2 ± 36.2 abc</td>
<td>950.7 ± 56.2 abc</td>
<td>887 ± 56.2 abc</td>
</tr>
<tr>
<td></td>
<td>(-34)</td>
<td>(-43)</td>
<td>(-23)</td>
</tr>
<tr>
<td>Chlorpromazine (5)</td>
<td>364.9± 24.2 abc</td>
<td>1084.2± 44.2 abc</td>
<td>415.08 ± 44.2 abc</td>
</tr>
<tr>
<td></td>
<td>(-29)</td>
<td>(-35)</td>
<td>(-64)</td>
</tr>
<tr>
<td>Clozapine (5)</td>
<td>413.2± 25.0 a</td>
<td>1326.06± 75.0 a</td>
<td>4208.5± 275.0 a</td>
</tr>
<tr>
<td></td>
<td>(-19.6)</td>
<td>(-20.5)</td>
<td>(+265)</td>
</tr>
<tr>
<td>Risperidone(2)</td>
<td>436 ± 32.2 a</td>
<td>1203.29 ± 32.2 a</td>
<td>3781.8± 332.2 abc</td>
</tr>
<tr>
<td></td>
<td>(-15)</td>
<td>(-27.8)</td>
<td>(+228)</td>
</tr>
</tbody>
</table>

P ≤ 0.05 as compared to a control group, b clozapine group, and c risperidone group

[Values are mean± S.E. from 6 animals in each group. Figures in parentheses are % increase (+) or decrease (-) over control]
Discussion
Chronic administration of haloperidol and chlorpromazine is associated with increase in orofacial hyperkinetic movements (VCM’s, tongue protrusions and facial jerking) and that of atypical antipsychotics such as clozapine and risperidone are associated with low prevalence of extrapyramidal side effects. Multi-receptor action of atypical neuroleptics is supposed to play a role in these side effects. In the present study the neurochemical levels of dopamine, norepinephrine and serotonin were estimated in the rat forebrain after the chronic administration of typical and atypical neuroleptics and their relationship with development of chronic extrapyramidal side effect studied. The main result of the present study is that chronic administration of typical neuroleptics (haloperidol and chlorpromazine) led to significant increase in orofacial hyperkinetic movements which is associated with significant decrease in serotonin, dopamine and norepinephrine levels where as atypical antipsychotics showed less prevalence of extrapyramidal side effects. Similarly, clozapine and risperidone showed decrease in dopamine and norepinephrine levels but that was significantly less than the decrease in typical antipsychotics. Atypical antipsychotics showed an increase in the levels of serotonin in the extracellular space.

Chronic administration of neuroleptics are associated with proliferation of dopamine 2 receptors in caudate putamen and nucleus accumbens, selective tissue and neuronal increase in dopaminergic receptor site, increase in dopamine receptor binding as evident by increase in Bmax, increase in striatal D2 mRNA expression. Also chronic blockade of dopamine D2 inhibitory receptor located in the glutamatergic terminals in the striatum leads to persistent and enhanced release of glutamate that damages the striatal output neurons resulting in increased orofacial movements and oxidative damage which are the hallmark of tardive dyskinesia. Haloperidol and chlorpromazine showed much more decrease in dopamine levels as well much more increase in hyperkinetic movements suggesting that there is a relationship between decrease in extracellular levels of dopamine after chronic administration of neuroleptics and development of tardive dyskinesia. As decrease in dopamine levels were comparatively less in atypical antipsychotics they have less prevalence of extrapyramidal side effects.

Alteration of serotonergic transmission has long lasting influence on the activity of glutamatergic system. It results into persistent increase in the extra cellular glutamate levels. Glutamatergic system has major control on the striatal output neurons and therefore, on the basal ganglia function. The serotoninergic input has been shown to make direct synaptic contact with dopaminergic neurons in both substantia nigra and ventral tagmental area, areas with the major involvement in the movement disorders/control. Stimulation of dorsal raphe serotoninergic fibres releases serotonin in the substantia nigra and this is associated with the decrease in firing of dopaminergic neurons. Stimulation of dorsal raphe serotoninergic fibres releases serotonin in the substantia nigra and this is associated with the decrease in dopamine related behaviours. Serotonin depletion as resulted by haloperidol and chlorpromazine results into increase in the extracellular levels of glutamate further resulting into down regulation of glutamate transport expression. As glutamate and glutamate transporters play an important role in the development of tardive dyskinesia, results of the present study suggested the possible involvement of serotonin and glutamate interaction in the pathology of this debilitating motor dysfunction.

Recent data suggest that, 5HT may help in neuronal preservation and indeed have a neuroprotective action in the striatum and other brain areas. Apart from its neuromodulatory action, 5HT may have a major role in the preservation of the structural organization of CNS especially in neurodegenerative diseases such as tardive dyskinesia. Haloperidol and chlorpromazine showed decrease in serotonin levels as well as increase in hyperkinetic movements. Atypical antipsychotics increase the level of serotonin after chronic administration suggesting the possible involvement of serotonin in the development of tardive dyskinesia supported by neuroprotective effect of serotonin.

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. Recent receptor binding studies have confirmed that with the chronic administration of haloperidol increases the receptor binding sites as well as the Bmax of adrenergic receptors which was not seen in case of chronic
administration of clozapine suggesting the possible involvement of this in the pathophysiology of tardive dyskinesia. In the present study extracellular concentration of norepinephrine was decreased with the chronic administration of haloperidol and chlorpromazine where as in atypical anti-psychotics the decreases in NE levels were not significant as compared to typical one.

It is widely accepted that in tardive dyskinesia, glutamatergic transmission has been increased and high concentration of glutamate lead to oxidative damage as well as neuronal degeneration. Glutamatergic transmission has been modulated by the concentration of dopamine, serotonin and norepinephrine in the extracellular regions of brain. Hence, with change in the concentration of these neurotransmitters, typical neuroleptics either directly through themselves or indirectly via glutamate transmission result into increased orofacial hyperkinetic movements and oxidative damage, hallmark of tardive dyskinesia which can be prevented by atypical anti-psychotics as they resulted into less decrease in dopamine and norepinephrine levels and increase in serotonin levels suggesting prominent role of serotonin in preventing extrapyramidal side effects.

In conclusion this study provides an insight into neurochemical changes associated with tardive dyskinesia and suggests the development of therapeutic options based on these.

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References
7 Hauber W, Involvement of basal ganglia transmitter systems in movement initiation, Prog Neurobiol, 56 (1998) 507.