CONTENTS

Editorial

Pharmacologists of India: Shiv Prakash

Review Article

Therapeutic alternatives from venoms and toxins: Shivaji P. Gawade

Research Articles

Genotoxic evaluation of morphine, buprenorphine, pentazocine, and noscapine by micronucleus and comet assay in albino mice: Lakshman Kumar Puli, P. A. Patil

Age-related susceptibility to chronic haloperidol-induced orofacial dyskinesia: Biochemical and neurochemical evidence: Mahendra Bishnoi, Kanwaljit Chopra, Shrinivas K. Kulkarni

Effect of amlodipine on blood and aortic tissue concentration of endothelin in male rabbits receiving atherogenic diet: M. Mohammadi, F. Mirzaei, Reza Badalzadeh


Gastrointestinal permeability studies using combinations of rifampicin and nucleoside analogue reverse transcriptase inhibitors in rats: T.T. Mariappan, Saranjit Singh

Effects of meloxicam and rofecoxib on psychomotor performance: A randomized, double-blind, placebo-controlled cross-over study: Marwan S.M. Al-Nimer

Non-invasive evaluation of arterial stiffness in patients with increased risk of cardiovascular morbidity: A cross-sectional study: Yashmaina Sridhar, M.U.R. Naidu, P. Usharani, Y.S.N. Raju

Serum glucose and triglyceride lowering activity of some novel glitazones against dexamethasone-induced hyperlipidemia and insulin resistance: B.R. Prashantha Kumar, T.K. Praveen, M.J. Nanjan, M.D. Karvekar, B. Suresh

Workshop Report

The basic concepts of scientific research and communication: (A Report on Preconference Workshop Held in Conjunction with the 40th Annual Conference of the Indian Pharmacological Society-2007): Pitchai Balakumar, Sreekant Murthy, Gowraganahalli Jagadeesh

Author Index, 2007

Title Index, 2007

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Age-related susceptibility to chronic haloperidol-induced orofacial dyskinesia: Biochemical and neurochemical evidence

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ABSTRACT

Objective: Aging is a continuous and intrinsic process of systems deterioration with time. Although the mean prevalence of tardive dyskinesia (TD), a neurological disorder associated with chronic haloperidol administration, is 20-25%, the cumulative rate of this disorder increases significantly in patients aged 55 years or more. The present study investigated the effect of aging on spontaneous development of orofacial dyskinesia and tried to find out the interactive substrate which is activated by chronic neuroleptic treatment and results in premature emergence of TD in adult animals.

Materials and Methods: Various behavioral (orofacial dyskinetic movements, stereotypy, locomotor activity, and percent retention); biochemical (lipid peroxidation, reduced glutathione levels, and antioxidant enzyme levels: SOD and catalase); and neurochemical (neurotransmitter levels) parameters were assessed in young (60-80 gm), matured adult (180-200 gm), and aged (380-400 gm) rats.

Results: Aging resulted in significant increase in hyperkinetic motor activities, vacuous chewing movements (VCMs), tongue protrusions, facial jerking, and development of dopamine supersensitivity (increased locomotor activity and stereotypy); there was also associated oxidative damage in all regions of the brain. The extracellular dopamine levels were also significantly decreased (45%) in the forebrain of aged animals. Chronic administration of haloperidol to aged animals further significantly increased all the parameters as compared to age-matched control animals. Chronic administration of haloperidol to matured adult animals showed similar changes, especially hyperkinetic movements, and oxidative damage in different parts of the brain. There was no significant change in young animals on chronic administration of haloperidol.

Conclusion: The findings of the present study suggest that free radical generation and development of dopamine supersensitivity are the prime interactive substrates that are activated by chronic neuroleptic treatment in matured animals and are responsible for the development of TD, whereas these paradigms are increased with aging and result in spontaneous orofacial hyperkinetic movements.

KEY WORDS: Aging, hyperkinetic, neurochemical, neurological disorders

Tardive dyskinesia (TD) is a hyperkinetic movement disorder that involves involuntary choreoathetoid repetitive movements, such as chewing and tongue protrusions; it usually involves the face, mouth, and tongue.[1,2] While the mean prevalence of TD is 20-25%, the cumulative rates of TD increase significantly with age, with patients above the age of 55 showing greater incidences.[1] The prevalence and status of spontaneous dyskineticias, clinically defined as hyperkinetic movements of no apparent cause, remain controversial in aging. Spontaneous dyskineticias in aged humans and animals suggest that the aging process involves a gradual loss of critical neuromodulatory factors in the motor regions of the brain.

The pathophysiological basis of TD remains unclear but several lines of evidence suggest that the neuronal changes in the basal ganglia produced by increased oxidative stress and glutamate excitotoxicity may play a role, especially in elderly.[3,4] The free radical hypothesis of aging asserts that the generation and accumulation of reactive oxygen species (ROS) increases with aging, which results in oxidative damage to critical biological molecules.[5-7] Similarly, other studies have reported that orofacial movements increased with aging.[8] The free radical hypothesis might offer a satisfactory explanation for the increase in TD with aging. Chronic administration of haloperidol stimulates glutamate transmission as well as inhibits glutamate uptake in different regions of the brain. This results in elevation of striatal ROS in vivo. The increase in ROS and glutamatergic...
activity lead to excitotoxicity and increased degeneration of neurons. Although no single explanation is enough for the pathological basis of the development of TD, different collective pathologies, such as receptor system abnormalities (dopamine receptor hypersensitivity, GABAergic loss in the striatum, and loss of cholinergic neurons), have been implicated.

Considering the importance of the possible role of aging in the development of orofacial hyperkinetic movement disorders and associated behavioral, biochemical, and neurochemical changes, we studied the effect of chronic administration of haloperidol in rats of different age-groups and investigated the possible inter-mechanistic relationship between aging and chronic neuroleptic treatment in the development of dyskinetic movements.

Materials and Methods

Animals
Male Wistar rats of three age-groups—young (60-80 gm), matured adult (180-200 gm), and aged (360-400 gm)—bred in the Central Animal House facility of Panjab University were used. The animals were housed under standard laboratory conditions, maintained on a 12:12 light-dark cycle, and had free access to food and water. The 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 h. The experimental protocols were approved by the institutional animal ethics committee and the study was conducted according to the guidelines of the Indian National Science Academy for the use and care of experimental animals.

Drugs and treatment schedule
The animals were divided in 6 groups: the first group (young) received vehicle, the second group (young) received haloperidol (1 mg/kg) plus vehicle, the third group (matured adult) received vehicle, the fourth group (matured adult) received haloperidol (1 mg/kg), the fifth group (aged) received vehicle, and the sixth group (aged) received haloperidol (1 mg/kg). Haloperidol (Serenace, Searle India, India) was diluted with distilled water. Haloperidol was administered intraperitoneally in a constant volume of 0.5 ml per 100 gm of bodyweight of rat. Haloperidol was administered once daily (0900) in the morning for a period of 21 days and behavioral assessments were done every week before drug administration. The final behavioral assessment was done 24 h after the last dose. Drug doses were based on our previous studies and also as reported in the literature.

Induction of orofacial dyskinesia
Haloperidol (1 mg/kg, i.p.) was administered chronically to rats for a period of 21 days to induce oral dyskinesia. All the behavioral assessments were carried out every week, and the last behavioral quantification was done 24 h after the last dose of haloperidol.

Behavioral assessment
The animals were behaviorally assessed for orofacial dyskinetic movements followed by assessment of memory and locomotor activity. On the test day, the rats were placed individually in a small (30 × 20 × 30 cm) Plexiglas cage for the assessment of oral dyskinesia. The behavioral 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. Locomotor activity was monitored using a photoactometer (IMCROP, India). The elevated plus maze was used to evaluate spatial long-term memory, following the procedure described in our earlier study/article. Percent retention was calculated by the formula:

\[
\text{Percent retention} = \frac{\text{transfer latency (day 1)} - \text{transfer latency (day 2)}}{\text{transfer latency (day 2)}} \times 100
\]

Biochemical assessment
On day 22, after behavioural quantification, the animals were sacrificed by decapitation. The brains were removed, the forebrain was dissected out and the cerebellum was discarded. The brains were put on ice and the cortex, striatum, and subcortical regions were separated and weighed. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). Whole brains (excluding the cerebellum) from each group were separately stored at -80°C for HPLC studies. The homogenates were centrifuged for 20 min at 15000 rpm and the supernatant was used for estimation of lipid peroxidation and reduced glutathione levels. The postnuclear fractions for catalase assay were obtained by centrifugation of the homogenate at 1000 g for 20 min at 4°C and for other enzyme assays, by centrifugation at 12,000 g for 60 min at 4°C. The subcortical region of the brains comprised all the remaining parts of the forebrain, including the hippocampus, thalamus, hypothalamus, amygdala, and other subthalamic structures. The quantitative measurements of lipid peroxidation and reduced glutathione in the forebrain were performed according to the methods of Wills and Ellman, respectively. The antioxidant enzymes, SOD and catalase, were assayed according to Kono and Luck, respectively. The protein content was measured according to the method of Lowry using bovine serum albumin as standard.

Neurotransmitter estimation
Biogenic amine (dopamine) was estimated by HPLC with an electrochemical detector by the method of Church. Waters standard system, consisting of a high-pressure isocratic pump, a 20 µl sample injector valve, C18 reversed-phase column, and an electrochemical detector, was used. Mobile phase consisted of 2% citric acid, 2% KHPO4, 1 mM EDTA, 1.2% MeOH, and 70 mg/ml of sodium octyl sulphate. The pH of the mobile phase was adjusted to 3 with the help of HCl (6N). Electrochemical conditions for the experiment were +0.800 V, with 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 the experiment, the forebrain frozen samples were taken out and thawed. They were homogenized in homogenizing solution containing 0.1 M perchloric acid. The samples were then centrifuged at 12000 g for 15 min. The supernatant was further filtered through 0.25 micron nylon filters before being injected into the HPLC injection pump. Data was recorded and analyzed with the help of Empower software.

Statistical analysis
One specific group of rats was assigned to one specific
drug treatment condition; each group comprised six rats \((n = 6)\). All the values are expressed as means ± SEM. The data were analyzed by using analysis of variance (ANOVA) followed by Tukey’s test. In all tests, \(P < 0.05\) was considered as statistically significant.

Results

Behavioral assessment

**Assessment of orofacial dyskinesia:** All the hyperkinetic orofacial movements (VCMs, tongue protrusion, and facial jerking) were found to be significantly increased with aging. Chronic administration of haloperidol (1 mg/kg, i.p.) resulted in time-dependent increase in VCMs, tongue protrusion, and facial jerking in all the age-groups, with similar effects in matured and aged animals. Although young animals also showed increases in these movements, it was significantly less than in matured and aged animals. Onset of severe hyperkinetic orofacial movements was most rapid in aged animals followed by matured animals [Figure 1 a-c].

![Figure 1: (a) Vacuous chewing movements (VCMs), (b) tongue protrusions, (c) facial jerking recorded on day 7, 14, and 22 (test day) in rats chronically treated with vehicle (matured animals), haloperidol (1 mg/kg, i.p., 21 days) (matured animals), vehicle (young animals), haloperidol (1 mg/kg, i.p., 21 days) (aged animals). Data is expressed as mean ± SEM. *\(P \leq 0.05\) as compared to control matured group (on the day of behavioral assessment); †\(P \leq 0.05\) as compared to control young group (on the day of behavioral assessment); ‡\(P \leq 0.05\) as compared to control aged group (on the day of behavioral assessment); ‡‡\(P \leq 0.05\) as compared to haloperidol treated matured group (on the day of behavioral assessment); ‡§\(P \leq 0.05\) as compared to haloperidol treated young group (on the day of behavioral assessment)
**Locomotor activity:** Total locomotor activity (ambulatory and rearing) was observed to be significantly higher in aged as compared to young and matured adult animals. Haloperidol (1 mg/kg, i.p.) treatment decreased the total locomotor activity (ambulatory and rearing) up to the 14th day; thereafter, it increased at the time of the last behavioural quantification in both matured and aged animals, but not in young animals, suggesting the development of supersensitivity in matured and aged animals [Figure 2a].

**Elevated plus maze test:** Aging resulted in significant decrease in percent retention time on elevated plus maze paradigm. Chronic haloperidol (1 mg/kg, i.p.) treatment resulted in further decrease in percent retention in matured animals as compared to age-matched control matured animals; however, there was no change in the percent retention in the case of young and aged animals as compared to their age-matched controls [Figure 2b].

**Biochemical assessment**

**Lipid peroxidation assay:** Lipid peroxidation was increased in all the three regions of the brain with aging. Chronic haloperidol treatment (1 mg/kg, i.p.) further resulted in significant increase in lipid peroxidation in both the regions

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**Figure 2:** (a) Total locomotor activity recorded before drug administration (base line), day 7, 14, and 22 (test day) (b) percent retention recorded on day 22 (test day) in rats chronically treated with vehicle (matured animals), haloperidol (1 mg/kg, i.p., 21 days) (matured animals), vehicle (young animals), haloperidol (1 mg/kg, i.p., 21 days) (young animals), vehicle (aged animals), haloperidol (1 mg/kg, i.p., 21 days) (aged animals). Data is expressed as mean ± SEM. *P ≤ 0.05 as compared to control matured group (on the day of behavioral assessment); †P ≤ 0.05 as compared to control young group (on the day of behavioral assessment); ‡P ≤ 0.05 as compared to control aged group (on the day of behavioral assessment); §P ≤ 0.05 as compared to haloperidol treated matured group (on the day of behavioral assessment); §P ≤ 0.05 as compared to haloperidol treated young group (on the day of behavioral assessment)
(cortex and striatum) of the brain but not in the subcortical regions in all the three age-groups as compared to the respective age-matched control animals. The maximum increase in lipid peroxidation was found in aged animals [Figure 3a].

**Estimation of reduced glutathione:** Reduced glutathione was significantly decreased with aging in the striatum and subcortical regions but not in the cortical region. Chronic haloperidol treatment (1 mg/kg, i.p.) resulted in significant decrease in reduced glutathione in both the regions (cortex and striatum) of the brain, but not in the subcortical regions, as compared to the respective control animals [Figure 3b].

**Catalase activity:** Catalase enzyme activity was significantly decreased in all the three regions of the brain with aging. Chronic haloperidol treatment (1 mg/kg, i.p.) resulted in further significant decrease in catalase in all the three regions (cortex, subcortical region, and striatum) of the brain of matured animals and in the subcortical region of aged animals as compared to the respective control animals [Figure 3b].

**Superoxide dismutase (SOD) activity:** SOD activity was decreased only in the cortical region with aging. Chronic haloperidol treatment (1 mg/kg, i.p.) resulted in significant decrease in SOD in the cortex and the striatal regions of the brain in all the three age-groups of animals as compared to the respective control animals [Figure 3c].

**Neurotransmitter estimation:** Aging resulted in significant decrease in dopamine levels in the forebrain. Chronic administration of haloperidol resulted in further decrease in the levels of dopamine in the extracellular spaces of aged animals as well as in matured animals; however, a slight decrease in the dopamine level was observed in young animals [Table 1].

**Discussion**

Age-related physiological changes make older persons more sensitive to the therapeutic and toxic effect of antipsychotics. There is a paucity of controlled studies on the efficacy of antipsychotics and their side effects, especially extrapyramidal side effects, in older persons.[17] The results of the present study indicate that age amplifies the incidence of VCMs, tongue protrusions, and facial jerking, as well as other behavioral parameters, in animals. Age also resulted in increased lipid peroxidation, decreased glutathione levels, and decreased antioxidant enzyme levels in different brain regions; these are all parameters of oxidative stress. Several studies have indicated that orofacial dyskinesia increases with aging and the associated increased oxidative damage.[10-20] Cumulative free radical damage is connected with aging in the brain and it can further increase with chronic administration of haloperidol.
significantly in aged and matured animals. In our study, administration of haloperidol in all the age-groups, but most parameters; these effects were potentiated by the chronic orofacial dyskinesia and associated behavioral and biochemical study confirmed a significant action of aging on spontaneous movement. Of particular importance was the effect of aging on the different behavioral, biochemical, and neurochemical parameters in animals of different age-groups. Further, we studied the effect of chronic haloperidol treatment on animals of different age-groups. Of particular importance to the free radical hypothesis of TD, the results of the present study confirmed a significant action of aging on spontaneous orofacial dyskinesia and associated behavioral and biochemical parameters; these effects were potentiated by the chronic administration of haloperidol in all the age-groups, but most significantly in aged and matured animals. In our study, it was found that aging increases the severity as well as the likelihood of development of TD. Haloperidol-treated aged animals showed significant increase in the severity on day 7, whereas in young and matured animals, though the onset was on day 7, the severity of the hyperkinetic orofacial movements was much less as compared to aged animals. Twenty-four hours after the last dose of haloperidol, the VCMs, tongue protrusions, and facial jerking were significantly higher in all the three treated groups as compared to controls, but it was much higher in aged and matured adult animals than in the young animals. Here, it can be argued that there is a possible neurobiological mechanism which is initiated in aged animals in the first week of the treatment, but in case of matured adults and young animals, it took some time to initiate the damage. Biological aging is associated with oxidative damage, and aged animals, due to increased free radical formation and decreased levels of antioxidant enzymes, are in a compromised state, which leads to the early onset of hyperkinetic movements. It is also relevant clinically, because it is well studied that aged patients are more prone to TD.

We also studied different oxidative damage markers such as lipid peroxidation, reduced glutathione levels, and different antioxidant enzyme (catalase and SOD) levels. lipid peroxidation was increased with aging, whereas the other oxidative damage parameters were decreased significantly. Lipid peroxidation was increased with aging in all the three regions of the brain, with the maximum effect seen in the striatal region. Reduced glutathione was significantly decreased in the subcortical regions with aging. Catalase enzyme activity was significantly decreased in all the three regions of the brain, whereas in the case of SOD it was significantly decreased in the cortical regions as age progressed. On chronic administration of haloperidol, lipid peroxidation increased significantly in the cortical as well as the striatal regions in both matured adult and aged animals, but in case of young animals there was no significant change observed in any of the regions. After chronic administration of haloperidol, reduced glutathione was also significantly decreased in the striatal and cortical regions, but not in the subcortical, in aged as well as matured adult and young animals. Chronic haloperidol administration resulted in a further significant decrease in catalase and SOD levels in aged animals. After chronic administration of haloperidol, the levels of SOD were decreased in young and matured animals, whereas catalase levels were significantly decreased in matured animals but not in young animals. Previous reports have indicated that out of the different monoamine systems the dopamine system is especially vulnerable to aging, whereas those of norepinephrine and 5-HT are more resistant. Aging results in decreased levels of dopamine in the extracellular spaces in different regions. On chronic haloperidol administration, dopamine receptor levels in different brain regions increased significantly, resulting in increased dopamine sensitivity and decreased dopamine turnover in the extracellular spaces. There might be a correlation between chronic haloperidol administration and aging, as there is significant decrease in dopamine levels in extracellular spaces with aging. This is also supported by our study as there was a 40% decrease in dopamine levels in aged animals when compared to matured animals, which was further increased to 65% with the chronic administration of haloperidol. On chronic administration of haloperidol, dopamine levels were decreased up to 35–40% in matured adult as well as young animals. Aging also resulted in a decrease in percent retention, which was further accentuated by chronic administration of haloperidol. Chronic administration of haloperidol resulted in significant decrease in percent retention in both young as well as matured adult animals.

In conclusion, the aging process leads to increased

**Table 1**

<table>
<thead>
<tr>
<th>Treatment (mg/kg i.p.)</th>
<th>Dopamine (pg/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (matured)</td>
<td>550 ± 45.2</td>
</tr>
<tr>
<td>Control (young)</td>
<td>643.2 ± 36.2</td>
</tr>
<tr>
<td>Control (aged)</td>
<td>402.5 ± 44.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Haloperidol (matured) (1)</td>
<td>382.4 ± 15.0&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Haloperidol (young) (1)</td>
<td>484.2 ± 22.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Haloperidol (aged) (1)</td>
<td>302.1 ± 22.2&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>P ≤ 0.05 as compared to control matured group (on the day of behavioral assessment); <sup>b</sup>P ≤ 0.05 as compared to control young group (on the day of behavioral assessment); <sup>c</sup>P ≤ 0.05 as compared to control aged group (on the day of behavioral assessment); <sup>d</sup>P ≤ 0.05 as compared to haloperidol treated matured group (on the day of behavioral assessment); <sup>e</sup>P ≤ 0.05 as compared to haloperidol treated young group (on the day of behavioral assessment)

In conclusion, the aging process leads to increased...
dopamine sensitivity and increased generation of free radicals. Both are important pathophysiologic causes for the initiation of TD and perhaps important reasons why spontaneous orofacial hyperkinetic movements are characteristic of old age. Chronic administration of haloperidol in matured adult animals resulted in the same pathophysiologically changes and aggravated them in aged animals.

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This study was supported by the UGC grant under Centre with Potential for Excellence in Biomedical Sciences (CPEBS). Mahendra Bishnoi is a project associate.

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