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Differential striatal levels of TNF-α, NFκB p65 subunit and dopamine with chronic typical and atypical neuroleptic treatment: Role in orofacial dyskinesia

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ABSTRACT

Long term use of typical neuroleptics such as haloperidol may be limited by unwanted motor side effects like tardive dyskinesia characterized by repetitive involuntary movements, involving the mouth, face and trunk. Atypical neuroleptics, such as clozapine and risperidone are devoid of these side effects. However the precise mechanisms of the neuronal toxicity induced by haloperidol are poorly understood. It is possible that typical and atypical antipsychotic differently affects neuronal survival and death and that these effects considerably contribute to the differences in the development of TD. The aim of the present study is to investigate the role of TNF-α and NFκB on the toxicity induced by chronic haloperidol administration in an animal model of tardive dyskinesia. Rats were treated for 21 days with: haloperidol (5 mg/kg), clozapine (5 and 10 mg/kg), risperidone (5 mg/kg) or saline. Orofacial dyskinetic movements and total locomotor activity was evaluated. Striatal levels of dopamine were measure by HPLC/ED whereas striatal levels of TNF-α and NFκB p65 subunit were measured by ELISA technique. Haloperidol increased orofacial dyskinetic movements and total locomotor activity (on day 22) ($P \leq 0.05$). Clozapine and risperidone also increased the orofacial dyskinetic movements but that significantly less than haloperidol ($P \leq 0.05$). Differential effect of haloperidol and atypical neuroleptics on striatal dopamine levels and striatal levels of TNF-α and NFκB p65 subunit was found out. Haloperidol significantly decreased the striatal dopamine levels whereas clozapine and risperidone did not. Haloperidol but not clozapine and risperidone significantly increased the levels of TNF-α and NFκB p65 subunit ($P \leq 0.05$). The present study suggests the impossible involvement of striatal TNF-α and NFκB p65 subunit in haloperidol-induced orofacial dyskinesia in rats, an animal model for human tardive dyskinesia.

1. Introduction

Neuroleptic drugs are used in the treatment of psychiatric disorders especially schizophrenia. However their long term use may be limited by unwanted motor side effects like tardive dyskinesia, Parkinsonism and akathisia (Kane, 1995). TD is characterized by repetitive involuntary movements, involving the mouth, face and tongue and sometimes, limb and trunk musculature (Casey, 1993, 2000; Tsai and Coyle, 2002). A peculiar characteristic of TD syndrome is that TD generally persist after haloperidol withdrawal and occasionally becomes irreversible indicating that the typical antipsychotic, haloperidol has produced long lasting changes in brain function that are no longer related to the presence of the drug (Meshul et al., 1992; Meshul and Tan, 1994).

The molecular mechanism related to the pathophysiology of tardive dyskinesia is not completely known. Increase in the density of striatal dopaminergic D_2 receptors observed in humans and in experimental rodent models of tardive dyskinesia coincide with the appearance of extrapyramidal side effects (Klawans and Rubovits, 1972). Various other receptor level hypotheses have been proposed, including development of dopaminergic receptor supersensitivity (Burt et al., 1977), a dopamine D_1/D_2 receptor imbalance (Casey, 1995; Waddington, 1997; Ikeda et al., 1999), a disturbed balance between dopaminergic and cholinergic system and dysfunction of Gamma Amino Butyric Acid (GABA) neurons (Gale, 1980). The participation of free-radicals derived from the metabolism of dopamine and/or from an enhancement of the glutamatergic transmission, secondary to presynaptic dopamine receptors blockade has gained ample experimental support (Casey, 2000; Kulkarni and Naidu, 2001; Tsai et al., 1998). Various animal studies have demonstrated an enhancement to glutamatergic participation as well as inhibition in the glutamate uptake after the chronic administration of haloperidol (Burger et al., 2005a). High density of serotonin receptors in the basal ganglia region and their interaction with the dopaminergic system has led to the hypothesis that serotonin might play role in movement

Abbreviations: TD, Tardive Dyskinesia; TNF-alpha, Tumor Necrosis Factor alpha; NFκB, Nuclear Factor Kappa Beta; HPLC, High Performance Thin Layer Chromatography; ED, Electrochemical Detector; ELISA, Enzyme Linked Immuno Sorbent Assay; DAT, Dopamine Transporter; TH, Tyrosine hydroxylase; VCM, Vacuous chewing movements; NMDA, *N*-Methyl-D-Aspartate; TP, Tongue protrusions; FJ, Facial jerkings.

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disorders associated with basal ganglia (Cara et al., 2001; Prehn et al., 1993; Semkova et al., 1998).Chronic administration of typical neuroleptics produced significant decrease in the levels of serotonin in different brain regions (Bishnoi et al., 2007a,b).

Recently the emphasis on experimental models of TD studies has changed from neurotransmitter model to an approach that considers haloperidol-induced TD as a consequence of a direct neurotoxic effect mediated by haloperidol itself (Andreassen and Jorgensen, 2000). However the precise mechanisms of the neuronal toxicity induced by haloperidol are poorly understood. It is possible that typical and atypical antipsychotic differently affects neuronal survival and death and that these effects considerably contribute to the differences in the development of TD.

Lines of evidence point to a role of decreased mitochondrial respiration and oxidative stress in haloperidol-induced toxicity (Cadet and Kahler, 1994). Haloperidol has been shown to induce the expression of TNF-α and NFκB, cellular factors which responds directly to oxidative stress (Post et al., 1998, 2002). NFκB p65 subunit is crucially involved in haloperidol-induced toxicity (Saldaña et al., 2006). It is supposed that these cellular factors are the mediators of the neuronal death pathway induced by haloperidol but their exact role still remains unknown.

The aim of the present study is to investigate the role of TNF-α and NFκB on the toxicity induced by chronic haloperidol administration in an animal model of tardive dyskinesia.

2. Material and methods

2.1. Animals

Male Wistar rats (180–220 g; 10–12 rats/group) bred in the Central Animal House facility of Panjab University were used. The animals were housed under standard laboratory conditions, maintained on a normal light–dark cycle and free access of 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 h. 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.

2.2. Drug treatment

Haloperidol (Serenace (Ampoule), Searle, Mumbai, India) (5 mg/kg), and risperidone (Sigma-Aldrich, St. Louis, MO, USA) (5 mg/kg) were dissolved in distilled water Clozapine (Sun Pharmaceuticals, Mumbai, India) (5 and 10 mg/kg) was dissolved in few droops of 0.1 M HCl (pH = 1–2) and volume was make up with distilled water. All the drugs were administered intra-peritoneally in a constant volume of 0.5 ml per 100 g of bodyweight of rat once daily at 0900 for a period of 21 days. Behavioural assessments were done 24 h after the last dose (Naidu et al., 2003).

2.3. Behavioural studies

2.3.1. Orofacial dyskinetic movements

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 given 10 min to get acclimatized to the observation chamber before behavioural assessments. To quantify the occurrence of oral dyskinesia, hand operated counter was used employed to score number of tongue protrusions, facial jerkings 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 or 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. The observation chamber has mirrored flooring and the back wall of the chamber also has a mirror so that no observation is missed when the animal was faced away from the observer. The behavioural parameters of oral dyskinesia were recorded continuously for a period of 5 min. In all the experiments, the scorer was unaware of the treatment given to the animals (Naidu et al., 2003).

2.3.2. Total locomotor activity

Total locomotor activity (ambulatory and rearing) was monitored using activity meter (IMCORP, India). Animal were individually placed in activity meter and the total activity count was registered for 5 min. The locomotor activity was expressed in terms of total photo beam counts/5 min per animal (Reddy and Kulkarni, 1998).

2.4. Dissection and biochemical studies

After behavioural assessment, animals were divided in two groups and sacrificed. Striatum was dissected out and weighed. A 10% (w v^{-1}) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). Cytoplasmic and nuclear fractions were prepared for the quantification of striatal TNF-α and NFκB active p65 subunit respectively. In another set of animals, the brains were removed; striatum was dissected out and was stored at −80 °C for HPLC studies for the estimation of dopamine. The quantification of TNF-α in rat striatum was done by the help and instructions provided by R&D Systems Quantikine Rat TNF-α immunoassay kit (Guerin-Marchand et al., 2001). The NF-κB/p65 ActivELISA (Imgenex, San Diego, USA) kit was used to measure NF-κB free p65 in the nuclear lysate of the rat striatum (Schaaf et al., 2006). The nuclear levels of p65 may correlate positively with the activation of NF-κB pathway. Protein content was estimated by the help of Lowry (1951).

2.5. Striatal dopamine levels

Dopamine was estimated by HPLC with electrochemical detector (ED). Waters standard system consisting of a high pressure isocratic pump, a 20 µl sample injector valve, C18 reverse phase column (type: Waters symmetry C18 (5 µm); length and diameter: 4.6×250 mm) and electrochemical detector were used. Data was recorded and analyzed with the help of Empower software. Mobile phase consisting of 2% citric acid, 2% $KHPO_4$, 1 mM EDTA, 1.2% MeOH, and 70 mg/ml of sodium octyl sulphate. pH 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. Samples (20 µl) were injected manually. On the day of experiment, frozen samples were thawed and they were homogenized in homogenizing solution containing 0.1 M perchloric acid. After that the samples were centrifuged at 12,000 ×g for 5 min. The supernatant was further filtered through 0.25 µm nylon filters before injecting in the HPLC injection pump. Data was recorded and analyzed with the help of empower software (Church, 2005; Bishnoi et al., 2008).

2.6. Statistical analysis

All the values are expressed as mean ± S.E.M. The data was analyzed by using analysis of variance (ANOVA) followed by Dunnett's test. In all tests, the criterion for statistical significance was $P \leq 0.05$. Pearson correlation test was used to correlate two groups. The criterion for significance was $P \leq 0.001$.

3. Results

3.1. Behavioural studies

Chronic administration of haloperidol (5 mg/kg, i.p. for 21 days) resulted in significant ($P \leq 0.05$) increase in orofacial dyskinetic