Dopamine and Serotonin Metabolism in the Dorsal and Ventral Striatum of Haloperidol-induced Tardive Dyskinesia Model in Rats

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Summary: The main candidate brain regions for abnormalities in schizophrenia have been striatum, frontal cortex and amygdala. Typical antipsychotics exert their effects via their actions in striatum. It is suggested that treatment with haloperidol consistently induce 70-80 % occupancy of the striatal D2 receptors. A serious side effect of long term use of traditional neuroleptic is the precipitation of tardive dyskinesia (TD). Long term administration of haloperidol produces vacuous chewing movements (VCMs) in rats. VCMs in animals following long term use of haloperidol is often taken as a model of tardive dyskinesia. Previous studies from our laboratory showed that long term administration of haloperidol increases the responsiveness of pre- and postsynaptic 5-hydroxytryptamine (5-HT; serotonin) -1A receptors. Therefore, the present study was aimed to monitor the metabolism of dopamine (DA) and serotonin (5-HT) in the dorsal striatum and ventral striatum of rats exhibiting TD. The results showed that repeated administration and withdrawal from haloperidol increases dopamine metabolism in both dorsal and ventral striatum. The levels of dopamine but not their metabolites were smaller in dorsal striatum of rats repeatedly injected with haloperidol. 5-HT metabolism increases in the dorsal striatum following withdrawal from haloperidol. The results suggest that increase in 5-HT metabolism particularly in dorsal striatum is involved in the inhibition of VCMs following haloperidol withdrawal in rats.

Introduction

Schizophrenia is a chronic, severe and disabling brain disease that affects approximately 1 % of the population. The symptoms of the illness are classified as positive symptoms (hallucination and delusions) and negative symptoms (apathy and social withdrawal). Although first generation of antipsychotics such as chloropromazine and haloperidol have been widely used for the treatment of schizophrenia but unfortunately, their use is often associated with a high incidence of extrapyramidal side effects (EPS) that include acute parkinsonism [1] and delayed tardive dyskinesia [2].

Tardive dyskinesia (TD) manifested by involuntary movements particularly in the oral lingual area [3] of rats chronically treated with typical neuroleptic such as haloperidol [4,5]. Vacuous chewing movements (VCMs) in rats are widely accepted as a rat model of TD. It has been shown that rats repeatedly treated with haloperidol develop VCMs [6]. It has been well established that blockade of dopaminergic neurotransmission results initially in Parkinsonism while sustained blockade of dopamine (DA) receptors after repeated haloperidol treatment results in DA receptor supersensitivity [7]. Therefore, TD may represent the DA hyperactivity in the striatum that accounts for increased output of abnormal behavior [8].

In addition to DA, serotonergic mechanisms are also involved in the elicitation of EPS. Recently, postmortem studies have shown that postsynaptic 5-HT-1A receptors are upregulated in the brain of schizophrenic patients [9]. Animal studies show that 5-HT-1A agonist could reverse haloperidol-induced catalepsy [10-11]. Neurochemical research on relationship between serotonin and motor activity showed that decrease in striatal serotonin metabolism following the administration of clozapine is involved in its ability to produce fewer EPS than haloperidol [12]. It is hypothesized that release of dopamine system from the inhibitory influence of serotonin following the stimulation of somatodendritic 5-HT-1A receptors could alleviate acute parkinsonian like

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effects of typical neuroleptics. Conversely, an increase in the responsiveness of somatodendritic and postsynaptic 5-HT-1A receptors following long term administration of haloperidol is involved in the elicitation of late-appearing dyskinesia [13-15].

Dorsal region of striatum is principally involved in motor control while ventral striatum is a region of brain known to be involved in the emotional control [16]. To understand the neurochemical involvement in the antischizophrenic effects of haloperidol and that in the precipitation of EPS, the present study was aimed to monitor the metabolism of dopamine and serotonin in the dorsal and ventral striatum respectively, of rats exhibiting VCMs following long term administration of haloperidol.

Results and Discussion

Intensity of VCMs in Rats Following 1, 2, 3 and 4 Weeks of Treatment with Haloperidol

The effects of haloperidol injections on vacuous chewing movements (VCMs) in rats are shown in Fig. 1. Two-way ANOVA showed that effects of drug administration ($F = 272.78$ df 1, 50 $p < 0.01$), repeated monitoring ($F = 17.04$ df 4, 50 $p < 0.01$) and interaction between two factors ($F = 28.25$ df 4, 50 $p < 0.01$) were significant. Posthoc analysis showed that the administration of haloperidol elicited VCMs 24 hours after the 7th, 14th, 21st and 28th but not after single injection. Animals repeatedly injected with haloperidol exhibited more VCMs 24 hours after 21st and 28th injection compared to chewing scores after 7th injection.

Several recent studies have indicated significant rhythmic variables in behavioral phenomena in the central nervous system (CNS) [17-19]. Marked daily rhythms in catalepsy response to single haloperidol administration have been also reported [20] suggesting the importance of dosing time to drug responses. The possible explanation for the changes in the haloperidol-induced behavioral responses is pharmacodynamics (DA receptor availability or sensitivity). Changes in hepatic metabolism of many drugs, with the greatest activity during mid-dark and the smallest activity during mid-light [21-22] have been also reported.

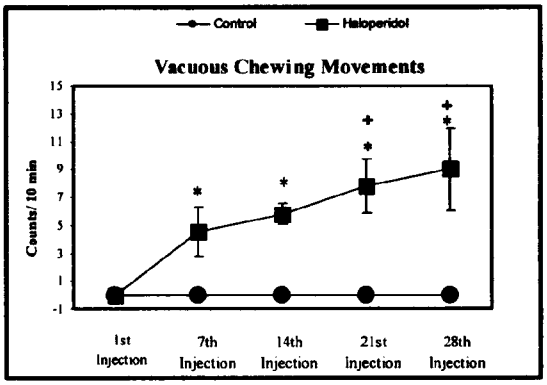

Fig. 1: Haloperidol-induced VCMs. Animals were injected with haloperidol or saline daily for 28 days and VCMs were monitored 22 hours after 1st, 7th, 14th, 21st and 28th injection. Values are means ± S. D. (n = 6). Significant differences by Newman-Keuls test. $*p < 0.01$ from respective day saline injected rats, $+ p < 0.01$ from 7th day value of similarly injected animals following two-way ANOVA.

Other authors have shown that under a 12: 12-h light/ dark cycle, there was a diurnal rhythm in the catalepsy responses to haloperidol [23]. The maximum and minimum catalepsy responses were detected at mid-light and mid-dark respectively. Therefore decreases in catalepsy responses may result from increases in the activity of rodent during the active period [20]. Repeated administration of haloperidol tends to produce tolerance in drug-induced EPS in rats [24]. Other authors have reported that greater development of tolerance in the cataleptic effects of haloperidol occur in the group of mice treated at 9:00 a.m. [23] *i.e.*, during the mid-light period. In the present study the behavioral and neurochemical effects of the drug and drug administration were therefore all performed during the mid-light period.

It is known that antagonism of DA receptors in the EPS region such as striatum result in the development of catalepsy, animal model of Parkinsonism. On the other hand DA receptors supersensitivity arising from the upregulation of DA D2 receptors following neuroleptic therapy accounts for the development of TD [25]. Other authors have reported that direct impairment of nigrostriatal

dopaminergic neurons act as a substrate for the occurrence of VCMs in rats [26]. We report that repeated administration of haloperidol for 4 weeks induces VCMs in rats. Our finding confirms those reported previously [27]. The increase in VCMs following repeated administration of haloperidol (Fig. 1) is explainable in terms of D2 receptor supersensitivity.

VCMs in Rats Following 4 Weeks of Treatment with Haloperidol and Withdrawal

Fig. 2 shows the effects of haloperidol injections and haloperidol withdrawal on VCMs in rats. ANOVA (df 2, 15) showed significant effects of haloperidol ($F = 36.06$ $p < 0.01$) on chewing movements. Posthoc comparisons showed that VCMs were elicited following repeated haloperidol administration as well as withdrawal from repeated administration. The intensity was greater following repeated administration than withdrawal from repeated administration.

Haloperidol induced VCMs were attenuated following withdrawal from repeated haloperidol administration (Fig. 2). Previously DA receptor supersensitivity alone was considered to be the explanation of TD [25]. Serotonergic neurons represent one another neurochemical system that prominently regulates oral activity behavior. The serotonergic system modulates the activity of dopaminergic neurons [28]. The nature of this modulation appears to be inhibitory. Pharmacological manipulations that tend to increase serotonergic functions diminish DA mediated behaviors or vice versa [29-30]. Animal studies show that repeated treatment of haloperidol increased responsiveness of somatodendritic as well as postsynaptic 5-HT-1A receptors [13-14]. An attenuation of VCMs following haloperidol withdrawal is explainable in terms of reversal of inhibitory influence of 5-HT on motor activity which appeared to decrease during repeated haloperidol treatment [14].

Effects of Repeated Haloperidol Administration and Haloperidol Withdrawal on Dopamine Metabolism in Rats

Fig. 3 shows the effects of haloperidol administration and haloperidol withdrawal on the levels of DA, DOPAC and HVA in the dorsal and ventral striatum. ANOVA (df2, 15) showed

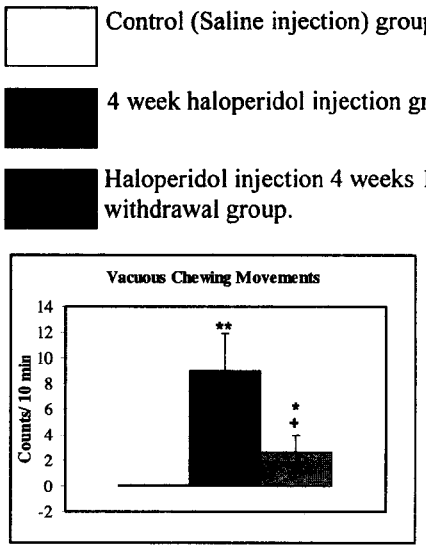

Fig. 2: Haloperidol-induced VCMs after 4 weeks haloperidol administration and 1 week drug withdrawal following 3 weeks haloperidol administration. VCMs were monitored 22 hours after 28 days of saline or haloperidol injection and 1 week after drug withdrawal following 21 days of haloperidol injections. Values are means $\pm$ S. D. ($n = 6$). Significant differences by Newman-Keuls test. $*p < 0.05$, $**p < 0.01$ from saline injected, $+p < 0.01$ from 4 week haloperidol injected animals following ANOVA.

significant effects on DA ($F = 12.24$ $p < 0.01$) and HVA ($F = 9.15$ $p < 0.01$) in the dorsal striatum. Effects on DOPAC ($F = 1.024$ $p > 0.01$) were not significant. Effects on the levels of DA ($F = 5.14$ $p < 0.05$) DOPAC ($F = 7.19$ $p < 0.01$) and HVA ($F = 5.16$ $p < 0.05$) in the ventral striatum were significant. Newman-Keuls test showed that repeated administration of haloperidol decreased DA levels in the dorsal striatum while withdrawal from haloperidol increased DA levels in both dorsal and ventral striatum. Haloperidol injections and withdrawal from haloperidol increased the concentration of HVA in the dorsal and ventral striatum. The levels of DOPAC increased following haloperidol withdrawal but not administration in the ventral striatum.

Dopamine is synthesized and released not only from the terminals of nigrostriatal dopaminergic pathway, but also from the dendrites in the cell body

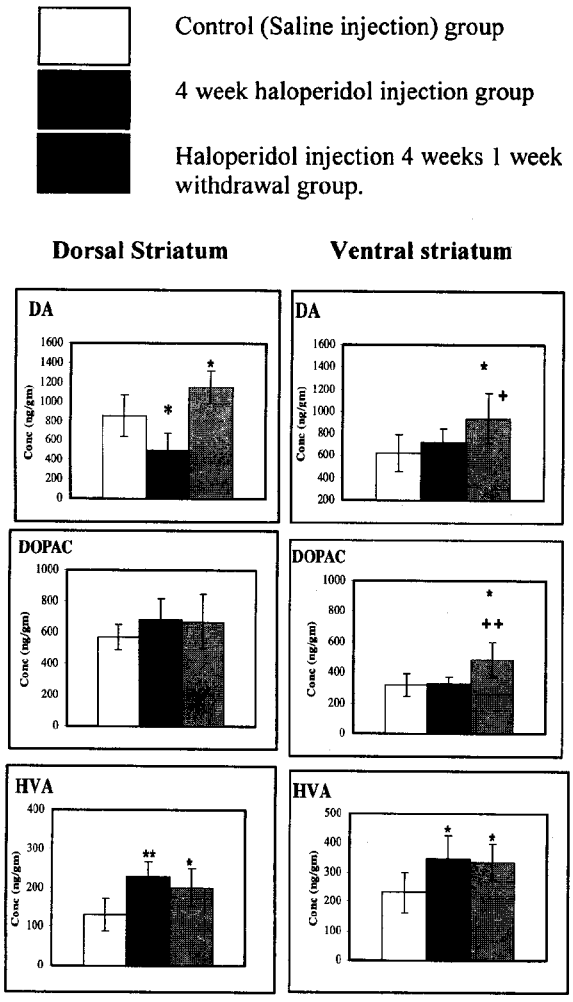

Fig. 3: Effects of 4 week repeated haloperidol administration and 1 week withdrawal following 3 weeks repeated haloperidol administration on the levels of DA, DOPAC and HVA in the dorsal striatum and ventral striatum of rats. Values are means ± S. D. (n = 6). Significant differences by Newman-Keuls test. $*p < 0.05$, $**p < 0.01$ from saline injected, $+p < 0.05$, $++p < 0.01$ from 4 week haloperidol injected animals following ANOVA.

region *i.e.*, substantia nigra. It is noted that haloperidol withdrawal increases DA in the dorsal striatum and ventral striatum which is more than that observed in chronically haloperidol injected rats (Fig. 3). This response in haloperidol withdrawn rats has been interpreted to be an adaptive increase in the postsynaptic receptor sensitivity to DA in response to its decreased availability.

It is well documented that DOPAC and HVA are quantitatively the most significant metabolites of DA of these, DOPAC is often considered to be an index of intraneuronal DA catabolism [31]. Many studies have shown that acute administration of haloperidol produces a dose dependent increase in DOPAC [32] and HVA [33] levels which are of greater magnetitude and longer duration in striatum [2]. Previous study from our laboratory showed the repeated administration of haloperidol increased levels of HVA but not DOPAC in striatum of rats [34]. The present results (Fig. 3) on the effect of haloperidol on DOPAC levels tend to show that in the ventral striatum of haloperidol withdrawal rats a large fraction of DA catabolized interneuronally. On the other hand, increase in HVA levels were also observed in the dorsal striatum and ventral striatum of repeatedly haloperidol injected and haloperidol withdrawal rats is explicable in terms of increased DA release in these brain regions.

Effects of Repeated Haloperidol Administration and Haloperidol Withdrawal on Serotonin Metabolism in Rats

Fig. 4 shows the effects of repeated haloperidol and haloperidol withdrawal on the levels of 5-HIAA and 5-HT in the dorsal and ventral striatum of rats. ANOVA (df 2, 15) showed significant effects of haloperidol on the levels of 5-HIAA ($F = 13.07$ $p < 0.01$) in the dorsal striatum. The effects of haloperidol on 5-HIAA ($F = 2.04$ $p > 0.01$), 5-HT ($F = 2.66$ $p > 0.01$) in the ventral striatum and 5-HT ($F = 1.043$ $p > 0.01$) in the dorsal striatum were not significant. Newman-keuls test showed that levels of 5-HIAA increased in the dorsal striatum following haloperidol withdrawal but not administration.

Striatum, a region of the brain involved in the control of motor activity, is a projection area of dorsal raphe neurons [35]. Manipulations that modulate brain serotonin metabolism have been shown to produce little effect in this brain region. Thus, immobilization stress resulted in an increase in 5-HT synthesis in many brain regions except the striatum [36]. Animal studies showed that repeated administration of haloperidol for 2 weeks resulted in

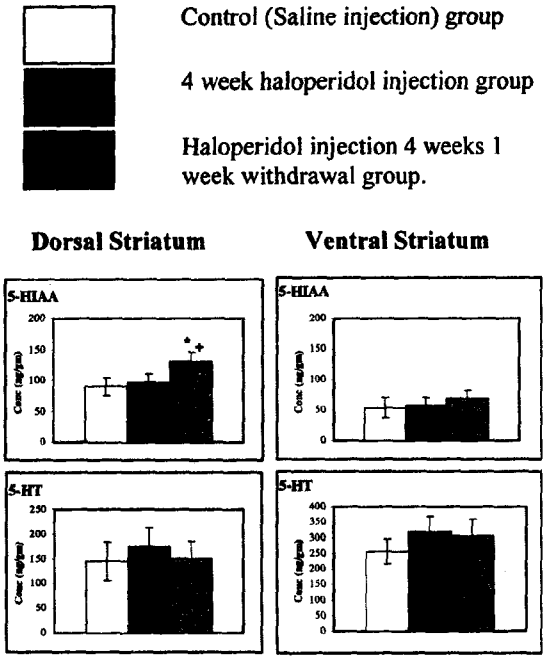

Fig. 4: Effects of 4 week repeated haloperidol administration and 1 week withdrawal following 3 weeks repeated haloperidol administration on the levels of 5-HIAA and 5-HT in the dorsal striatum and ventral striatum of rats. Values are means ± S. D. (n = 6). Significant differences by Newman-Keuls test. *$p < 0.01$ from saline injected, +$p < 0.01$ from 4 week haloperidol injected animals following ANOVA

increased accumulation of 5-HTP in the striatum [14]. The present results show that 5-HT metabolism was not affected in the ventral striatum following repeated administration or withdrawal from repeated administration. In the dorsal striatum 5-HT metabolism increased following withdrawal from haloperidol may be explicable in terms of normalization in the effectiveness of somatodendritic 5-HT-1A receptors.

Experimental

Animals

Locally Bred male albino Wistar rats weighing 180-200g purchased from the Agha Khan University, Pakistan were housed individually with free access to cubes of standard rodent diet and tap water 3 days before experimentation. All experiments were performed according to a protocol approved by the local care committee.

Drugs

Haloperidol (Serenace; Searle, USA), purchased as injectable ampoules of 5 mg/ ml, was injected intraperitoneally at a dose of 1 mg/ ml body weight. Control animals were injected with saline in volumes of 1 ml/ kg body weight.

Experimental Design

18 rats were randomly assigned to 3 groups of six each as: Control group (Normal group), 4 week haloperidol injected group (test group 1) and 1 week withdrawal from 4 week haloperidol injected group (test group 2). Haloperidol was injected repeatedly to the animals of test group 1 and test group 2 at a dose of 1 mg/ kg twice daily for 3 weeks between 10: 30-11: 00 a.m (1^{st} injection) and 5: 00 to 5: 30 p.m.(2^{nd} injection). Control animals were injected with saline 1 ml/ kg at the same time. During 4^{th} week animals of test group 2 were injected with saline while animals of test group 1 received haloperidol. Control animals received saline. VCMs were monitored weekly 22 hours after first injection at 8:30-9:30 a.m. After 4 weeks of the treatment animals were killed 1 hour after the respective saline or haloperidol injection. Brains were removed quickly. Dorsal striatum and ventral striatum taken out and stored at -70° C for the estimation of monoamines and their metabolites by HPLC-EC.

Vacuous Chewing Movements (VCMs) Quantification

VCMs were measured in Perspex activity cages. The animals were transferred to the cages ($26x26x26cm^3$) with saw dust covered floor. Rats were allowed to acclimate to new environment for at least 15 minutes. VCMs were counted for 10 minutes. Each burst of purpose less chewing was counted as 1 if its duration was atleast 3 seconds. VCMs were scored on 24 hours after the saline or drug injection.

Dissection of Striatum

The dissection procedure was same as described earlier [12]. A fresh brain was dipped in

ice cold saline and placed with its ventral side up in molded cavity of a brain slicer. Fine finishing wire was inserted into the slots of the slicer to give slices of 2 mm thickness. The slices containing striatum was transferred to a slide kept on ice. Dorsal and ventral striatum were collected from the slice of brain region.

HPLC-EC Determination of Dopamine, Serotonin and their Metabolites

A 5μ shim-pack ODS separation column of 4.5 mm internal diameter and 15 cm length was used. The mobile phase was 0.1 M sodium phosphate buffer (pH 2.9) containing 14 % HPLC grade methanol, 0.023 % OSS, 0.005 % EDTA. Electrochemical detection was done at an operating potential of 0.8 V (glassy carbon electrode Vs Ag/ AgCl reference electrode).

Statistical Analysis

Data on repeated drug administration was analyzed by two-way ANOVA. Data after drug withdrawal was analyzed by one-way analysis of variance (ANOVA). Posthoc comparisons were done by Newman Keuls test. p values less than 0.05 were considered significant.

Conclusions

The present study shows that an increase in 5-HT metabolism particularly in the dorsal striatum may be involved in the suppression of TD following haloperidol withdrawal. On the other hand, it is tempting to relate the greater increase of DA in the dorsal striatum with the suppression of TD following haloperidol withdrawal. A greater increase of DA metabolism in the ventral striatum following drug withdrawal from repeated administration of haloperidol may be more relevant to the emotional effects of drug because ventral striatum is related to emotional control.

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