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Reversal of haloperidol-induced tardive vacuous chewing movements and supersensitive somatodendritic serotonergic response by imipramine in rats

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Abstract: The study was designed to test the hypothesis that a decrease in the responsiveness of somatodendritic 5-HT-1A receptors by co-administration of imipramine could reverse the induction of VCMs and supersensitivity at 5-HT-1A receptors by haloperidol. Rats treated with haloperidol 0.2mg/rat/day for 2 weeks induced VCMs with twitching of facial musculature that increased in a time dependent manner as the treatment continued to 5 weeks. Co administration of imipramine (5mg/kg/ml) attenuated haloperidol-induced VCMs after 2 weeks and completely reversed it after 5 weeks. The intensity of 8-hydroxy -2-di(n-propyleamino)tetraline (8-OH-DPAT)-induced locomotion and forepaw treading were greater in saline+haloperidol injected but not in imipramine+haloperidol injected animals. 8-OH-DPAT induced decreases of 5-HT metabolism were greater in saline+haloperidol injected animals but not in imipramine+haloperidol injected animals. The mechanism involved in reversal/attenuation of haloperidol-induced tardive dyskinesia by imipramine is discussed.

Key words: Haloperidol, Imipramine (IMI), Tardive dyskinesia (TD), Somatodendritic 5-HT-1A receptors, Postsynaptic 5-HT-1A receptors, Vacuous chewing movement (VCMs)
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INTRODUCTION

Neuroleptics are used extensively in the treatment of schizophrenia and other affective disorders. Unfortunately typical antipsychotics such as haloperidol and chlorpromazine often cause distressing side effects involving extrapyramidal tract. These adverse reactions comprise of a variety of movement disorders[1,2] including tardive dyskinesia (TD), which occurs in 20-40 % of the patient population[3,4]. Tardive dyskinesia, a syndrome of potentially irreversible, involuntary hyperkinetic disorder that occurs during chronic neuroleptic treatment, is a major limitation of neuroleptic therapy[3,5]. 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[6,7,8].

In addition to dopamine, 5-hydroxytryptamine (5-HT; serotonin) and particularly 5-HT-1A receptors have important in the etiology of schizophrenia and the elicitation of extrapyramidal symptoms (EPS)[9,10]. Because postsynaptic 5-HT-1A receptors were upregulated in postmortem schizophrenic brain[11], 5-HT-1A receptor-mediated endocrine responses were determined in female patients with schizophrenia compared with normal control participants[12].

Clozapine and other antipsychotic drugs with substantial affinity for 5-HT-1A receptors[13,14] produced negligible levels of EPS although still controlling psychotic symptoms effectively[15,16].

A role of 5-HT-1A receptors is important in the treatment of TD and Parkinsonian-like effects of neuroleptic drugs, because reserpine-induced dyskinetic movements in a rat model of TD were reversed by the co-administration of buspirone[17], a partial agonist at 5-HT-1A receptor[18]. 8-Hydroxy-2-(di-n-propylamino)tetraline (8-OH-DPAT), a selective 5-HT-1A agonist, inhibited haloperidol-induced VCMs dose dependently[19]. Acute parkinsonian-like symptoms also attenuated by prior administration of buspirone and 8-OH-DPAT[20,21].

In a previous study, we have shown that administration of haloperidol at a dose of 1mg/kg for only 2 weeks elicited an increase in the responsiveness of somatodendritic as well postsynaptic 5-HT-1A receptors[22].

It was suggested that a resultant decrease in the normal inhibitory serotonergic influence on motor activity may be involved in the precipitation of TD in patients on haloperidol therapy. Other authors have shown that chronic administration of imipramine, a tricyclic antidepressant resulted in a decrease in the effectiveness of somatodenedritic 5-HT-1A receptors[23].

The present study was designed to test the hypothesis that coadministration of imipramine could reverse the induction of VCMs and supersensitivity at somatodendritic 5-HT-1A receptors by haloperidol in a rat model of TD.

MATERIALS AND METHODS

Animals

Locally bred male albino Wistar rats weighing 180-220 g purchased from HEJ Research Institute, University of Karachi, Pakistan were housed individually with free access to cubes of standard rodent diet and tap water 3 days before starting the experiment.

Drugs

Haloperidol (Serenace, Searl; USA) purchased as oral drops of 2.0mg/ml was given orally in drinking water at a dose of 0.2mg/rat/day[24]. Imipramine purchased from Sigma, was dissolved in saline and injected subcutaneously at a dose of 5mg/ml/kg/day.

Experimental protocol

Twenty four animals were divided into four groups (six animals in each group) (i) saline+saline; (ii) saline+Imipramine (iii) haloperidol+saline; (iv) haloperidol+Imipramine were received respective treatment for 5 weeks. The animals were injected accordingly saline (1mg/kg), Haloperidol at a dose of 0.2 mg/rat/day and IMI at a dose of 5 mg/ml/kg once daily at 10:00-10:30 hours for 5 weeks. Behavioral assessment of tardive VCMs was carried out weekly at 9:00-9:30 hours i.e. 1 hour before the drug administration. Behavioral and neurochemical effects of 8-OH-DPAT were monitored after a drug washout period of 2 days so that the presence of drug may not interfere with the effects of the drug. Animals of each of the above groups divided in to saline or 8-OH-DPAT injected subgroups were injected accordingly with saline (1 mg/kg or 8-OH-DPAT at a dose of (0.5mg/kg) selected on the basis of previous study[25,26]. Hyperlocomotion and forepaw treading elicited by the drug were scored for 25 minutes starting 5 minutes post injection. Behavioral data were collected by a blind observer. Animal were decapitated 1 h after the drug or saline injection to collect striatum as describe by Haleem and Khan[26]. The samples were stored at a set temperature of 78°C for the estimation of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA).

Vacuous chewing movements (VCMs) quantification

Animals were placed individually in an activity box (26x26x26cm) with sawdust-covered floor and were allowed to adapt the observation cage for a period of 15 minutes. VCMs were monitored during 10 minutes observation period. For calculation purposes, each burst of purposeless chewing was counted as one, if its duration was at least 3 seconds[25,26].

8-OH-DPAT elicited 5-HT syndrome

Animals were placed individually in rectangular Perspex activity cages (26x26x26cm) with sawdust covered floor 15 minutes before injecting 8-OH-DPAT. Forepaw treading and locomotion elicited by the drug were scored as described earlier by Haleem and Khan [26]. The number of cage crossings (movement in any direction with all four paws) and forepaw treading were scored for 1 minute, every 5 minutes up to 30 minutes i.e. in 5 sessions of 1 minute each. A total of 5 scoring periods was later determined.

HPLC-EC determination of 5-HT and 5-HIAA

5-HT and 5-HIAA levels were determined by HPLC-EC as described before[26]. A 5µ Shim-Pack ODS separation column of 4.0mm internal diameter and 150mm length was used separation was achieved by mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1M phosphate buffer of pH 2.9 at an operating pressure 2000-3000psi on Schimadzu HPLC pump. Electrochemical detection was achieved on Schimadzu L-ECD-6A detector at an operating potential of 0.8V. Calculations were done by an in line Shimadzu C-R6A Chromatopac.

Statistical analysis

Effects of imipramine on the time course of haloperidol-induced VCMs were analyzed by three way ANOVA with imipramine and haloperidol as between subject factors and weeks as within subject factor. Effects of 8-OH-DPAT on 5-HT and 5-HIAA levels in the striatum were also analyzed by three way ANOVA. Data on 8-OH-DPAT-induced hyperactivity and forepaw treading, monitored only in 8-OH-DPAT injected animals were analyzed by two way ANOVA. Post-hoc comparisons were done by Newman Keuls test. P values only of < 0.05 were taken as significant.

RESULTS

Figure 1 shows the intensity of VCMs in saline and imipramine injected animals. Three-way ANOVA revealed significant effects of haloperidol ($F=400.85$ df=1,100 $p<0.01$), imipramine ($F=130.56$ df=1,100 $p<0.01$) and weeks ($F=59.67$ df=4,100 $p<0.01$). Interactions between imipramine *haloperidol ($F=147.23$ df=4,100 $p<0.01$), weeks*haloperidol ($F=41.96$ df=1,100 $p<0.01$), weeks*imipramine ($F=14.69$ df=4,100 $p<0.01$) and haloperidol*imipramine*weeks ($F=13.98$ df=4,100 $p<0.01$) were also significant. Post-hoc analysis showed that administration of haloperidol elicited VCMs in saline injected animals after the 2nd week of treatment. Saline injected animals exhibited an increase in the intensity of haloperidol-induced

VCMs after the 5th week of treatment. Imipramine injected animals exhibited a significant increase in VCMs after 4th and 5th weeks. An attenuation of haloperidol-induced VCMs in imipramine injected animals was observed following 2nd to 5th weeks.

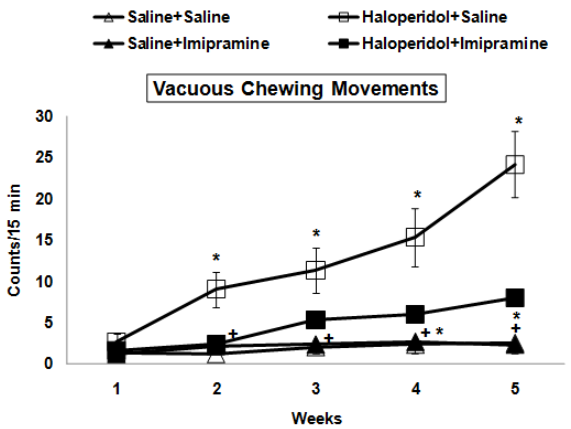

Figure 1: Intensity of haloperidol-induced VCMs in animals treated with saline and imipramine. Values are means±SD. (n=6). Significant differences by Newman-Keuls test: *p<0.01 from saline+saline and saline+imipramine treated animals, +p<0.01 from haloperidol+saline treated animals following three-way ANOVA.

Figure 2 shows 8-OH-DPAT-induced hyperactivity and forepaw treading in saline+saline, saline+haloperidol, imipramine+saline, imipramine+haloperidol treated animals Data on number of cage crossings analyzed by two-way ANOVA showed significant effect of haloperidol (F=3.87 df=1,8 p<0.05), imipramine (F=52.99 df=1,8 p<0.01) and interaction between haloperidol*imipramine (F=13.83 df=1,8 p<0.01). Data on forepaw treading analyzed by two-way ANOVA showed significant effects of haloperidol (F=6.0 df=1,8 p<0.01), imipramine (F=14.51 df=1,8 p<0.01) and interaction between haloperidol*imipramine (F=7.40 df=1,8 p<0.01). Post-hoc analysis showed that 8-OH-DPAT-induced cage crossings and forepaw treadings were greater in saline+haloperidol than saline +saline injected animals. Cage crossings and forepaw treadings were smaller in imipramine +haloperidol than saline+haloperidol injected animals. The results suggested an increase in 8-OH-DPAT-induced hyperactivity and forepaw treading in haloperidol injected animals and reversal of this response in rats cotreated with imipramine.

Figure 3 shows the effects of administration of 8-OH-DPAT on 5-HT and 5-HIAA concentration in the striatum of rats pre-treated with saline+saline, imipramine+saline, saline+haloperidol and imipramine+haloperidol. Data on 5-HT concentration analyzed by three-way ANOVA revealed significant effects of imipramine (F=79.94 df=1,16 p<0.01) and 8-OH-DPAT (F=61.27 df=1,16 p<0.01). Effect of haloperidol (F=1.99 df=1,16 p>0.05) was not significant. Interactions between imipramine*8-OH-DPAT (F=1.02 df=1,16 p>0.05) was not significant. Interactions between imipramine*haloperidol (F=8.80 df=1,16 p<0.01), 8-OH-DPAT*haloperidol (F=53.45 df=1,16 p<0.01) and haloperidol*imipramine*8-OH-DPAT (F=12.80 df=1,16 p<0.01) was significant.

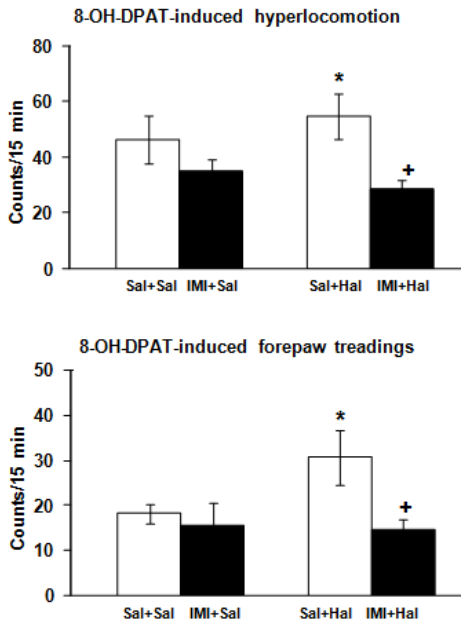

Figure 2: Intensity of 8-OH-DPAT-induced hyperlocomotion and forepaw treading in saline+imipramine, imipramine+saline, saline+haloperidol and imipramine+haloperidol treated animals. Values are means±SD. (n=3). Significant differences by Newman-Keuls test: *****p<0.01 from saline+saline treated animals, +p<0.01 from saline+imipramine treated animals following two-way ANOVA.

Data on 5-HIAA concentration analyzed by three-way ANOVA showed significant effects of imipramine (F=14.88 df=1,16 p<0.01). Effects of haloperidol (F=0.02 df=1,16 p>0.05) and 8-OH-DPAT (F=0.02 df=1,16 p>0.05) were not significant. Interaction between imipramine*8-OH-DPAT (F=6.05 df=1,16 p<0.05) was significant. Interactions between imipramine*haloperidol (F=0.08 df=1,16 p>0.05), 8-OH-DPAT*haloperidol (F=0.43 df=1,16 p>0.05) and haloperidol*imipramine*8-OH-DPAT (F=0.48 df=1,16 p>0.05) was not significant. Post-hoc analysis by Newman-Keuls test showed that administration of 8-OH-DPAT decreased 5-HT levels of saline+saline, saline+haloperidol and imipramine+saline treated animals. The levels of 5-

HT increased in saline+haloperidol pre-treated animals than saline+saline pre-treated animals. Imipramine+haloperidol pre-treated animals exhibited a decrease of 5-HT levels than imipramine+saline pre-treated animals.

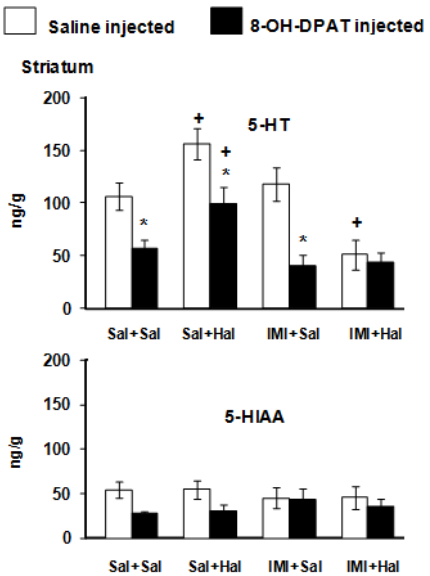

Figure 3: Effects of administration of 8-OH-DPAT on 5-HT and 5-HIAA levels in the striatum of animals treated with saline+saline, saline+haloperidol, imipramine+saline and imipramine+haloperidol treated animals. Values are means±SD (n=3). Significant differences by Newman-Keuls test: *p<0.01 from saline treated animals, +p< 0.01 from saline+saline and imipramine+saline treated animals following three-way ANOVA.

DISCUSSION

Other authors have reported that induction of orofacial dyskinesia by haloperidol at a dose of 1.5 mg/kg/day for 3 weeks could be reversed by the co-administration of 5-HT-1A agonist such as 8-OH-DPAT[19] and sarizotan[27]. Reserpine induced orofacial dyskinesia were also reversed by the co-administration of buspirone[17], a partial agonist at 5-HT-1A receptor [28]. Previously it has been reported that imipramine (a tricyclic antidepressant) could reduce the severity of catalepsy[29]. In the present study treatment with haloperidol at a dose of 0.2mg/rat/day induced tardive VCMs in 2 weeks that increased in a time dependent manner as the treatment continued for 5 weeks. Co-administration of IMI at a dose of 5mg/ml/kg/day attenuated and completely reversed the induction of VCMs in a time dependant manner (Figure 1).

A role of somatodendritic 5-HT-1A receptors in the onset of VCMs was proposed[26] because administration of haloperidol for 2 weeks elicited VCMs[4,5,7,20] and increased the responsiveness of somatodendritic 5-HT-1A receptors in rats[26,30]. An important finding of the present study is that reversal of haloperidol-induced VCMs in rats co-treated with IMI was associated with the reversal of haloperidol-induced increase in the responsiveness of somatodendritic 5-HT-1A receptors. The present study therefore suggests that somatodendritic 5-HT-1A receptors have an important role in the precipitation and alleviation of haloperidol induced-VCMs.

Numerous investigations of interactions between brain 5-HT and dopamine system, have demonstrated both cooperative[31] and antagonistic interactions[32,33]. The dopamine system has traditionally been considered crucial to control of motor activity[34,35]. With respect to anatomical site of action a view has developed that striatum is involved in the control of motor behavior. The serotonergic system is known to play a role in the modulation of activity of dopaminergic neurons. The nature of modulation seems to be inhibitory and at the level of origin of dopamine system in the midbrain as well as in the terminal region [36]. Serotonin antagonists with selectivity towards 5-HT-2C receptors could release dopamine neurotransmission from the inhibitory influence of 5-HT to alleviate Parkinsonian like effects of neuroleptics[33,37,38].

5-HT-1A receptors are known to be located both presynaptically, where they functions as somatodendritic autoreceptors, and postsynaptically. Behavioral responses produced by the activation of 5-HT receptors may arise from receptors with either a presynaptic or a postsynaptic localization. It has been reported that electrical stimulation of the dorsal raphe inhibited a subpopulation of nigrostriatal neuron termed " slow firing" because they are normally under the tonic inhibitory influence of 5-HT[39]. Since 8-OH-DPAT is known to suppress the firing rate of the dorsal raphe, it may inhibit 5-HT synthesis and release. 8-OH-DPAT could increase the firing rate of the "slow firing" dopamine neurons by releasing the tonic inhibitory influence of 5-HT[39].

Elicitation of 5-HT-Syndrome by administration of 8-OH-DPAT was reported by Hjorth and coauthors[40]. The behavior is independent of presynaptic machinery, as it was not blocked by inhibition of 5-HT synthesis. Increase in motor activity, forepaw treading, head weaving and flat body posture are some of the distinct behavioral components of the syndrome[41]. Stimulation of somatodendritic 5-HT-1A receptor resulting in a decrease in the inhibitory influence of 5-HT on dopaminergic neurons[42], as well as postsynaptic 5-HT-1A, is known to be involved in hyperlocomotion[43]. Stimulation of somatodendritic

5-HT-1A receptors by 8-OH-DPAT also resulting in a decrease in the inhibitory influence of serotonin at 5-HT-2C receptors could release dopamine neurotransmission from inhibitory influence of 5-HT to attenuate neuroleptic-induced catalepsy[21]. An increase in the effectiveness of somatodendritic 5-HT-1A receptors in rats treated with haloperidol for 5 weeks (Figure 1) would be expected to decrease the inhibitory influence of serotonin on the activity of dopaminergic neurons. The results are therefore consistent with the notion that decrease in the serotonin influence on the activity of dopaminergic neuron is involved in the elicitation of VCMs while a normalization of serotonergic influence in rats co-treated with imipramine could reverse the induction of VCMs by haloperidol.

Imipramine is an antidepressant drug that preferentially inhibits 5-HT reuptake[44,45]. It is previously suggested that antidepressant effects of imipramine are mediated by some of 5-HT receptor subtypes. The drug has been to reduce the sensitivity of catalepsy and functional activity of 5-HT-1A receptors[29] and is clinically used as effective treatment of depression[46]. A decrease in 5-HT turn over has been reported to occur in the striatum[47] and hippocampus and frontal cortex[48] following administration of imipramine. In the present study imipramine was injected at a dose of (5mg/kg) that was found to decrease 5-HT-turnover in the striatum (Figure 3). The mechanism by which imipramine could reverse haloperidol induced increase in the effectiveness of somatodendritic 5-HT-1A receptor (Fig 1) may involved in a decrease in the effectiveness of somatodendritic 5-HT-1A receptors by repeated administration of the drug[49,50].

Stimulation of the dorsal raphe nucleus (DRN) somatodendritic 5-HT-1A receptors inhibits the electrical activity of the serotonergic neurons leading to a reduction of the serotonergic transmission in the forebrain region[51]. The activation of postsynaptic 5-HT-1A receptors in the fronto-parietal cortex would diminish the function of the DRN 5-HT-1A autoreceptors by a negative feed-back mechanism leading to the inhibition of the firing activity of the DRN serotonergic neuron[52]. In relation to this effect, there are evidences indicating that these cortical 5-HT-1A receptors are postsynaptic receptors[53,54] located on glutamatergic pyramidal output neurons which form synapses preferentially with gamma-aminobutyric acid neurons in the DRN[53,54]. As acute imipramine induces an increased 5HT-1A receptor density in the frontal cortex and increased serotonin levels in the brain while chronic imipramine was correlated to a reduction of frontal cortex 5-HT-1A receptor density and to an increment of the hippocampus 5-HT-1A receptor density[55]. This is also evident in the present study (Figure 3) where the effects of 8-OH-DPAT in decreasing 5-HT concentration were smaller in imipramine+saline than saline+saline injected animals. The results suggest that a decrease in the responsiveness of 5-HT-1A receptors could reverse the induction of supersensitivity by haloperidol.

Administration of 8-OH-DPA elicits a hyperactivity syndrome often describe as serotonin syndrome. Increase in motor activity, forepaw treading and flat body posture are some of the distinct behavioral components of the syndrome[41]. Administration of haloperidol for 2 weeks[26] and 5 weeks[25a,b] elicited a significant increase in motor activity while intensity of forepaw treading was not altered has been shown previously. The present study shows that both hyperlocomotion and forepaw treading increased following 5 weeks administration of haloperidol (Figure 2). In addition the present study shows that long term administration of imiparmine at a dose of 5 mg/kg for 5 weeks did not alter 8-OH-DPAT-induced motor activity or forepaw treading.

Evidence suggests that serotonin also has stimulatory influence on motor activity[21]. These effects of serotonin are possibly mediated via postsynaptic 5-HT-1A receptors. Thus systemic administration of 8-OH-DPAT, a selective 5-HT-1A agonist acting via somatodendritic as well as postsynaptic receptors, attenuated haloperidol-induced catalepsy and decreased 5-HT concentration in the striatum. Although administration of imipramine also produced similar effects but anticataleptiogenic and not the serotonin metabolism reducing effects of 8-OH-DPAT were greater than imipramine, suggesting a role of postsynaptic 5-HT-1A receptors in the anticataleptiogenic profile of 8-OH-DPAT.

In conclusion, the present results on the reversal of haloperidol-induced VCMs by imipramine are largely explainable in terms of the reversal of supersensitivity of somatodendritic receptors. It is suggested that drugs that preferentially affect somatodendritic 5-HT-1A receptors may be use in extending therapeutics in schizophrenia.

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