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Effect of haloperidol administration on GABA and glutamate dehydrogenase activity in Albino Rat Brain

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Abstract
Objective: The objective of our study was to investigate the effects of chronic haloperidol administration on glutamate dehydrogenase activity and GABA level.
Methods: We used ex vivo magnetic resonance spectroscopy along with high performance liquid chromatography to analyze forebrain tissue from rats administered oral haloperidol for 40 days.
Results: Haloperidol administration for 40 days decreases the receptor binding of GABA and GABA levels at dose of 5mg/kg b.w., 1.0 mg/kg b.w., and 0.5 mg/kg b.w. and the activity of glutamate dihydrogen are increases significantly at the dose of 1.0 mg/kg b.w., and 0.5 mg/kg b.w.
Conclusion: Chronic haloperidol administration in rats appears to increase forebrain GABA and glutamate dehydrogenase activity. Studies exploring these processes in subjects with schizophrenia should take into account the potential confounding effects of antipsychotic medication treatment.

Keywords: Haloperidol, GABA, Glutamate dehydrogenase.

1. Introduction
Haloperidol is a potent antipsychotic and antianxiety agent with a strong antiemetic effect. Acute administration of haloperidol to rats leads to blockage of pre and post synaptic dopamine receptors increasing the rate of dopamine turnover due to feedback activation of the dopamine neurons [1, 2]. The chronic haloperidol administration has also been reported to increase the striatal dopamine receptor binding due to super sensitivity of dopamine receptors sites [3, 4, 5].
The inter relationship between GABAergic and dopaminergic systems and the possibility that former modulates the activity of the dopaminergic neurons have attracted great interest [6, 7]. The levels of GABA in CNS are regulated by glutamate which is synthesized glutamate dehydrogenase. Since alteration in this enzyme significantly influence the GABA synthesis, drugs and chemicals affecting the activity of this enzyme could also influence the GABAergic system. The involvement of GABAergic system, in the action of haloperidol believe to exert its clinical action by blocking the dopamine receptors is indicated by inhibition of the glutamate dehydrogenase, by haloperidol under in vitro condition [8]. In depth studies has not been conducted on the involvement of GABAergic neurotransmission in the action of haloperidol through significant amount of data is available on the interaction of this drug with dopamine receptor. Therefore to further understand the role of GABAergic system in the action of haloperidol the candidate has attempted to study the levels of GABA receptor binding in the animals exposed to this drugs.

2. Material and Methods
Ethics
All procedures were in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals, as well as the guidelines of the Animal Welfare Act.

Methods
Adult male wistar albino rats (150 ± 20 grams) of industrial toxicology Research Centre, breeding colony, Lucknow were used in this study.

Mode of Treatment
Group I: Haloperidol was administered intraperitoneally at doses equivalent to 0.5 mg/kg, 1.0 mg/kg and 5 mg/kg body weight for 24 hours. The control animals received an equal volume of Ethanol (1 mg/kg body weight) in an identical manner.

Group II: Haloperidol was administered intraperitoneally at doses equivalent to 0.5 mg/kg, 1.0 mg/kg and 5 mg/kg body weight for 48 hours. The control animals received an equal volume of ethanol (1 mg/kg body weight) in an identical manner.

Group III: Haloperidol was administered intraperitoneally at doses equivalent to 0.5 mg/kg, 1.0 mg/kg and 5 mg/kg body weight for 40 days. The control animals received an equal volume of ethanol (1 mg/kg body weight) in an identical manner.

Preparation of crude synaptic membrane and neurotransmitter receptor binding assay.
A crude membrane fraction was prepared from brain regions by homogenisation of tissue in 19 volumes of 0.32 M sucrose followed by centrifugation for 14 minutes [9].

Determination of glutamate dehydrogenase activity
The enzyme activity was measured by method of Rajlaxmi *et al* [10].

Estimation of GABA level
The GABA levels were assayed using a HPLC modified method described by Dravid *et al* [11].

Protein Estimation
The protein was estimated by lowry method [12]

Statistical Analysis
The experimental data were analysed by student 't' test. Differences from controls were considered significant at $p < 0.05$.

3. Result

Effect of haloperidol on the receptor binding of GABA
Haloperidol caused a significant decrease in the binding of ^{3}H – Mucimol to cerebellar membrane at the doses of 1 and 5 mg/kg body weight (table-1) and had no significant effect on the binding of ^{3}H – Mucimol at a dose of 0.5 mg/kg body weight in ratssacrified 48 hours after the haloperidol treatment. The animals exposed to haloperidol (0.5, 1.0 & 5.0 mg/kg body weight) for 24 hours showed a significant decrease in the cerebellar binding in a dose dependent manner. Scatchard analysis revealed that the decrease in the binding of ^{3}H – Mucimol was due to a decrease in maximum number of binding sites (Bmax) without any alteration in the binding affinity (Kd) except at the dose of 0.5 mg/kg body weight (24 hours treatment) where both the affinity and Bmax were altered.

Table 1: Shows Significant Decrease in the receptor binding of ^{3}H – Mucimol in the cerebellum

Treatment	24 hours exposure	48 hours exposure	40 days exposure
Controls	128±6.1	124±3.6	125±4.8
5 mg/kg b. w.	82±4.6***	92±5.2***	89±3.2***
1 mg/kg b. w.	91.5±2.6**	102±1.4**	102±2.1**
0.5 mg/kg b. w.	105±0.5*	118±6.8	109±0.4*

Binding is expressed in terms of p mole bound/g protein. * p <.05, ** p <.01, *** p <.001

There was a significant reduction in the receptor binding of ^{3}H – Mucimol in the cerebellum of the animals exposed to haloperidol at the dose of 0.5, 1.0 & 5.0 mg/kg for 40 days (table-1). Scatchard analysis revealed a significant reduction in the maximum number of binding sites without any change in the affinity of the receptor. No change in the binding of ^{3}H – Mucimol was observed in the striatal region following haloperidol treatment after 24 hours, 48 hours and 40 days at any of the dose given (table-2).

Table 2: Shows Significant Decrease in the receptor binding of ^{3}H – Mucimol in the striatal Region

Treatment	24 hours exposure	48 hours exposure	40 days exposure
Controls	72.1±3.4	68±4.6	65±4.9
5 mg/kg b. w.	79±19.6	64±13.9	69.5±2.4
1 mg/kg b. w.	62±7.4	72±3.9	79±3.1
0.5 mg/kg b. w.	76±6.8	61±6.1	64±3.1

Binding is expressed in terms of p mole bound/g protein. Data are expressed as mean ± S.E

Effect of the Activity of Glutamate Dehydrogenase on Exposure to Haloperidol
The glutamate dehydrogenase activity was significantly increased in cerebellum in a dose dependent manner. When the rats were exposed to haloperidol for 24 hours or 48 hours there was no change in striatal GDH activity. At 40 days of treatment, the enzyme activity was significantly increased at all doses except 5 mg/kg in cerebellum without any alterations in the corpus striatum (table -3).

Table 3: Shows effect of haloperidol on glutamate dehydrogenase activity in the cerebellum.

Treatment	Activity of GDH	% increase
Controls	14.6±2.8	
5 mg/kg b. w.	26.2±12.9	44.2
1 mg/kg b. w.	31.9±1.4**	54.7
0.5 mg/kg b. w.	29.2±2.1*	49.3

Activity expressed in terms of U/mg of protein. Data are expressed as mean ± S.E.
* p <.05, ** p <.01

Effect of haloperidol on the GABA levels
The alteration induced by haloperidol exposure on the levels of GABA in cerebellum shown in table -4. After 24 or 48 hours of haloperidol treatment there was a significant increase in the levels of GABA at the doses of 0.5, 1.0 & 5.0 mg/kg body weight, the maximum induction being at doses of 5.0 mg/kg body weight.
The animals exposed to haloperidol for 40 days showed a significant dose dependent increase in the levels of GABA in brain cerebellum (table-4). The reduction in the GABA levels was maximum when the rats were exposed to haloperidol for 24 hours.

Table 4: Shows Effect of Haloperidol on GABA Level in the Cerebellum.

Treatment	24 hours exposure	48 hours exposure	40 days exposure
Controls	3.2±0.3	3.8±0.3	3.6±0.12
5 mg/kg b. w.	4.7±0.2**	4.8±0.04**	5.0 ±0.19**
1 mg/kg b. w.	3.9±0.05**	4.2±0.04 **	4.7 ± 0.09 **
0.5 mg/kg b. w.	3.75 ± 0.3 *	3.95 ± 0.02	4.15 ± 0.12 *

Data are expressed as mean ± S.E. * p<.05, ** p<.01

4. Discussion

Haloperidol, known to exert its antipsychotic effects by affecting dopaminergic system, has also been found to increase

the activity of glutamate dehydrogenase responsible for the synthesis of glutamate, a precursor of GABA. Involvement of GABAergic system in haloperidol induced neurotoxicity has been reported by [8, 13]. However, no directly affects the level of GABA and GABA receptors under *in vivo* conditions.

The decrease in the GABA receptors in cerebellum (37%) and striatum (11%) suggests disturbances in GABAergic system after haloperidol appears to be due to increase in GABA levels, since receptor is often modified by a change in the a availability of neurotransmitter within the synapse [14, 15, 16, 17].

The specificity of haloperidol in causing a marked increase in the levels of GABA in cerebellum than the other brain regions, supported by the studies showing increased activity of glutamate dehydrogenase, further suggest the role of GABAergic neurotransmission in the action of haloperidol.

Evidence for localization of dopamine receptors on GABAergic nerve terminals and retina and a functional link between basal ganglia and cerebellum have been suggested by several investigators [16, 18, 19, 7]. However no anatomical evidence for the presence of dopamine receptors on the gABA terminals, in cerebellum has presents so far. It has been reported by starr *et al* [7] that neurotransmitter species interact in a complex manner so that disturbance in one circuit will ultimately affect a wider range of transmitters. A particular transmitter may have a regulatory effect upon a synapse of another transmitter. GABA may thus stimulate the synaptic release of dopamine. This, however does not seem to be the case since GABAergic input in the corpus striatum is minimal and as evident in the present study, no change in ^{3}H – Mucimol binding in corpus striatum was observed. Also the decreased binding observed in cerebellum in animals treated with haloperidol is unlikely to be the secondary response mediated by dopamine.

The fact that haloperidol is a potent inhibitor of the brain GABA neurotransmission suggest that the antipsychotic drugs of this group could also involve GABA neurotransmitter, its receptors and enzyme glutamate dehydrogenase, might be of pharmacological significance.

5. Conclusion

The present data clearly indicate significant increase in Glutamate dehydrogenase & GABA levels and a significant decrease in the binding of ^{3}H – Mucimol to cerebellar membrane at the doses of 1 and 5 mg/kg body weight. GABAergic neurotransmission in rat brain on haloperidol treatment and suggest that other drugs of this group may also be involving GABAergic system in their action. It is hoped that these studies will eventually help in suggesting better therapeutic measures against neurotoxicity of these drugs. Studies exploring these parameters in subjects with schizophrenia should take into account the possible effects of antipsychotic medications, especially potent D2 antagonists like haloperidol.

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