Effect of single prenatal haloperidol exposure on hippocampus and striatum of developing rat brain

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Poor development and differentiation of three layered cytoarchitectural pattern of brain, degenerating pyramidal cells with pyknotic nuclei and substantial loss of both large and small pyramidal cells of the hippocampal CA1 region were observed in fetuses of pregnant Charles-Foster rats exposed to single high dose of haloperidol (50 mg/kg body weight) on day 12 of gestation. In treated striatum, reduction in size, complete degeneration of multipolar cells with fragmented nuclei and increased extracellular spaces were observed. Unsacrificed group of day 12 haloperidol treated rat offsprings at 9 weeks of age exhibited cognitive behavioural dysfunctions in passive avoidance (behaviour) test. These findings indicate that a single (high dose) prenatal haloperidol exposure during critical period of CNS development not only induces micromorphological alterations in foetal hippocampus and striatum but also lasting cognitive impairment in adult rat offsprings.

Haloperidol (HAL), a typical antipsychotic drug, is frequently prescribed for the treatment of severe manic and schizo-affective neural disorders. The critical action of neuroleptics is blockade of cerebral dopamine receptors DA2 in particular.12

In rodents, prenatal HAL exposure during critical period of brain development5 induces neurochemical and structural alterations in various foetal brain areas especially in dopaminergic rich sites like limbic system and basal ganglia.3-7 Scalzo et al.5,8 reported that prenatal HAL exposure during CNS development alters neurotransmitters level particularly that of dopamine in various foetal brain areas which may be responsible for long-lasting abnormal behavioural responses of young and adult rat offsprings9. Engel and Lundborg10 found that prenatal exposure of pentfluridol, a neuroleptic, caused a decrease in the synthesis and release of dopamine from the mesolimbic dopaminergic system. Prenatal exposure to another neuroleptic agent, chlorpromazine causes significant reduction in nor-adrenalin turnover in striatum, hippocampus and other brain areas resulting into cognitive behavioural deficit11 in adult rats. Kellogg and Lundborg12 have documented abnormal behaviour of rat offsprings treated prenatally with HAL. Scalzo et al. found that prenatal exposure to HAL significantly reduces the dopamine receptor binding (D1 and D2) in caudate nucleus and nucleus accumbens. Such dopaminergic deficit in typical dopamine enriched areas of brain were accompanied by overt behavioural dysfunctions in young rat offsprings. Experimental studies have indicated that prenatal exposure to HAL not only induces alterations in dopamine level in caudate nucleus (a dopamine enriched area) but also expressed long-term behavioural alterations in anxiety13-16, depression16, and other behavioural responses8,17.

Besides, a typical role of HAL to alter the dopamine metabolism at least in caudate nucleus is able to change the acetylcholine levels in the hippocampus18,19 which may also be responsible for prolonged cognitive dysfunctions in rat/mice offsprings20,21. A modulating role of haloperidol has been suggested in hippocampal dopaminergic activity and their overt behavioural responses in mammalian offsprings22. Acetylcholine is one of the important neurotransmitters in the hippocampus23. Several studies have suggested that cholinergic mechanism might be involved in mediating hippocampus related behavioural functions.

Jurand24 and Jurand and Martin25 reported that prenatal administration of single high dose of antipsychotic drugs like haloperidol (23-35 mg/kg) on very beginning of 9th day of gestation induced exencephaly, ectopia of neural tube, dilation and retardation of choroid plexus in fourth brain ventricle including growth retardation in mice embryos. No information is available on prenatal exposure of HAL and its effects on micromorphology of hippocampus.
and striatum of rat fetuses. The present study has been planned to elucidate the effect of a single (high) dose of HAL administered prenatally during critical period of neuronal development to assess the micromorphological aberrations in hippocampus and striatum in rat fetuses as well as impaired cognitive dysfunction in adult rat offsprings.

Materials and Methods

Male and female Charles-Foster rats (150-200 g) were placed together overnight for mating and presence of sperm in vaginal swab was taken as day zero (GD 0) of gestation. Haloperidol decanoate (50 mg/kg, Senorm L.A., Sun Pharmaceutical Industries Ltd. Vapi, Gujarat) was administered, ip once in a single dose at 9 hr on gestation day 12 (GD 12) of pregnant rats. Another group of pregnant control rats was treated similarly with equal volume of vehicle (0.9% normal saline with 1.5% benzyl alcohol). Approximately one half of the dams of both control and treated groups were sacrificed on day 21 of gestation (GD 21) by intracardial perfusion with 10% neutral formalin and their fetuses were collected, weighed and examined malformations, if any. Their brains were quickly removed, blotted dry, weighed individually, examined for overt malformations and then fixed in the same fixative. After fixation, the brains were further processed for histological studies by staining with Haematoxyline and Eosine. Simultaneously, the other one half of the pregnant dams of both treated and control groups were allowed to deliver and after 16 hr of delivery, the litters were culled to 8 pups per dam and foster nursed by normal lactating mothers. The pups were weaned at 3 weeks of age. At 9 weeks of age, young rat offsprings were subjected to passive avoidance behaviour test.

Passive avoidance behaviour test — The apparatus was a rectangular box (45 × 30 × 40 cm). It had two walls of steel and the remaining two walls of transparent plexiglass to permit observations. The box had a grid floor to deliver the electric current and a sliding transparent plexiglass cover. A 8 cm high plastic platform (17 × 12 cm) was fixed to the grid floor at the centre of the apparatus. Testing began by placing the rat on the raised platform. Latency to step down (SDL<sub>i</sub>) was recorded. Electric shock was not delivered on day 2. If the animal remained on the raised platform for 5 min test period, the maximum score of 300 sec was assigned. At day 9 (after a gap of one week) latency to step down (SDL<sub>2</sub>) was again recorded to test the retention of the passive avoidance learning.

Statistical analysis — First, mean ± SD values for each group were calculated and the significance of difference between corresponding vehicle and haloperidol treated groups were analysed by applying Student's t test. The obtained step down latency scores (time in sec) for passive avoidance test was found to be heterogeneous. As such these time scores were subjected to log X transformation to reduce the heterogeneity following the assumptions for homogeneity/heterogeneity and then subjected to unpaired Student's t test.

Results

Histological observations — On histological evaluations of GD 12 treated specimen, the laminar architecture of typical three layered hippocampal cortex was found to be less developed and poorly differentiated in comparison to corresponding control (Fig. 1 A and B). There was substantial deficit in the number of both large and small pyramidal cells as compared to control. The thickness of the pyramidal cell layer was reduced with granular look of molecular and polymorphic layers (Fig. 1 C and D). The pyramidal cells were found to be degenerating, depicting irregular shape with increased granular cytoplasm and pyknotic nuclei (Fig. 1 E and F).

In the HAL treated specimen, the striatum was found to be markedly reduced in size and cell population as compared to controls (Fig. 2 A and B). Only a few large multipolar (ganglionic) cells were observed which were severely degenerated containing debris of fragmented nuclei (Fig. 2 C and D). On higher magnification small cells were also observed to be deficit in population with marked frequency of pyknotic nuclei giving granular appearance. At few places, the cells were observed to be crowded together due to spongy degeneration of extracellular matrix (Fig. 3 A and B).

Behavioural observation — On passive avoidance learning and retention behaviour of cognitive paradigm, GD 12 HAL treated rat offsprings showed significant retention deficit (P < 0.001) of passive avoidance at 24 hr but not at one week retention interval in comparison to vehicle treated rat offsprings.
Fig. 1—Histopathological findings of CA1 region of developing hippocampus (HPC) prenatally treated with haloperidol on GD 12 (A. control hippocampus with typical laminar structure; B. disturbed laminar architecture of treated hippocampus, H & E. × 50; C. CA1 region of control HPC with pyramidal cell layer; D. thin pyramidal cell layer with scattered cells in CA1 region of treated HPC, H & E. × 150; E. control pyramidal cells in CA1 region; F. reduced number of pyramidal cells with pyknotic nuclei. Note the spongy degeneration of intercellular matrix (↑). H & E. × 600. P - polymorphic layer, Py - pyramidal layer, M - molecular layer).
Fig. 2—Histopathological observations of foetal striatum prenatally treated with HAL on GD 12 (A, control striatum; B, treated striatum showing marked reduction in size, H & E, × 50; C, control striatum with large and small multipolar (ganglionic) cells; D, details of B showing marked degeneration of both small and large ganglionic cells. Note the fragmented nuclei (↑), H & E, × 150).
(Table 1). There was no significant difference on step down latency between control and HAL treated group on day 1 of testing (before electric shock delivery).

**Discussion**

Prenatal administration of single dose of haloperidol to pregnant rats induced alteration of three layered laminar cytoarchitecture and neuronal deficit in hippocampus. The neuronal loss was severe in both large and small pyramidal cells of CA1 region of hippocampus. In striatum, severe neuronal degeneration was also observed. It has been reported from this laboratory that prenatal single HAL exposure during critical period of brain growth induces congenital gross malformations of body and brain as well as micromorphological deformity of cerebral cortex.

The role of hippocampus is in cognitive performances i.e. related to learning and memory retentions. The results obtained from the behavioural paradigm indicate significantly impaired acquisition and retention of learning in young adult rat offsprings treated prenatally to single dose of HAL on GD 12, this being the critical period of neural vulnerability in hippocampus. Earlier reports indicate that prenatal administration of CNS acting drugs like benzodiazepine, chlordiazepoxide and PHB may induce substantial learning and retention deficits in various paradigms. The present findings are in agreements with these reports at least in cognitive impairment in rat offsprings. The present findings also support the hypothesis that an interaction between monoaminergic and cholinergic neurotransmitter systems may be involved in the actions of neuroleptic drugs like HAL on cognitive functions. Administration of neuroactive drugs like HAL and PHB, at doses which are not teratogenic, may express overt behavioural dysfunction without causing any structural deformities.

Jurand and Jurand and Martin have reported dilation of IVth brain ventricle and retardation of choroid plexus in rats induced with a single high dose of HAL (23-35 mg/kg) on day 9 of gestation. Lipska and Weinberger reported that early hippocampal damage affects the development of cerebral cortex which in turn is involved in the regulation of striatal DA function. The present results indicated that prenatal exposure to dopamine antagonist, HAL.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age (in week)</th>
<th>n</th>
<th>SDL0: Retention intervals at 1 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>13</td>
<td>SDL0: 0 hr (log sec.)</td>
</tr>
<tr>
<td>Haloperidol GD 12</td>
<td>9</td>
<td>10</td>
<td>SDL0: 24 hr (log sec.)</td>
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<td></td>
<td></td>
<td></td>
<td>SDL1: 24 hr (log sec.)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>SDL2: 1 week (log sec.)</td>
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SDL = stepdown latency; GD 12 = gestational day 12.
induces not only hippocampal injuries but also disturbs the development of the frontal cortex and striatum with their behavioural sequelae.

From the available literature, it is difficult to draw any conclusion concerning cause of such behavioural changes following exposure to haloperidol in intrauterine life. In all probability, the drug has an indirect effect on the development of different neurotransmitter systems in the rat brain. The drug metabolites may also be selectively toxic as the rate of metabolism and excretion of such a metabolite is liable to be delayed in the foetus. It is also expected that HAL or its metabolites may remain in the maternal plasma concentration for extended period of time, thus affecting the developing CNS.

Several monoamine neurotransmitters are thought to have trophic effects during morphogenesis prior to their function as neurotransmitters. It is also presumed that changes in neurotransmitter functions, coupled with reported changes in cellular energy metabolism may induce long-lasting changes in neuronal systems regulating particular behaviour.

The present study concludes that HAL has substantial effect on developing CNS and long-lasting cognitive behavioural alteration in rat offsprings if administered during the critical period of cortical and hippocampal neurogenesis since, proliferating, migrating or differentiating nerve cells are more susceptible than mature cells. The process of hippocampal neurogenesis, if exposed to PHB gets severely affected but the severity of adverse affect is reduced if PHB is administered during late pregnancy, i.e. after completion of the pyramidal cells neurogenesis. The present findings of substantial reduction in pyramidal cell population and alteration in cytoarchitecture of hippocampus are in agreement with those workers whose exposure time of teratogenic agents corresponds to the period of neurogenesis.

It has generally been thought that the dopamine system in the rodent brain does not even begin to develop prior to day 12 of gestation. Immunocytochemical studies show that dopaminergic fibres from substantia nigra/ventral tegmental area do not reach the forebrain before GD 15, while HPLC studies suggest that dopamine and dopamine metabolite levels are not detectable much before GD 17. Similarly, receptor binding studies and studies measuring the expression of m-RNA for DA1 and DA2 receptors indicate that these receptors do not appear in embryonic forebrain specially in caudate putamen prior to GD 14. Indeed, receptor number and dopamine content are still only a fraction of adult levels by the time of birth in the rat. Further, mid brain dopamine regions undergo differentiation over day 11-15 of gestation in rat.

There is increasing evidence that a number of neurotransmitters can play a trophic role to regulate brain growth and development. Dopamine may be one of the candidates for this role. The evidence for a trophic role of dopamine is indirect. A number of studies indicate that early exposure to compounds which block dopaminergic transmission may block cellular proliferation or growth. The present study supports the hypothesis of trophic role of dopamine in early development of CNS. Role of other neurotransmitters in regulation of brain growth is not clearly understood as yet. Thus, results obtained from the present study reveal that a single (high dose) prenatal HAL exposure may induce morphomorphological alterations in hippocampus and striatum leaving lasting imprint on cognitive behaviour of rat offsprings. In the present study, the dose of the drug used closely corresponds to the recommended therapeutic doses in human beings. Therefore, it is hypothesized that HAL treatment given on GD 12 may alter the development and differentiation of DA receptors which induce structural deformities and cognitive impairment. Clinical use of HAL for treatment of various CNS disorders during human pregnancy should again be scrutinized.

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