Effect of phenobarbitone administration to pregnant rats on anxiety in offsprings

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Phenobarbitone (PHB), an anticonvulsant drug is used chronically during pregnancy and early neonatal period for convulsive disorders, epileptic seizures and stressful labour pain\(^1\). There is a very sensitive balance between potential risks and beneficial uses (therapeutics) of long term exposure of PHB during pregnancy. Experimental studies on rodents also documented that prenatal and early neonatal chronic administration of PHB induces neurotrophological, neuromicromorphological, neurochemical and neurobehavioural alterations in rat offsprings\(^2\). In utero exposure to PHB may also induce behavioural alterations particularly deficit in cognitive functions\(^3\), slower habituation to new environment\(^4\) as well as alterations in open-field activity\(^5\) in rat offsprings. More recently, it has been shown that administration of anticonvulsant drugs like PHB, at doses which are not teratogenic, can cause subtle changes in the behaviour of offsprings\(^6\). In rodents, literature on prenatal administration of PHB and changes in anxiety patterns in various test situations is scanty and often inconclusive. Middaugh et al.\(^7\) found that rodent offsprings exposed prenatally to PHB exhibited enhanced open-field activity. Therefore, the present study has been planned to elucidate the effect of prenatal PHB exposure during critical period of fetal brain development on anxiety patterns in young rat offsprings.

Materials and Methods

Male and female Charles-Foster rats (175-200g) were placed together overnight for mating and presence of sperm in the vaginal swab was taken as day 0 (zero) of gestation. Phenobarbitone sodium (10 mg/kg body weight) was administered intraperitoneally daily at 09.00 hrs from day 12 to day 21 of gestation (GD12-GD21) to one group of pregnant rats. Another group of pregnant rats were similarly treated with vehicle (normal saline). The PHB and vehicle treated pregnant rats were allowed to deliver and after 16 hrs 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 and weighed weekly. At 8-9 weeks of age, young rat offsprings were subjected to open-field exploratory behaviour, elevated plus-maze behaviour, elevated zero-maze behaviour tests of anxiety pattern in the laboratory. All animals were housed in colony cages at 25±1°C and 45-55 % RH, with 12 hr L : D cycle. Animals were fed ad libitum with standard pellet chow and allowed to free access to drinking water. Experiments were conducted between 09.00 and 14.00 hrs.

Behavioural tests

(i) Open-field exploratory test—An open-field apparatus similar to that of Brönstein\(^8\) was used to test the exploratory behaviour of rats. Detail description of the design was explained in our
In novel test situation, each animal was centrally placed in the test apparatus for maximum 5 min and following behavioural aspects were noted.

1. Ambulation—measured by counting the number of squares crossed by rat.
2. Rearing—measured by counting the number of times the rat stood on its hind limbs (supported and unsupported).
3. Self-grooming—by counting the number of responses of grooming, scratching, licking and washing made by individual rat and
4. Faecal pellets—counted by number of faecal pellets excreted by individual rat.

(ii) Elevated plus-maze test—This model is used to test the anxiety in rodents. Detailed description of the design was also explained in our earlier reports. In brief, it consists of two opposite arms, 50x10 cm, giving the apparatus shape of a plus sign. The maze was kept in dimly lit room and elevated 50 cm above the floor. Animals were placed individually in centre of the maze facing an enclosed arm. Number of entries and time spent on the open and enclosed arms were recorded during the next 5 min for each rat. An arm entry was defined when all four paws of the rat were on the arm.

(iii) Elevated zero-maze test—This test is used to test the anxiety in rodents. The maze comprised of a black annular platform (105 cm in diameter, 10 cm width) elevated 65 cm above the ground level, divided into equally into four quadrants. The two opposite quadrants were enclosed by a black perspex wall (27 cm high) on both the inner and outer edges of the platform, while other two opposite quadrants were surrounded by perspex "lip" (1 cm high) which served as a tactile guide to animal on these open areas. The apparatus was illuminated by dim light arranged in such a manner as to provide similar lux levels in open and enclosed quadrants. Rats were placed on one of the enclosed quadrants for a 5 min test period. During the 5 min test period, time spent on open arms, number of head dips over the edge of platform and number of 'stretched attend postures' from closed to open quadrants were recorded. Animals were scored as being in the open area when all four paws were in the open quadrants and in the enclosed area when all four paws had passed the open-closed divide.

Statistical analysis—First, mean±S.E. values for each group were calculated and significance of difference between vehicle and PHB treated groups were analysed by applying Student’s ‘t’ test.

Results

Open-field behaviour—Prenatally PHB treated rat offsprings displayed significantly increased ambulation and rearing in open-field behaviour when compared to those of control (Table 1). In open-field arena there was no significant alteration in self-grooming and faecal droppings in PHB treated rat offsprings.

Elevated plus-maze behaviour—The result obtained from the various indices of elevated plus-maze behaviour indicate that rat offsprings exposed to PHB during critical period of gestation significantly spent less time on open arms and spent more time in enclosed arms and made significantly more number of entries in enclosed arms when compared to those of controls (Table 2). The ratio between open and enclosed arms entries and ratio of time spent on open and enclosed arms was also significantly different from controls.

Elevated zero-maze behaviour—The rat offsprings treated prenatally with PHB exhibit significantly less time on open arms as well as made significant less number of head dips and 'stretched attend postures' in comparison to control rat offsprings (Table 3).

Discussion

In rodents, prenatal and neonatal exposure of

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Ambulation (N)</th>
<th>Rearing (N)</th>
<th>Self-grooming (N)</th>
<th>Faecal pellets (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Vehicle)</td>
<td>8</td>
<td>61.13±5.22</td>
<td>22.38±1.69</td>
<td>5.88±3.69</td>
<td>1.25±1.75</td>
</tr>
<tr>
<td>PHB (10 mg/kg)</td>
<td>7</td>
<td>71.00±6.71**</td>
<td>14.29±5.38**</td>
<td>4.29±0.76</td>
<td>0.57±0.79</td>
</tr>
</tbody>
</table>

P value: ** < 0.01 in comparison to control.
phenobarbitone induces micromorphological alterations, changes in neurotransmitters levels in fetal brain and long-lasting altered behavioural responses in adult offsprings.\(^5,6,11\). Prenatal administration of PHB to pregnant rats induced structural changes in offsprings.\(^7\). Yanai et al.\(^20\) found that exposure to PHB from gestation day 6 to parturition induced significant reduction of brain weight in rat offsprings. Rats exposed to PHB (10 mg/kg) during late prenatal period exhibited significant loss of cerebellar neurons in 4 months old rat offsprings.\(^21\). Bargman et al.\(^7\) reported that prenatal phenobarbitone exposure during last two trimesters of pregnancy (days 9-18) or part of this period (days 9-13, 13-16 or 16-18) had a long lasting substantial neuronal deficit in cerebellum and hippocampus. Neonatal rats exposed to PHB (60 mg/kg) from postnatal day 3 to 20 exhibited microencephaly, loss of purkinje and pyramidal cells and extensive loss of cortical neurons.\(^22,23\). Fishman et al.\(^24\) also documented mitochondrial and myelin degenerations in molecular and granular cell layers of cerebellum. In rodents, prenatal and/or neonatal PHB exposure not only induces the structural deformities in CNS but also expressed overt long-lasting altered behavioural responses in adult offsprings.\(^2,13\). Prenatally PHB (2.5 mg/kg) treated rat offsprings displayed deficit in cognitive functions in passive avoidance test.\(^9\). Such type of functional deficit in rodents treated prenatally or neonatally to PHB have been reported earlier on maze learning paradigms.\(^24,26\). Mice exposed prenatally from day 14 to 21 of gestation to PHB (20 or 40 mg/kg) exhibited increased open-field activity when compared with controls\(^10\) and this effect persists into adulthood. The result obtained from the present study in the open-field arena corroborate well with findings of other worker\(^10\). This abnormal increase in locomotor activity of PHB treated animals may be attributed to slower habituation to the novel environment as has been also suggested by other workers.\(^7,10\). Abnormally increased activity such as ambulation, rearings, defecation and urination represents more primitive responses on the novel test situation and is considered to be an index of increased emotionality.\(^26\). Prenatal HAL treated rats also exhibited enhanced anxiety state on other paradigms of anxiety viz. elevated plus-maze, and elevated zero-maze tests. Unlike the open-field exploratory behaviour, these rats showed hypoactivity in centre and hyperactivity in the periphery of the tunnel-board entry test indicating increased state of anxiety (Unpublished data). The literature on the influence of prenatal PHB exposure on the offsprings' emotional behaviour is scanty. However, in our laboratory increased state of anxiety have been reported in offsprings treated prenatally with diazepam\(^15\), haloperidol\(^16,37\).

### Table 2—Effect of prenatal phenobarbitone administration on elevated plus-maze behaviour in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time spent on (sec)</th>
<th>Responses on elevated plus-maze</th>
<th>Ratio of open/enclosed arms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Open arms (N)</td>
<td>Enclosed arms (N)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Time (sec)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Entries (N)</td>
</tr>
<tr>
<td>Control (vehicle)</td>
<td>7</td>
<td>71.14±22.64</td>
<td>140.70±28.00</td>
<td>4.43±1.27</td>
</tr>
<tr>
<td>PHB (10 mg/kg)</td>
<td>8</td>
<td>32.54±13.19</td>
<td>186.95±20.79</td>
<td>3.63±1.06</td>
</tr>
</tbody>
</table>

P value: *<0.05; **<0.01; ***<0.001 in comparison to control respectively.

### Table 3—Effect of prenatal phenobarbitone treatment on elevated zero-maze responses in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time spent on open arms (N)</th>
<th>No. of head dips (N)</th>
<th>Stretched atlanal postures (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Vehicle)</td>
<td>7</td>
<td>87.50±8.33</td>
<td>13.00±2.58</td>
<td>5.00±1.41</td>
</tr>
<tr>
<td>PHB (10 mg/kg)</td>
<td>7</td>
<td>63.09±9.94</td>
<td>8.14±2.19</td>
<td>2.43±1.44</td>
</tr>
</tbody>
</table>

P value: **<0.01; ***<0.001; in comparison to control respectively.
The paradigms used in this study have been subjected to through critical appraisal and validated as animal models of anxiety.

Thus, the available reports both on prenatal or postnatal exposure to PHB indicate that it may adversely affect the central nervous system if administered during critical period of brain development. Day 12 to 21 of pregnancy in rats appears to be most vulnerable to action of neuroactive drugs because this is the critical period for neurogenesis, organogenesis, synaptogenesis and formation of specific neural circuits, rapid cell proliferation and functional maturation of central dopaminergic system. It is therefore likely that phenobarbitone or its metabolites directly interfere the neurogenesis of the developing brain regions thus resulting in long-lasting overt behavioural responses.

The mechanism by which anticonvulsants decrease neuronal survival and in otherways adversely affect the developing nervous system is perhaps through blocking the action of a trophic substance. These trophic molecules or growth factors play an important role in development of CNS.

The relationship between behavioural abnormalities and possible alterations in neurotransmitters level is not clearly understood. However, brain levels of nucleic acids and proteins are slightly reduced in young offsprings of mice injected with phenobarbitone. The prenatal PHB exposure to pregnant dams did not alter brain concentrations of dopamine or norepinephrine in the adult mice offsprings, but at age of day 21 (PND 21) induced alterations in whole brain levels of dopamine and norepinephrine. Iser and Yanai found long-lasting alteration in dopamine receptors after prenatal exposure to phenobarbitone. Mice exposed to phenobarbital during prenatal period (GD 9-18) had a long term reduction in the level of hypothalamic NE and DA at the age of PND 50. Since these results are contradictory, further studies required to correlate the prenatal barbiturate induced neurochemical changes with behavioural and neurornorphological alterations which may explain the possible mechanism(s).

However, these biochemical and morphological alterations may lead to functional deficits in CNS resulting in behavioural changes in rat offsprings. Although caution must be taken in extrapolation of data from animal studies to human beings, however, clinical studies have also shown that prenatal drug exposure results in behavioural characteristics of a hyperkinetic child, excitement, irritability, tearfulness and aggression.

The present study indicates that prenatal exposure to PHB during critical period of brain development may adversely affect the behaviour of the offsprings. Hence, PHB and possibly other anticonvulsant drugs can be said to induce psychotraumatological effects in offsprings if administered during critical period of gestation.

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References
