Effect of prenatal alprazolam exposure on anxiety patterns in rat offspring

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Prenatal alprazolam (APZ) treatment in 0.1 and 0.2 mg/kg/day doses during 13-20 days of gestation induced significant increase in open-field ambulation, rearings, self-grooming and faecal pellets in rat offspring. Prenatal APZ treated rats displayed significantly increased anxiogenic behaviour on elevated plus maze (spent less time on open arms, more time on enclosed arms and made less number of entries on open arms) and increased anxiogenecity on elevated zero maze (APZ treated rats spent less time on open arms and made less number of head dips and stretched attend postures in comparison to control rat offspring). The results indicate persistent behavioural alterations in the rat offspring after prenatal exposure to APZ.

The benzodiazepines (BDZs) anxiolytics are the most widely prescribed psychoactive drugs and rank among the most commonly used class of therapeutic agents. Also, BDZ anxiolytics are widely prescribed during pregnancy, with 30 to 40% of all pregnant women being given antianxiety drugs at some stage of pregnancy. Prenatal exposure to BDZs induces a variety of functional disturbances. In general, motor activity, exploratory behaviour, learning, anxiety and operant responses may be altered in adult animals exposed in utero to BDZs like diazepam, chlordiazepoxide, and lorazepam, etc.

Alprazolam (APZ) is a triazolobenzodiazepine, which like other BDZs has antianxiety, anticonvulsant and muscle relaxant properties. In contrast to other BDZs, it has been shown in placebo-controlled trials to have antidepressant properties. Sedation is the most common side effect of APZ and it is directly related to its dosage. Now-a-days APZ is a drug of choice for the management of anxiety disorder and panic disorder with or without agoraphobia. There are some reports, which indicate that APZ use during pregnancy may induce teratogenic effects like cleft palate, pyloric stenosis, moderate tongue-tie, umbilical hernia and ankle inversion and clubfoot. Mice exposed prenatally to APZ have been reported to exhibit increased individual rather than group activity and male aggression.

The literature on the behavioural teratological effects of prenatal exposure to APZ is almost scanty though the available reports indicate that prenatal APZ exposure is not devoid of teratological effects. As such, the present investigation has been planned to assess the effects of prenatal APZ treatment in rats on the behaviour of rat offspring on various parameters of anxiety.

Materials and Methods

Animals and general procedure—Primiparous Charles Foster (CF) rats were used. Rats were acclimatized to the laboratory for a minimum of 20 days prior to drug administration and were maintained on a 12 hr light:dark cycle with rodent chow (Brook Bond, Lipton, India) and tap water available ad libitum. They were housed in standard polypropylene cages in groups of 4-5, at 25 °C ± 1 °C; 45-55 % RH.

All the pregnant rats (presence of sperm in vaginal swab was taken as day one of conception) were randomly assigned to four maternal drug treatments: Alprazolam 0.1, 0.2 mg/kg and control groups. Alprazolam (Alprax, Torrent Pharmaceuticals, Ltd, India) solution was prepared in 0.2% carboxy methyl cellulose (CMC) suspension. Pregnant females were administered APZ 0.1 or 0.2 mg/kg/day orally with the help of an orogastric cannula during gestation days 13 to 20 this being the critical period for neural development in rats. Pregnant control rats were similarly treated with vehicle i.e., 0.2% CMC. Beginning with the morning of day 21, the gravid rats were checked twice daily for deliveries. Animals were allowed to deliver normally and newborns were culled to 8 pups/dam and were foster nursed. The pups were weighed weekly and weaned at three weeks of age. Thereafter, at eight weeks of age, one male rat pup from each litter was randomly selected to form different
treatment groups to control the possible litter effects on behavioural measure. These rat offspring were subjected to following behavioural tests of anxiety at 8 weeks of age.

**Behavioural testing**

1. **Open-field test (OFT)** —The open-field apparatus was made of plywood and consisted of squares (61 x 61 cm) with high walls (61 x 61 cm). The entire apparatus was painted black except for 6 mm thick white lines, which divided the floor onto 16 squares. Open-field was lighted by a 40W bulb focussing onto the field from a height of about 100 cm. The entire room, except the open-field, was kept dark during the experiment. Each animal was centrally placed in the test apparatus for 5 min and the following behavioural aspects were noted:
   
   (a) Ambulation: this was measured in terms of the number of squares crossed by the animal;
   
   (b) Rearings: number of times the animal stood on its hind limbs;
   
   (c) Self groomings: number of times the animal groomed its facial region, and licked / washed / scratched various parts of its body;
   
   (d) Activity in centre: number of central squares crossed by the animal; and,
   
   (e) Faecal droppings: number of faecal droppings excreted during the period.

2. **Elevated plus-maze test (EPM)** —The maze had two opposite open arms, 50 x 10 cm, crossed with two enclosed arms of the same dimension but having 40 cm high walls. The arms were connected with a central square, 10 x 10 cm, giving the apparatus shape of a plus sign. The maze was kept in a dimly lit room and elevated 50 cm above the floor. Naive rats were placed individually in centre of the maze, facing an enclosed arm. Thereafter, number of entries and time spent on the open and enclosed arms were recorded during the next 5 min. An arm entry was defined when all four paws of the rat were in the arm. A neutral 'blind' observer made observations.

3. **Elevated zero-maze test (EZM)** —The maze comprised a black perspex annular platform (105 cm in diam, 10 cm width) elevated to 65 cm above the ground level, divided 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 the remaining two opposite quadrants were surrounded by perspex "lip" (1 cm high) which served as a tactile guide to animals on these open areas. The apparatus was illuminated by dim white 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. The maze was cleaned with 5% ethanol/water solution and dried thoroughly between test sessions. During the 5 min test period, time spent on open arms, number of 'head dips' over the edges 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 only when all four paws had passed the open-closed divide.

**Statistical analyses** —The data are expressed as means ± SD for each treatment group. The data obtained from each response measures were subjected to Kruskal-Wallis one way analysis of variance (ANOVA) and inter group comparisons were made by Mann-Whitney U-test for only those responses which yielded significant treatment effects in the ANOVA test.

**Results**

*Open-field behaviour* —Rat offspring treated prenatally with APZ displayed significantly increased open-field ambulation, self groomings and faecal pellets in comparison to control rat offspring (Table 1). However, open-field rearing behaviour in rat offspring was found to be significantly increased only by APZ (0.1mg) exposure.

*Elevated plus maze behaviour* —The results of the elevated plus maze behaviour test indicate that rat offspring exposed to APZ (0.1 and 0.2 mg) during gestation spent significantly less time on open arms and more time on enclosed arms and made significantly less number of entries on open arms in comparison to control rat offspring (Table 2). However rat offspring treated with APZ did not differ significantly from controls with respect to enclosed arm entries.

*Elevated zero maze behaviour* —Rat offspring treated with APZ (0.1 and 0.2 mg) spent significantly less time on open arms as well as made significantly less number of head dips and stretched attend postures in comparison to control rat offspring (Table 3). Moreover, behaviour of rat offspring of APZ (0.1 mg/kg) treatment group on all the parameters did not differ significantly from those of APZ (0.2 mg/kg) treatment group.
Discussion

There is only one report on the psychotrophic effects of prenatal APZ treatment in mice: APZ exposure on gestation day 18 increased the tendency in offspring for individual rather than group activity and male aggression\textsuperscript{15}. Direct comparisons of the findings of the present study are not possible because of paucity of studies on behavioural effects of prenatal APZ exposure in both experimental and clinical settings. However, prenatal APZ treatment in higher doses (3 mg/kg and above) has been found to increase embryo mortality and fetal development in rabbits\textsuperscript{22}. Further, APZ exposure (0.32 mg/kg) on gestation day 18 in mice has been found to affect reproductive performance in the offspring\textsuperscript{23}.

Prenatal exposure to diazepam, another BDZ anxiolytic, has been reported to increase open-field behaviour\textsuperscript{1} and reduced locomotion in rodents\textsuperscript{24}. Prenatal exposure to lorazepam during days 13 to 20 of gestation increased open-field activity at 3 weeks of age in mice\textsuperscript{3}. Prenatal phenobarbitone, alcohol\textsuperscript{25} and haloperidol\textsuperscript{26} treatments have also been found to increase open-field activity in rodent offspring. This abnormal increase in open-field activity of treated animals may be due to slower habituation to the novel environment\textsuperscript{27}.

Contradictory reports are also available on the effects of prenatal benzodiazepine exposure on anxiety patterns in rodent offspring i.e., it reduced and increased social interaction in rat offspring respectively in familiar and unfamiliar environments in comparison to vehicle treatment\textsuperscript{28}. However, increased anxiety state in rat offspring on elevated plus maze, elevated zero maze and social interaction tests due to prenatal exposure to diazepam (10 mg/kg/day) during gestation days 13 to 20 is also on record\textsuperscript{1}.

There is little information on prenatal exposure to BDZs and changes in neurotransmitter activity. However, it is now clear that early developmental exposure to BDZs can induce long-lasting effects on CNS and on behavioural functions in animal models. From both neurochemical and behavioural observations following BDZs exposure, the most sensitive

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Ambulation</th>
<th>Rearing s</th>
<th>Self-Groomings</th>
<th>Faecal pellets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>8</td>
<td>70.1±3.5</td>
<td>21.3±4.5</td>
<td>4.1±1.4</td>
<td>1.9±1.1</td>
</tr>
<tr>
<td>APZ (0.1mg)</td>
<td>8</td>
<td>85.6±3.7</td>
<td>27.5±3.7</td>
<td>5.1±2.1</td>
<td>3.9±1.3</td>
</tr>
<tr>
<td>APZ (0.2mg)</td>
<td>8</td>
<td>88.2±4.2</td>
<td>25.0±4.3</td>
<td>8.7±0.9</td>
<td>5.4±1.4</td>
</tr>
</tbody>
</table>

Superscripts a and b indicate statistical significance respectively in comparison to control and APZ (0.1 mg). a, b and aa, bb denote P<0.05 and 0.01 respectively (Mann-Whitney U-).

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Time spent on (in sec)</th>
<th>Entries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>8</td>
<td>26.0±8.4</td>
<td>214.9±13.9</td>
</tr>
<tr>
<td>APZ (0.1mg)</td>
<td>8</td>
<td>11.8±3.9</td>
<td>241.3±13.0</td>
</tr>
<tr>
<td>APZ (0.2mg)</td>
<td>8</td>
<td>8.7±6.3</td>
<td>260.8±17.8</td>
</tr>
</tbody>
</table>

Superscripts a and b indicate statistical significance respectively in comparison to control and APZ (0.1 mg). a, b and aa, bb denote P<0.05 and 0.01 respectively (Mann-Whitney U-).

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Time spent on open arms</th>
<th>Entries on open arms</th>
<th>Head dips</th>
<th>Stretched attend postures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>8</td>
<td>26.8±8.2</td>
<td>3.6±1.6</td>
<td>4.5±1.4</td>
<td>6.0±0.7</td>
</tr>
<tr>
<td>APZ (0.1mg)</td>
<td>8</td>
<td>8.9±2.3</td>
<td>2.2±0.8</td>
<td>2.2±1.0</td>
<td>5.8±1.6</td>
</tr>
<tr>
<td>APZ (0.2mg)</td>
<td>8</td>
<td>12.0±2.9</td>
<td>1.8±0.5</td>
<td>1.8±0.8</td>
<td>4.7±0.9</td>
</tr>
</tbody>
</table>

Superscripts a and b indicate statistical significance respectively in comparison to control and APZ (0.1 mg). a, b and aa, bb denote P<0.05 and 0.01 respectively (Mann-Whitney U-).
period for inducing long-term effects of prenatal drug exposure appears to be the 3rd week of gestation in rats. This period appears to be the most vulnerable to the action of neuroactive drugs because this is the critical period for synaptogenesis and formation of neural circuits. Developmentally, BDZ receptors have been identified by the beginning of the third week of gestation in the rat. Autoradiographic studies indicate that BDZ receptors appear earliest in the brain stem and hypothalamus in the rat, but by birth BDZ binding is present in all areas of the CNS. Similarly development of GABA and catecholamine receptors also takes place during the 3rd week of gestation in rats. Acetylcholine, y-aminobutyric acid and catecholamine neurotransmitters also appear during this period. GABA is the major inhibitory transmitter in the CNS and alteration in neurotransmission of such a widely distributed system during critical stages of development may considerably affect the developing CNS. Prenatal BDZ exposure during third week of gestation appears to induce altered binding of BDZ in the offspring and a supersensitivity phenomenon at the level of BDZ-GABA complex has been consistently reported. Gestational exposure to diazepam has been reported to reduce cerebellar and cortical norepinephrine levels. The effects of early diazepam exposure on the NE projection to the hypothalamus were not apparent until after 5 weeks of age and functional activity within the hypothalamic NE system is normally delayed. Various functional deficits have been reported in rodent offspring following prenatal BDZ exposure, including alterations in stress responses, EEG synchronization, startle response and complex maze learning tasks. Studies have also documented reduced number of glial cells in the cortex of the offspring following prenatal diazepam exposure, indicating neural degeneration. These observations suggested that prenatal BDZ exposure interferes with the normal organization or development of specific neural systems or mechanisms responsible for mediation of various behavioural functions.

The results of the present study indicate that the effects of prenatal APZ treatment or early life may be different from those of anxiolytic/antistress/anti-depressant effects in animals. The clinical studies have also shown that prenatal drug exposure results in behavioural characteristics of a hyperkinetic child, a syndrome that includes excessive motor activity, excitement, irritability, tearfulness and aggression. It has been suggested that because of early insults, appropriate neural changes that should normally take place during adolescence may not occur, thereby interfering with the acquisition of mature behaviours.

In support of this suggestion, an influence of prenatal alcohol exposure in the development of schizophrenia almost 20 years after birth has been suggested.

The present investigation indicates that prenatal exposure to APZ during critical developmental period of brain in rat can adversely affect the behaviour of the progeny and hence APZ can be said to induce behavioural teratological effects.

References