Hyperglycaemia in pregnancy: Effects on the offspring behaviour with special reference to anxiety paradigms

M. Ramanathan, Arun K Jaiswal & Salil K Bhattacharya*

Neuropharmacology Laboratory, Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, 221 005, India.

Received 31 May 1999; revised 1 December 1999

Maternal hyperglycemic effect was studied on the offspring behaviour. Offspring were obtained from diabetic rats by mating a normal father with a diabetic mother (NFDM), diabetic father with normal mother (DFNM) and diabetic father with diabetic mother (DFDM). Rats were rendered diabetic by injecting streptozotocin (STZ, 50 mg/kg IP) in citrate buffer.

Offspring were subjected to various anxiety parameters including open field exploratory behaviour, elevated plus maze and zero maze behaviours, and the social interaction tests at the age of 8 weeks. The results indicate that offspring of NFDM and DFDM showed anxiogenic activity on the elevated plus maze zero maze and the social interaction test. Offspring of NFDM and DFDM exhibited hyper and emotional activity in the open field behaviour test. The behavioural alterations observed in the offspring were comparable to the behavioural alterations noted in STZ diabetic rats as reported earlier. Further offspring of NFDM and DFDM exhibited mild hyperglycaemia. No significant behavioural alterations in the offspring of DFNM were observed. It may be concluded, that exposure of offspring to diabetic environment in their foetal life can lead to anxiogenic/emotional behaviours in adult life.

Although there are number of experimental studies and clinical reports concerning teratogenic effect of diabetes mellitus during pregnancy, there is little information on effects on postnatal development and behaviour. Recently Seshiah1 reviewed maternal hyperglycemic effects on the fetus. Apart from development of foetal pancreatic beta cell mass and congenital malformations, impaired intellectual performance, macrosomia, foetal cardiac arrhythmia, intrauterine death etc., have been noted. In experimental conditions, prenatal exposure to a glucose rich environment produced congenital deformities such as incomplete sacral ossification, caudal dysgenesis, retarded growth, hyperglycaemia and delayed lung maturation in the offspring2-4. In addition, diabetic environment led to increased antioxidant profile in the embryos5. However, the available reports do not document behavioural alterations in the offspring of diabetic parent animals, though some studies have indicated hyperglycaemia, increased antioxidant profile and congenital deformities. We recently reported decreased, dopamine (DA), increased serotonin (5HT) and norepinephrine (NE) levels in the diabetic rats6. Administration of DA receptor antagonists, anxiolytics7,8 and 5HT neuronal re-uptake antidepressants9 during pregnancy were found to affect rat offspring behaviour in later life. Forty percent offspring of non-insulin dependent diabetes mellitus parents develop diabetic complications in their adult age10. In a clinical study, 71% of diabetic patients had a life time history of atleast one psychiatric episode (increased risk of affective and anxiety disorders) and 33% of the patients had major depression11. The present study was planned to assess the anxiety behaviour patterns in the offspring of diabetic rats. Diabetic rats exhibit anxiogenic behaviour12. The point of reference was to investigate the effect of prenatal hyperglycaemia on anxiety pattern in the progeny.

Materials and Methods

Subjects and induction of diabetes—Adult Charles Foster albino rats, of either sex (150-200 g), procured from Central Animal House of the Institute, were housed in groups of 4-5 in colony cages at an ambient temperature of 25±2°C and 45-55% relative humidity with 12 hr light/dark cycle. They had free access to pellet chow (Brook Bond Lipton, India) and water. Rats were rendered diabetic by injecting streptozotocin (STZ, Sigma USA) (50 mg/kg IP) in citrate buffer (pH 4.5). Control animals received equal volume of citrate buffer only. Rats were kept for mating after one week of STZ Injection. Only those animals, which showed blood glucose level between 14-16 mmol/L, were used. Offspring from diabetic
rats were obtained by mating diabetic father with normal mother (DFNM), normal father with diabetic mother (NFDM) and diabetic father with diabetic mother (DFDM). Control offspring were collected from normal father and normal mother (NFNM). Female rats were separated on day 1 of gestation as evidenced by the presence of sperm in vaginal swab. At birth, the litters were culled to 8 pups per dam and were foster nursed by non-diabetic materialized lactating mothers. The pups were weaned at 21-22 days of age and at 8 weeks of the age the offspring of control and diabetic rats were subjected to behavioural testing. Blood glucose levels of offspring and diabetic parents were measured in tail vein blood sample by the GOD/POD method. All the experiments were performed between 0900 and 1400 hrs.

**Behavioural experiments**

**Open field exploratory behaviour test**—An open field apparatus similar to that of Bronstein was used to study the open field exploratory behaviour of rats. It was made of plywood and consists of a square (61x61cm) with high walls (61 cm). The entire apparatus was painted black except for 6 mm white lines that divided the floor into 16 squares. The open field was lighted by a 100 W bulb focusing onto the field from a height of about 100 cm from the floor. 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 of anxiety were recorded:

i) Ambulation—this was measured in terms of the number of squares crossed by the animal,

ii) Rearing—by counting the number of times the animal stood on its hind limbs,

iii) Self grooming—number of times the animal made these responses viz. grooming of the face, licking/washing and scratching the various parts of the body,

iv) Defecation—the number of fecal boli excreted during the period,

v) Activity in center—number of central squares crossed by the animal. Wherever required ratio (central squares crossed / peripheral squares crossed) between the central and peripheral squares crossed were calculated.

**Elevated plus maze behaviour test**—The plus maze consisted of two opposite arms (50x10 cm) crossed with two opposite enclosed arms of the same dimension with 40 cm high. The arms were connected with a central square (10x10 cm) to give the apparatus a plus sign appearance. The maze was kept elevated 50 cm above the floor in a dimly lit room. The rats were individually placed on the central square of the plus maze facing an enclosed arm. The time spent and number of entries made by the rat, during the next 5 min, on open and closed arms were recorded. An arm entry was defined when all the four limbs were on the arm.

**Elevated zero maze behaviour test**—The maze comprised of an annular black perplex platform (105 cm diam. 10 cm width) elevated 65 cm above the ground level and divided equally into four quadrants. The two opposite quadrants were enclosed by black perplex walls (27 cm high) on both the inner and outer edges of the platform, while the remaining two opposite open quadrants were surrounded only a perplex “lip” (1 cm high) which serve as a tactile guide to animals on these open arms. The apparatus was illuminated by indirect dim white light arranged in such a manner to provide similar lux levels in open and closed quadrants. Rats were placed in one of the closed quadrants for a 5 min test period. During the 5 min test period, time spent on open quadrants, number of ‘head dips’ over the edge of the platform, and number of ‘stretched attend postures’ from enclosed to open quadrants were recorded. Animals were scored as being in the open area when all the four paws were in the open quadrants and in the enclosed area only when all four paws had passed the open-closed divide.

**Social interaction test**—Rats were housed singly for 5 days prior to the test. The social interaction arena was a dimly lit wooden box (60x60x35 cm) with a solid floor. The rats received two 7.5 min familiarization sessions individually, at an interval of 1 hr, 24 hr before final testing. The next day, rats of same sex were paired on weight basis and placed on the test arena for 7.5 min. The time spent by the rat pair in active social interaction characterized by sniffing, following, grooming, kicking, boxing or crawling over or under the partner was scored.

All the apparatus were cleaned with 5% ethanol/water solution and dried thoroughly between the sessions. A neutral blind observer made observations unaware of the nature of treatment given to the animals.

**Statistical analyses**—The data are expressed as mean ± SE and were subjected to the non-parametric Kruskal-Wallis one way ANOVA followed by Mann-Whitney U-test for inter groups comparisons.
Results

STZ (50 mg/kg) produced hyperglycaemia after 72 hr. Offspring of NFDM and DFDM experienced significant mild hyperglycaemia and it maintained up to a recorded 6 months of age. Offspring of DFNM were found to be euglycemic (Table 1).

Open field exploratory behaviour test—Offspring of diabetic rats NFDM and DFDM exhibited significantly increased ambulation, urination and no alteration in the grooming behaviour and fecal pellets level in comparison to control and STZ diabetic rats. No significant change was noticed in the central square activity in all the groups. However, peripheral ambulation, and the ratio between peripheral and central ambulation were found to be significantly increased and decreased, respectively, in all the groups. No significant change in the open field behaviour of DFNM rats was observed in comparison to control offspring. Unlike offspring, STZ diabetic rats exhibited decreased ambulatory/exploratory behaviour (Table 2).

Elevated plus maze behaviour test—Offspring of diabetic rats NFDM and DFDM exhibited decreased number of entries on open arms and total entries, more time spent on enclosed arms and decreased time spent on open arms in comparison to control rats. The time and entries ratio were also found to be reduced in DFDM and NFDM groups in comparison to control rats, which indicate anxiogenic behaviour in these rats. Further offspring of DFDM and NFDM groups did not differ significantly in the activity in comparison to STZ diabetic rats (Table 3).

Elevated zero maze behaviour test—Offspring of NFDM and DFDM groups spent significantly less time in the open quadrants and made less number of head dips in comparison to offspring of non-diabetic rats, which indicate anxiogenic behaviour in the offspring of diabetic rats. Stretched attend posture remained unaltered. Further, stretched attend postures and time spent in open quadrants in these groups did not differ significantly from STZ diabetic rats whereas number of head dips was significantly reduced. No significant difference was observed in the zero maze behaviour of DFNM rats offspring in comparison to control offspring (Table 4).

Social interaction test—Social interaction time in a novel environment of the paired offspring of diabetic rats was found to be significantly reduced (DFDM 175.98±11.75; NFDM 181.45±13.55) in comparison to control rats (216.09±10.24) indicating anxiogenicity. However, these offspring exhibited significantly more interaction time in comparison to STZ diabetic rats (81.69±8.89). The interaction time in DFNM rat offspring remains unaltered (198.96±12.75).

Discussion

The results indicate that offspring of diabetic rats exhibited anxiogenic patterns on elevated plus maze as indicated by decreased number of entries on open arms and total entries, more time spent on enclosed arms and decreased time spent on open arms in comparison to control rats. Further offspring of DFDM and NFDM groups did not differ significantly in the activity in comparison to STZ diabetic rats (Table 3).

Table 1—Blood glucose level and body weight of the offspring of STZ diabetic and non-diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Blood glucose level (mmol/L)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STZ diabetic rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Offspring</td>
<td>11</td>
<td>15.27±0.70</td>
<td>183.3±17.35</td>
</tr>
<tr>
<td>Control (NFNM)</td>
<td>15</td>
<td>4.44±0.72</td>
<td>217.8±9.9</td>
</tr>
<tr>
<td>DFNM</td>
<td>8</td>
<td>5.54±0.46</td>
<td>212.3±10.0</td>
</tr>
<tr>
<td>NFDM</td>
<td>13</td>
<td>6.94±0.57</td>
<td>175.0±15.6</td>
</tr>
<tr>
<td>DFDM</td>
<td>13</td>
<td>7.98±0.21</td>
<td>155.6±10.3</td>
</tr>
</tbody>
</table>

Table 2—Open field exploratory behaviour in offspring of STZ diabetic and non-diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Peripheral</th>
<th>Ambulation</th>
<th>Central</th>
<th>Ratio</th>
<th>Rearing</th>
<th>Grooming</th>
<th>Fecal boli</th>
<th>% Urination</th>
</tr>
</thead>
<tbody>
<tr>
<td>STZ diabetic rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Offspring</td>
<td>46.15±3.68</td>
<td>3.38±0.34</td>
<td>0.07±0.01</td>
<td>9.08±1.07</td>
<td>4.16±0.87</td>
<td>3.07±0.73</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Control (NFNM)</td>
<td>68.63±3.09</td>
<td>8.63±0.90</td>
<td>0.11±0.01</td>
<td>19.88±1.22</td>
<td>3.25±0.59</td>
<td>5.57±0.39</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>DFNM</td>
<td>79.63±6.63</td>
<td>11.60±1.63</td>
<td>0.11±0.02</td>
<td>24.60±4.02</td>
<td>4.20±1.24</td>
<td>5.84±0.37</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>NFDM</td>
<td>117.54±7.69</td>
<td>9.00±0.85</td>
<td>0.07±0.01</td>
<td>24.62±2.93</td>
<td>3.62±0.55</td>
<td>3.85±0.79</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>DFDM</td>
<td>109.42±5.77</td>
<td>6.17±0.52</td>
<td>0.06±0.03</td>
<td>29.25±1.68</td>
<td>4.08±0.57</td>
<td>4.17±0.96</td>
<td>0.58</td>
<td></td>
</tr>
</tbody>
</table>

Figures in parentheses indicate number of animals

Superscripts * and ** indicate statistical significance in comparison to STZ diabetic rats and control (NFNM) rats. a,b and a,b,b denote P<0.05 and P<0.01 respectively.
plus maze, zero maze, social interaction tests. These behavioural alterations have also been noted in diabetic rats. However, offspring of diabetic rats displayed increased locomotor/exploratory activity in the open field behaviour test unlike diabetic rats that exhibited decreased ambulatory behaviour in the open field. Though the offspring of DFDM and NFDM groups showed hyperactivity in the open field, their field activity was confined to the peripheral area of the open field. Further, urination was increased which can be interpreted as increased emotional/anxious behaviour. All these behavioural responses have been critically validated for congenital malformation, hypertropic cardiomyopathy, placental insufficiency, pre-eclampsia, macrosomia, foetal intrauterine death, caudal dysgenesis, defective sacral ossification, etc.

Earlier reports indicate that offspring of diabetic rats show congenital malformation, hypertropic cardiomyopathy, placental insufficiency, pre-eclampsia, macrosomia, foetal intrauterine death, caudal dysgenesis, defective sacral ossification, etc. No such congenital perturbations were noted in present study, possibly because of the mild levels of hyperglycaemia in the parents.

Abnormally increased activity in the open field such as ambulation, rearing, defecation and urination represent more primitive responses and are considered to be an indices of increased emotional/anxious behaviour. All these behavioural alterations may be attributed to altered neurotransmitter levels. Although no experimental data is available at present in the offspring of diabetic rats, it is possible that increased 5HT and NE levels may affect the regulation of foetal brain SHT levels, may affect the regulation of foetal brain SHT levels, may affect the regulation of foetal brain SHT levels, may affect the regulation of foetal brain SHT levels.
and consequently the normal maturation of brain 5HT pathways. These changes may thus produce neurochemical and functional alterations in pre- and post-synaptic components of brain 5HT pathways in the rat progeny. Prenatal treatment with a DA antagonist led to decreased number of DA neurons in substantia nigra and ventral tegmental areas in the offspring. Hence, decreased DA and increased NE in the substantia nigra and ventral tegmental areas in the offspring of diabetic rats may similarly contribute to the behavioural alterations in offspring.

The mild hyperglycaemia (6.96-7.98 mmol/L) observed in the offspring of diabetic rat persisted up to the age of 6 months. This chronic hyperglycaemia which is present fairly for a long duration in the life of offspring, may also cause neuronal changes leading to behavioural alterations. It has been suggested that, oxygen free radicals were involved in the high incidents of foetal dysmorphogenesis associated with diabetic pregnancies. Embryos exposed to glucose rich environment have shown increased antioxidant profile, indicating oxidative stress in these animals. The observed mild hyperglycaemia and altered antioxidant profile in the offspring of diabetic rats may also contribute the altered behaviour.

It can be concluded that, offspring of diabetic rats exhibit mild hyperglycaemia and anxiogenic behavioural pattern in various behaviour parameters tested. The anxiogenic behaviour of the offspring was comparable to that of STZ induced diabetic rats. The behavioural alterations observed in the offspring may be due to exposure to high glucose level, altered neurotransmitter activity and free radicals during their foetal and embryonic stages or may be due to the changes taking place during their development as a consequence of diabetes. Further studies are required to delineate the causes underlying behavioural alterations in diabetic offspring.

References


