Prenatal Phenobarbital Exposure Induced Developmental Changes In Rat Brain And Muscle

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ABSTRACT

Female pregnant rats were treated with Phenobarbital which is an antiepileptic drug and is used in the treatment of certain types of seizure and anxiety. Twenty rats were housed in standard cages, with 4 female and 1 male to a cage. A commercially prepared diet and clean drinking water were provided ad libitum. Female rats were divided into control and treated groups. The treated groups divided into two subgroups, were given 3g of phenobarbital with their feed in group1 and 4g of phenobarbital per kg of diet in group 2 from day 12 to 18 of the pregnancy (vaginal plug = 1 day). Control female rats were introduced equivalent amount of feed without phenobarbital. The offsprings from both control and treated groups were collected on day 21 and at the age of 12 weeks sacrificed with Co2. The body weight of each rat was determined at 3 and 12. The Biceps brachii, Triceps brachii, Soleus, Tibialis, Gastronemius muscle were dissected and were examined, weighed and further fixed in formalin for histomorphological examination. The brains were dissected out, on gross examination, they did not show any difference in morphology between control and treated groups. Histological observation of the treated brains showed dilatation of blood vessels and spongiform changes with scattered patches of gliosis around degenerated cellular mass. Phenobarbital caused widespread apoptotic neurodegeneration in the brains of rats. Subventricular zone was found to be thickened in comparison to the control brains. There was zenersk erosion and vacuoles in the muscle fibers. There were significant differences between the absolute weights and muscle mass indices of control and prenatally Phenobarbital-exposed groups. Serum Testestrone and estrogen was measured by a ELISA technique. There was marked reduction in serum Testestron level in rats in treatment groups. From the result obtained in this study its concluded that phenobarbital damaged the brain cells and subsequently affected the androgen level and muscle in rats prenatally fed with Phenobarbital.

Key words: Apoptosis, Brain, Antiepileptic, Rat.

Introduction

Seizure incidence during the neonatal period is higher than any other period in the lifespan [1], yet there is little knowledge about this period in terms of the effect of seizures or of the drugs used in their treatment. The fact that several antiepileptic drugs (AEDs) [2] induce pronounced apoptotic neuronal death in specific regions of the immature brain prompts a search for AEDs that may be devoid of this action [3]. Phenobarbital (previously known as phenobarbitone in the UK) belongs to a group of medicines called barbiturates. It is used to treat epilepsy and works by stabilising electrical activity in the brain [4]. The brain and nerves are made up of many nerve cells that communicate with each other through electrical signals. These signals must be carefully regulated for the brain and nerves to function properly. When abnormally rapid and repetitive electrical signals are released in the brain, the brain becomes over-stimulated and normal function is disturbed. This can result in fits or seizures [5]. Neurotransmitters are chemicals that are stored in nerve cells and are involved in transmitting messages between the nerve cells. GABA is a neurotransmitter that acts...
as a natural 'nerve-calming' agent. It helps keep the nerve activity in the brain in balance. Glutamate is a neurotransmitter that acts as a natural 'nerve-exciting' agent. It is released when electrical signals build up in nerve cells and subsequently excites more nerve cells. It is thought to play a key role in causing epileptic seizures [7].

Phenobarbital increases the activity of GABA and decreases the activity of glutamate in the brain. These actions help stabilise the electrical activity in the brain and prevent epileptic fits. Phenobarbital prevents epileptic fits by preventing the excessive electrical activity in the brain. It is thought to achieve this by affecting certain neurotransmitters in the brain [4].

The placenta offers no significant barrier to the passage of barbiturates to the foetus, Thus, these drugs become widely distributed in foetal tissues when consumed by dam during pregnancy [8] although the majority of children born to women with epilepsy are normal, they are at increased risk for malformations as well as for poor neuropsychological outcomes [9]. The risk of in utero exposure has to be measured against the risk of the underlying disease. Thus, understanding the magnitude and differential effects of AEDs on teratogenesis is important. Our studies are aimed at both of these issues.

Materials and Methods

Adult wistar rats of albino strain weighing 200 gms obtained from Animal house of the college of Urmia Veterinary school. Sixteen adult female rats and four adult male rats were used as breeding stock in this experiment. The rats were housed in standard cages, with 4 female and 1 male to a cage. A commercially prepared diet and clean drinking water were provided adlibitum. The female rats were divided into control and treated groups. The treated groups divided into two subgroups, were given 3g of phenobarbital with their feed in group 1 and 4g of phenobarbital per kg of diet in group 2 from day 12 to 18 of the pregnancy (vaginal plug = 1 day). This period was chosen based on earlier observation that the development of muscle and innervation of muscle fibers are completed on day 16th of gestation. From group 1 and group 2 females, a total of 24 male offsprings were randomly selected and equally allocated into 2 groups and constituted the prenatally phenobarbital exposed males. All the selected rats were maintained on commercially prepared diet. At 12 weeks of age the rats in all groups were sacrificed with CO2. The body weight of each rat was determined at 3 and 12. The Biceps brachii, Triceps brachii, Soleus, Tibialis, Gastronemius muscle were dissected and weighed. The brains were collected from both the groups and were subjected to histological study after staining the paraffin sections by haematoxylin and eosin stains. Serum Testestron and estrogen was measured by a ELISA technique.

Statistical Analysis:

The data obtained for the muscle were subjected to analysis of variance using F-ratio and Duncan’s New Multiple Range.

Results and Discussions

Hormonal Results:

The result of hormonal analysis is shown in Table 1. The normal range Testestron 5.4 and estrogen was 2.4ug/ml. The result of hormonal changes in different groups showed, marked suppression of serum Testestron to less than 60% of baseline values was a consistent and
highly significant predictor of androgen concentration (reduced to 2–7% that normal levels) and there was a correlation between the testosterone and estrogen deficiency.

Table 1. Comparison of hormonal changes in two groups.

<table>
<thead>
<tr>
<th>Hormonal Assay</th>
<th>Treatments</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ug/ml)</td>
<td>T1-Control</td>
<td>T2</td>
</tr>
<tr>
<td>4.60 ± 0.499^a</td>
<td>3.60 ± 0.168^b</td>
<td>2.80 ± 0.168</td>
</tr>
<tr>
<td>17-B Estradiol (ug/ml)</td>
<td>2.70 ± 0.213^a</td>
<td>233 ± 0.122^b</td>
</tr>
</tbody>
</table>

^a. Values in the same row and variable with no common superscript differ significantly. ^b: P<0.05, ^c: P<0.01, NS: Not Significant. ^1 Values are means of nine observations per treatment and mean ± S.E given for each measurement. ^2 T1 = control males, T2 = group I, T3=group II

Muscles:

The comparison of the absolute muscle weights of control and Phenobarbital exposed male rats showed significant differences between the groups. Comparison revealed that the absolute weights of soleus, tibialis cranialis and gastronemius muscles of the control males were significantly superior to those of the Phenobarbital exposed groups ( P<0.01). There were no significant findings between the absolute weight of the two treatment groups (p>0.05). The absolute weight of triceps brachii muscle of control males was significantly superior to that of the Phenobarbital exposed (P< 0.05) (Table 2). In this study pair of groups were compared using student "t" test. The results showed that, tibialis cranialis, (p<0.05) gastrocnemius (p<0.01) of control males weighed heavier than those of Phenobarbital-exposed males but the other muscles were no significant (p>0.05) (Table 3). The absolute muscle weights of control male rat was heavier than those of male rat in groups 1 and 2, triceps brachii (p<0.05), tibialis cranialis (p<0.01) gastrocnemius(p<0.01). This study also demonstrated the muscle mass indices of muscles were significant except bicep brachii. Histological examination of the muscle showed muscle fiber degeneration and regeneration. Some muscle fibers were normal in size and dark pink. Others, which were degenerating, were smaller and lighter pink. They were sometimes vacuolated and had central nuclei rather than the normal peripheral nuclei. Small fibers with a bluish tinge and enlarged nuclei were regenerating fibers. Some of the smaller fibers were cut into smaller pieces (fig.5).

Table 2. Comparison Of Body Weights (G) Of Three Groups Of Male Rats Of Phenobarbital-Exposed And Control, In Age Of 3 And 12 Week

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 Weeks</td>
</tr>
<tr>
<td>T1 Control</td>
<td>58.5 ± 0.29^a</td>
</tr>
<tr>
<td>T2- PhB</td>
<td>44.8 ± 0.22^a</td>
</tr>
<tr>
<td>T3</td>
<td>54.1 ± 0.2^b</td>
</tr>
<tr>
<td>SEM</td>
<td>0.245</td>
</tr>
<tr>
<td>Significance</td>
<td>*</td>
</tr>
</tbody>
</table>

^a. Values in the same row and variable with no common superscript differ significantly. ^b: P<0.05, ^c: P<0.01, NS: Not Significant. ^1 Values are means of six observations per treatment and their pooled SEM. ^2 T1 = control males, T2=PhB, Phenobarbital group 1., T3 = Phb group 2

Table 3. Comparison of muscle weights (mg) of three groups of male rats of control, phenobarbital-exposed, in age of 12 week.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1- Control</td>
<td>T2-phB</td>
</tr>
<tr>
<td>Biceps brachii</td>
<td>0.189 ± 0.78^a</td>
</tr>
<tr>
<td>Triceps brachii</td>
<td>0.468 ± 0.06^a</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.115 ± 0.02^a</td>
</tr>
<tr>
<td>Tibialis cranialis</td>
<td>0.433 ± 0.02^a</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>0.624 ± 0.31^a</td>
</tr>
</tbody>
</table>

^a. Values in the same row and variable with no common superscript differ significantly. ^b: P<0.05, ^c: P<0.01, NS: Not Significant. ^1 Values are means of six observations per treatment and their pooled SEM. Mean ± S.E given for each measurement. ^2 T1 = control males, T2 =PhB, phenobarbital group 1., T3 = Phb group 2
Brain:

Phenobarbital caused widespread apoptotic neurodegeneration in the brains of rats (Fig. 1). The microscopic examination of brain revealed various degree of neuron degeneration and necrosis to hemorrhages in different parts of the brain in phenobarbital exposed rats (Fig. 2). The area of necrosis in some area of the brain was large enough to see as vacuole (Fig. 4) included foci of hemorrhages accompanied with severe vasogenic and cytotoxic edema (Fig. 3), rarefaction and necrosis was observed in the cortical and medullary areas of brain hemisphere. The deteriorated regions were surrounded by gliosis were predominantly located in the white matter of the medullary layer more on the white mater (Fig. 1). Hemorrhages were visible either within the necrotic areas or were restricted to areas surrounding the cystic necrosis (fig. 3).

Fig. 1: Apoptotic neuronal cells in the thalamic region of the brain (H & E X 400)

Fig. 2. Congestion and perivascular cuffing (aggeregate of lymphocytes and glial cells in vrchaw robin space), necrotic neuronal cells in cortical region of the brain and hemorrhage in leptomeninge (H & E X 400)

Fig. 3: Vasogenic and cytotoxic edema in the brain of the rat exposed with Phenobarbital, (H & EX 400)
Fig. 4: Cavity and hemorrhage in the brain of rat exposed with Phenobarbital. (H & E X 400).

Fig. 5: The muscle fibers degenerated, vacuolated and some have central nuclei rather than the normal peripheral nuclei (H&Ex400).

Discussion:
Although there are a few reports about antiepileptic drug induced congenital malformations [1,14] but none of these authors reported brain and muscle anomalies induced by phenobarbital. Here we report that Phenobarbital one of the major AEDs cause sensitive neurons to undergo apoptotic death in the developing rat forebrain. These findings apply to compounds that block voltage-gated sodium channels, enhance GABAergic inhibition, or block glutamatergic excitation. Neurotoxicity of AEDs is age-dependent and is associated with impairment of neurotrophin-mediated survival promoting signals in the brain [7]. The combination of AEDs with different modes of action results in a substantially higher apoptotic response compared with monotherapy.

The primary pathological lesion that was observed was congestion and edema in the brain. It may be possible that via Na⁺ channels, it affects the osmolarity of blood vessels, which interacts with various factors responsible at the time of growth and development of the affected fetuses. This activity explains the mechanism of cellular toxicity of treated rat brains. Programmed cell death (apoptosis), in contrast to necrosis, is characterized by uniform internucleosomal DNA fragmentation, nuclear shrinkage, chromatin compaction as well as cytoplasmatic condensation, and disintegration [15-16].

Numerous studies have provided evidence of an association between antiepileptic drugs (AEDs) and muscle in persons treated with. As well, numerous biochemical abnormalities have been described including hypocalcemia, hypophosphatemia, reduced levels of biologically active vitamin D metabolites, hyperparathyroidism [17]. However few studies have evaluated the affect of Phenobarbital on androgen and muscle in rat.

Serum concentrations of testosterone and estrogen during the postoperative period was much lower than basal line. All of the serum hormonal concentrations were significantly affected by the operation. In this retrospective
analysis, we demonstrated a significant association between lower serum level of androgen and brain damage in male rats.

Reduction in muscle mass of males prenatally exposed to Phenobarbital was reported by [18]. The smaller muscle mass of the phenobarbital-exposed group as observed from the analysis of the soleus muscle was due to a smaller number of muscle fibres being present than in the control group, since the muscle fibre sizes were similar in both groups. This indicated that prenatal administration of phenobarbital inhibits normal hyperplasia of muscle fibres. This reduction may be attributed to loss of influence of testosterone on muscles. The reduction in the muscle mass may have resulted hypothalamic neuronal losses in the prenatally – phenobarbital exposed mice. The histopathological study of the brain showed neuronal losses and vacuolation in brain tissues, [19] reported neuron necrosis in different region of the brain in mice. It was assumed that the prenatal exposure of rat to Phenobarbital may have resulted the destruction of neurons at hypothalamic levels, hence disrupting the hypothalamus – pituitary – Testis regulatory mechanism. Impairment in the production of the releasing factor by the hypothalamus, may adversely affect the testosterone – synthesizing ability of the the interstitial cells of Leydig whose function has been shown to be dependent on the stimulation by pituitary interstitial cell stimulating hormone [20]. Thus it is concluded that the clinicians should very carefully justify and prescribe the therapy of Phenobarbital especially to pregnant women.

References

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