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Pathological investigation on pre-natal use of Phenobarbital in the Rat Nervous and Muscular system

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Abstract

Phenobarbital, a barbiturate, is used as an asleep aid and in the treatment of certain types of seizure and anxiety. The placenta offers no significant barrier to the passage of barbiturate to the foetus. This study was carried out to investigate the pathological affect of Phenobarbital on the nervous system and subsequent retardation of muscle of biceps brachii, triceps brachii, soleus, tibialis cranialis, gastroneumius. Prenatal exposure of rats to Phenobarbital was accomplished in utero by giving their mothers food to which was added 3g Phenobarbital per kg feed.The microscopic pathology of brain revealed various degrees of degenerative and necrotic changes in different parts of brain. Big vacuoles, edema and hemorrhage were also noted. There were significant differences between the absolute weights and muscle mass indices of control and prenatally Phenobarbital-exposed groups.

It is concluded that prenatal administration of Phenobarbital had detrimental effects on rat nervous and muscular system.

Key words: Androgen, phenobarbital, necrosis, prenatal, rat

INTRODUCTION

A Phenobarbital is a barbiturate Is the most commun drug in the global medicine [1] and the oldest still commonly used. It also has sedative and hypnotic properties but, as with other barbiturates, has been superseded by the bezodiazepine for these indications. In more developed countries it is no longer recommended as a first-line medication; however it is relied on as an alternate when a patient fails to respond to treatment with more modern AED's (Anti-Epileptic-Drugs) [2]. It is still commonly used around the world to treat neonatal seizures.

During pregnancy, The placenta offers no significant barrier to the passage of barbiturates to the fetus, Thus, these drugs become widely distributed in fetal tissues when consumed by mother during pregnancy [3] although the majority of children born from women with epilepsy are normal, they are at increased risk for malformations as well as have poor neuropsychological outcomes [4] The risk of in-utero exposinghas to be measured against the risk of the underlying disease.Thus, understanding the magnitude and differential effects of AEDs on teratogenesis is considerable.

In vitro studies [5] has demonstrated that the secretion of testicular testosterone is in response to the stimulatory action of gonadotropines . The release of these gonadotropines from the pituary has been shown to be in response to stimulation of gland by hypothalamic luteinizing hormone releasing factor (LHRF).

Since Phenobarbital diffuses to all parts of central nervous system. It is possible that the neurons of the hypothalamus may be destroyed by this drug. Neuronal losses in the hypothalamus will disrupt the hypothalamus-pituitary-testis regulatory axis which results in loss of or considerable reduction in the testosterone synthesis in testicular interstitial cells of Leydig.

In the early 1940s, Albright and Reifenstein [6] were among the first to refer to the ant osteoporotic and anabolic properties of androgens. Testosterone has an anabolic effect and stimulates the growth of muscle [7]. Several studies have confirmed the testosterone increase muscle mass [8]. Strength and endurance, other studies have reported even more specific effects of testosterone in skeletal muscle [9]. The levator ani muscle, for example disappears during development of female rats, but can be maintained with testosterone administration [10].

The aim of this study was answer to the question; whether Phenobarbital can damage the brain cells and subsequent affect on the androgen and how androgens may affect muscle strength and provide protection for muscle growth.

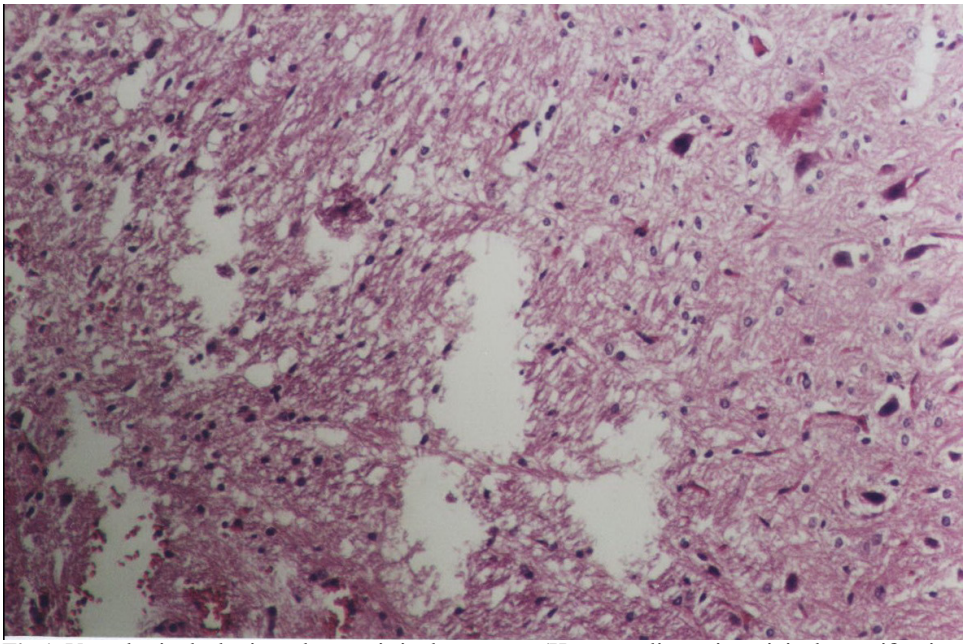

Fig 1. Vacuoles in the brain and necrosis in the neurons (Hematoxylin-eosin; original magnification &100).

MATERIAL AND METHODS

Twenty adult female rats and five adult rats were used as breeding stocks in this experiment. The rats were kept in standard cages, with 4 female and 1 male to a cage. A diet and drinking water were provided ad libitum.

Each Pregnant ratwere housed in cages, separately. Pregnancy diagnosis was based on the observation of vaginal plug in the morning following mating. Day one of pregnancy was assumed to correspond to the vagainal plug was observed. next the pregnant rats were divided into two groups. Twelve female rats comprised Group 1 and were given commercially prepared diet. Eight females made up group 2 and were given the same diet but 3g Phenobarbital was added per kg of diet from gestation day 8 to day 18. This period was chosen based on earlier observation that the development of muscle and innervations of muscle fibers are completed are completed on day 16th of gestation.

Following parturition, the litter size for each rat was limited to six until weaning at 21 days of age. From group 1 female, a total of 36 male offsprings were randomly selected and equally allocated into 2 groups. Control and casterated groups. Castration was done on all the males in the castrated group at 28 days of age. From group 2 females, 18 male offsprings were randomly selected and constituted the prenatally phenobarbital exposed males. All the selected rats were housed 3 to 4 cages according to study group and were maintained on commercially prepared diet. At 12 weeks of age the rats in all groups were sacrificed with CO2.

Quantitative Measurement

The body weight of each rat was determined at 3 and 12 wks of age. After sacrifice, the biceps brachii, soleus, and gastronemious , tibialis cranialis were carefully dissected out. The mean weight of the muscles was determined .Serum Testosterone was measured by ELISA kits.

Table 1. Comparison of body weights (g) of three groups of male rats of control, phenobarbital-exposed and castrated, in age of 3 and 12 week.

Groups	Age	
	3 week	12 week
T1- Control	60.5 ± 0.28[a]	199.25 ± 0.09[a]
T2- PhB	56.4 ± 0.24[b]	156.56 ± 0.05[b]
T3- C	54.1 ± 0.22[b]	160.15 ± 0.04[b]

[ab:] Values in the same row and variable with no common superscript differ significantly. P<0.05, P<0.01, NS: Not Significant. [1] Values are means of eighteen observations per treatment and their pooled SEM. [2] T1 = control males, T2 =PhB, phenobarbital male., T3 = C, castrated male

Table 2. Comparison of muscle weights (g) of three groups of male rats of control, phenobarbital-exposed and castrated, in age of 12 week.

Groups	Treatments				
	T1-Control	T2-PhB	T3-C	SEM	Significance
Biceps brachii	0.191 ± 0.047[a]	0 .081 ± 0.012[b]	0.115 ± 0.087[b]	0.724	**
Triceps brachii	0.620 ± 0.272[a]	0.234 ± 0.046[b]	0.229 ± 0.044[b]	0.080	*
Soleus	0.118± 0.043[a]	0.028± 0.008[b]	0.029 ± 0.008[b]	0.011	*
Tibialis cranialis	0.511± 0.063[a]	0.258± 0.014[b]	0.259 ± 0.016[b]	0.031	**
Gastrocnemius	0.740± 0. 116[a]	0.362 ± 0. 056[b]	0.366 ± 0.053[b]	0.342	**

[ab]: Values in the same row and variable with no common superscript differ significantly . * : P<0.05, **: P<0.01, NS: Not Significant. [1] Values are means of eighteen observations per treatment and their pooled SEM. Mean ± S.E giveu for each measurement. [2] T1 = control males, T2 =PhB, phenobarbital male., T3 = C, castrated male

Statistical Analysis

The data obtained for using SAS software by ANOVA test which were appropriate for a randomized complete block design (RCB) and when significant differences (p<0.05) were detected, means were compared post-hoc using the Duncan Multiple Range Test.

RESULTS

Hormonal Results

Serum Testosterone was measured inWistar rats on similar age and weight (12 wk old; n = 18) Compared with reported norms for male Wistar rats, serum testosterone levels showed for control group (5.40 ± 0.499 ug/ml), whereas serum testosterone levels measured for Phenobarbital- exposed group showed (3.30 ± 0.168 ug/ml) The result of hormonal changes showed significant differences in levels of Testosterone between Phenobarbital- exposed and control group (p<0.01). Serum Testosterone of the castrated male rats was measured (0.56 ± 0.09) because of to be sure that they are castrated.

Body Weight

The comparison of body weights of control, castrated or Phenobarbital exposed male mice showed significant differences, in groups at 3 weeks (P<0.05) and in groups of 12 weeks were presented (P<0.01) (Table1).

Muscles

The comparison of the absolute muscle weights of control and Phenobarbital exposed and castrated male rats significant differences between the groups. Comparison revealed that the absolute weights of bicep brachii, tibialis cranialis and gastronemius muscles of the control males were significantly superior to those of the Phenobarbital exposed and castrated group (P<0.01).The absolute weight of triceps and soleus muscle of control males was significantly superior to that of the castrated male and Phenobarbital exposed (P< 0.05) (Table 2).In this study pair of groups were compared using student “t” test. The results showed that , soleus (p<0.05) of control males weighted heavier than those of Phenobarbital- exposed males and the other muscles were significant (p<0.01) (Table 3). Absolute muscle weights of control male rat heavier than those of castrated male rat (Table 4). The

Table 3. Comparison of muscle weights (g) between two groups of male rats of control and phenobarbital-exposed with using student ‘T’ test. in age of 12 week.

Groups	Treatments			
	T1-Control	T2-PhB	T-test	Significance
Biceps brachii	0.191 ± 0.047[a]	0 .081 ± 0.012[b]	4.970	**
Triceps brachii	0.620 ± 0.272[a]	0.234 ± 0.046[b]	8	*
Soleus	0.118± 0.043[a]	0.028± 0.008[b]	4.569	*
Tibialis cranialis	0.511± 0.063[a]	0.258± 0.014[b]	8.849	**
Gastrocnemius	0.740± 0. 116[a]	0.362 ± 0. 056[b]	6.376	**

[ab]: Values in the same row and variable with no common superscript differ significantly . * : P<0.05, **: P<0.01, NS: Not Significant. [1] Values are means of eighteen observations per treatment and mean ± S.E giveu for each measurement. [2] T1 = control males, T2 =PhB, phenobarbital male

Table 4. Comparison of muscle weights (g) between two groups of male rats of control and castrated, in age of 12 week.

Groups	Treatments			
	T1-Control	T3-C	T-test	Significance
Biceps brachii	0.191 ± 0.047[a]	0.115 ± 0.087[b]	4.654	**
Triceps brachii	0.620 ± 0.272[a]	0.229 ± 0.044[b]	3.614	*
Soleus	0.118 ± 0.043[a]	0.029 ± 0.008[b]	4.494	*
Tibialis cranialis	0.511 ± 0.063[a]	0.259 ± 0.016[b]	8.748	**
Gastrocnemius	0.740 ± 0.116[a]	0.366 ± 0.053[b]	6.517	**

[ab:] Values in the same row and variable with no common superscript differ significantly . * : P<0.05, **: P<0.01, NS: Not Significant. 1 Values are means of eighteen observations per treatment and mean ± S.E giveu for each measurement. 2 T1 = control males, T2 =PhB, Phenobarbital male., T3 = C, castrated male

mean of significance varied for the muscles studied and these were as follows: triceps brachii and soleus (p<0.05) and the other muscles were (p<0.01) .There was no significant between Phenobarbital- exposed and castrated male (p>0.05)(Table 5) .This study also demonstrated the muscle mass indices of muscles differed significantly except bicep brachii (Table 6).

Brain

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. The area of necrosis in some area of the brain was large enough to see as vacuole (Fig1).

DISCUSSION

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 [11].

However few studies have evaluated the affect of Phenobarbital on androgen and muscle in rat. Serum concentrations of testosterone during the postoperative period were much lower than basal range. 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.

Results of the present study demonstrated that the body weights of castrated and Phenobarbital male rats treated were lower than those of controls. This is contrary to the observations Ihemelandu and Ibebunjo [12] reported the castration of male mice brought about faster growth rate, bigger body size and greater weight at maturity when compared to intact males. However, it has been shown that castration resulted in decreased growth rate in sheep [13], goats [14] and rats [15]. The reason for the disparity between their report with result of this study and other reports are showed is not known. The difference may be related to species variations such as age and nutrition.

The response of skeletal muscles to androgen deprivation and/or administration is muscle and species

Table 5. Comparison of muscle weights (g) between two groups of male rats of phenobarbital-exposed and castrated with using student 'T' test. in age of 12 week.

Groups	Treatments			
	T2-PhB	T3-C	T-test	Significance
Biceps brachii	0 .081 ± 0.012[b]	0.115 ± 0.087[b]	0.598	NS
Triceps brachii	0.234 ± 0.046[b]	0.229 ± 0.044[b]	0.146	NS
Soleu	0.028± 0.008[b]	0.029 ± 0.008[b]	0.610	NS
Tibialis cranialis	0.258± 0.014[b]	0.259 ± 0.016[b]	0.162	NS
Gastrocnemius	0.362 ± 0. 056[b]	0.366 ± 0.053[b]	0.058	NS

[ab:] Values in the same row and variable with no common superscript differ significantly . * : P<0.05, **: P<0.01, NS: Not Significant. 1 Values are means of eighteen observations per treatment and mean ± S.E giveu for each measurement. 2 T1 = control males, T2 =PhB, phenobarbital male, T3 = C, castrated male

specific. For example, the flexor carpi radialis muscle of the male frog Xenopus laevis atrophies in response to castration and returns to normal size within 7 days of testosterone administration [16], whereas these procedures have no discernible effect in the sartorius muscle of the same species. Similarly, in the guinea pig the temporalis muscle is highly sensitive to androgens [16]. The temporalis muscle of the female guinea pig contains type IIa fibers predominantly, whereas in the male, testosterone causes hypertrophy of the muscle and an increase in type IIb fibers [17], It is believed that the pectoral and shoulder girdle muscles are the most androgen-dependent muscles in humans [18].

Reduction in muscle mass of males prenatally exposed to Phenobarbital was reported by Ihemelandu [19]. 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 fibers. 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, Yannai and Bergman [20] reported neuron necrosis in different region of the brain in mice. It was assumed that the prenatal exposure of rat to Phenobarbital may cause destruction of neurons at hypothalamic levels, hence disrupting the hypothalamus –pituitary testis axis regulatory mechanism. Impairment in the production of the releasing factor by the hypothalamus, may adversely affect the testosterone –synthesizing ability of the the interstisial cells of Leydig whose function has been shown to be dependent on the stimulation by pituitary interstitial cell stimulating hormone.

Table 6. Comparison of muscle mass (mg/g) of three groups of male rats of control, phenobarbital-exposed and castrated, in age of 12 week.

Groups	Treatments				
	T1-Control	T2-PhB	T3-C	SEM	Significance
Biceps brachii	1.002 ± 0.02^{a}	0.656 ± 0.04^{b}	0.572 ± 0.01^{b}	0.033	NS
Triceps brachii	3.120 ± 0.16^{a}	2.168 ± 0.11^{b}	2.500 ± 0.10^{b}	0.131	*
Soleus	0.774 ± 0.04^{a}	0.250 ± 0.02^{b}	0.300 ± 0.01^{b}	0.038	*
Tibialis cranialis	2.466 ± 0.10^{a}	1.928 ± 0.04^{b}	1.860 ± 0.02^{b}	0.091	**
Gastrocnemius	3.088 ± 0.09^{a}	2.386 ± 0.07^{b}	2.282 ± 0.05^{b}	0.068	**

[ab:] Values in the same row and variable with no common superscript differ significantly . * : P<0.05, **: P<0.01, NS: Not Significant. 1 Values are means of eighteen observations per treatment and their pooled SEM. Mean ± S.E given for each measurement. 2 T1 = control males, T2 =PhB, phenobarbital male., T3 = C, castrated male

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