STRUCTURAL AND FUNCTIONAL CHANGES IN RAT OFFSPRING INDUCED BY PRENATAL PHENYTOIN ADMINISTRATION

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Summary

Phenytoin (sodium salt), a developmental neurotoxicant, was administered orally by gavage (150 mg/kg) to pregnant rats on days 7–18 of gestation. Various developmental and behavioural indices were evaluated. This study suggest that prenatal phenytoin exposure may result in developmental, behavioural deficits and neurochemical changes.

Introduction

Phenytoin (PHT) is a widely prescribed antiepileptic drug. Recent theory suggests that PHT teratogenicity is mainly initiated by adverse drug action on the embryonic heart at a sensitive stage of development, resulting in embryonic hypoxia/ischaemia (1) and via reactive PHT intermediates and production of reactive oxygen species (2). In the present study the model of PHT induced chronic intrauterine hypoxia was evaluated in rats on their reproductive outcome, development and behaviour. Dopamine (DA) levels in the rat brain regions (striatum, cortex, hypothalamus, hippocampus) were determined by a specific and highly sensitive bioanalytical HPLC method.

Material and Methods

Subjects were sperm positive (gestational day 0), nulliparous Wistar/DV rats. The animals were housed under standard laboratory conditions. The study design is depicted in Figure 1.

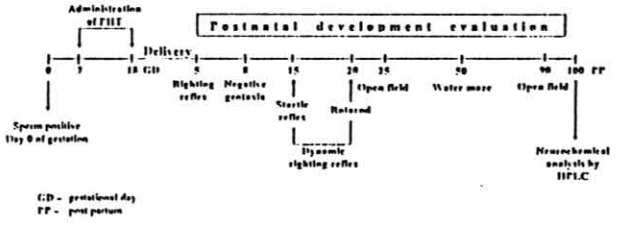

Fig. 1: Results and Discussion

No maternal death or abortion occurred either in the control or PHT group. The dose of 150 mg/kg PHT resulted in a significant reduction in maternal weight gain over the treatment period when compared to control (Table 1). In newborn pups in the PHT group, dark red colouration of the skin was observed. Litter characteristics and offspring mortality are shown in Table 1. There were no significant differences between groups with respect to the gestation period and sex ratio. The PHT group demonstrated a significant increase in offspring mortality (50.7 %) and decrease in litter weight compared to control, persisting during the preweaning period up to day 21 post partum (PP). The pattern of pup mortality showed that the majority of deaths occurred between days 0–4 PP. In our opinion, neither lack of milk in the mammary glands nor decreased ability of pups to suck maternal milk can be excluded in this phenomenon. McCartney et al. (3) also reported a decreased viability index of rat pups during the first four days of age. Causes of their death may result from insufficient care of mothers for their offspring, as no milk was found in the stomachs of dead pups. The individual variables of somatic growth and maturation (unfolding of external ear, ear and eye opening) in the pups from the PHT group were not significantly altered compared to controls, except the delay in emergence of the incisors from the gingiva on days 10 and 11 PP (data not shown). These changes may be due to an increase in either growth-affecting prenatal epidermal growth factor or thyroid function, or both (3). Soon after eye opening (14–15 day PP), however, a blood-stained secretion (chromodacryorrhea) was observed in the pups from the PHT group (71.43 %) and this persisted up to the end of the study at 100 days PP. The secretion pigment was identified spectrophotometrically as protoporphyrin (4).

Table 1

Litter characteristics and offspring mortality

Variables	Control	*PHT*
No. of litters	15	26
Maternal weight gain [g] GD 7-18	48.0±3.23	31.54±4.05**
Gestation period (day)	21.73±0.15	22.07±0.09
No. of live pups		
PP 0	9.13±0.59	6.77±0.81*
PP 4	9.07±0.59	4.85±0.87**
PP 21	8.73±0.56	4.04±0.83***
Litter weight [g]		
PP 0	54.60±3.19	45.74±3.82
PP 5	95.73±6.14	67.76±9.58*
PP 10	183.47±11.81	111.47±16.39**
PP 15	237.47±12.18	168.69±23.40*
PP 20	319.73±16.75	232.31±31.41***
PP 21		
- Male	43.31±0.92	38.84±0.85***
- Female	45.04±0.93	40.14±0.94***
Sex ratio ♂ / ♀	55/61	50/49

** p < 0.05, ** p < 0.01, *** p < 0.001 – compared to control (Student t-test), GD – gestational day, PP – post partum*

Animals in the PHT group showed an abnormal, stereotyped, spontaneous circling behaviour, which was first observed by Worhees (5). In our study, this abnormal behaviour was present with 35% in males and 40% in females. Circling behaviour was never seen in the control group. No statistically significant differences were found in neuromotor and reflex development (righting reflex, negative geotaxia, startle reflex, rotarod) of the PHT pups compared to control (data not shown). However, the ability of PHT pups to stay on the rotating rod, which requires hind limb coordination, was non-significantly decreased. The maturation of the dynamic righting reflex of the PHT pups was significantly delayed on day 15 and 18 PP (Figure 2). Succesful righting is dependent on the development of functional motor and sensory systems. The coordination of the systems required suggests that central as well as peripheral pathways must participate in a mature response. The cerebellum is involved in the coordination of types of locomotion that require utilization of multiple exteroreceptive and proprioceptive cues and it has been suggested that delay in the maturation of the rat cerebellar cortex contributes to delayed development of complex locomotor skills (6).

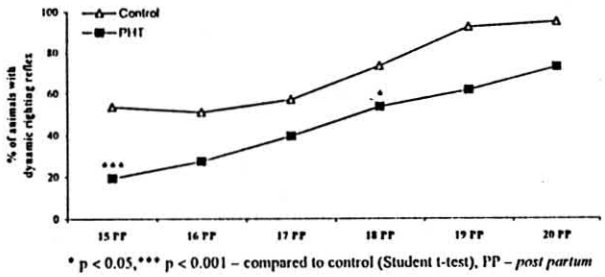

Fig. 2: Dynamic righting reflex

As for vertical exploratory activity of 25-day-old pups, significant differences were recorded only in PHT female pups compared to control (Figure 3). These changes may be explained by the influence of PHT on the development of hind limb balancing, which is first seen in the rat on day 13 PP and is the last postural response in the process of maturation (4). On day 90 PP an increase in motor activity was observed in both genders, but it was significant

only in PHT male adult animals (Figure 4). Vertical exploratory activity on day 90 PP was not affected; there were no differences in foecal bolus contents (data not shown). McCartney et al. (3) also described that motor activity was significantly higher after 150 mg/kg PHT administration for female pups and female adult animals. Cognitive ability of offspring was tested using modified Morris water maze (1.1 m in diameter) assay for 4 consecutive test days starting with 50 day PP. In this test the PHT offspring showed no differences in the time needed to escape from the water onto the hidden platform (data not shown). Our findings were different in comparison with data published by McCartney at al. (3). These authors observed increased time to escape onto the platform after prenatal administration of 150 mg/kg PHT to rats. The main difference may have been caused by the different diameter size of the water maze (1.4 m vs 1.1 m in our study). In 100-day-old male and female rats from the PHT group a significant decrease in the brain wet weight was found (Figure 5). Other authors (3, 4, 7) also detected lower brain weights in rats after prenatal administration of PHT. In our previous study with PHT, we did not find any morphological changes in the brain on histological evaluation (8).

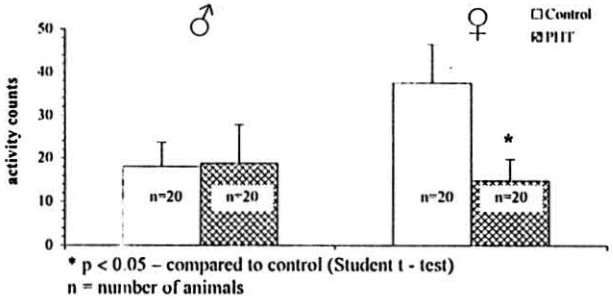

Fig. 3: Vertical exploratory activity of 25-day-old pups

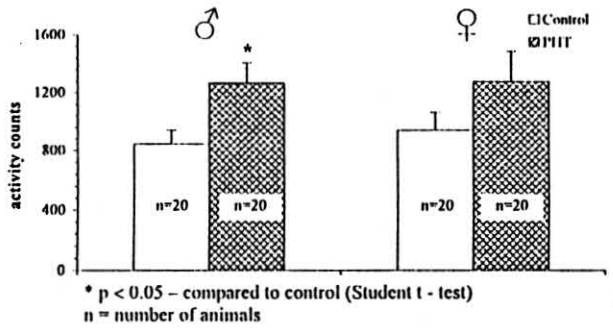

Fig. 4: Motor activity of 90-day-old adult rats

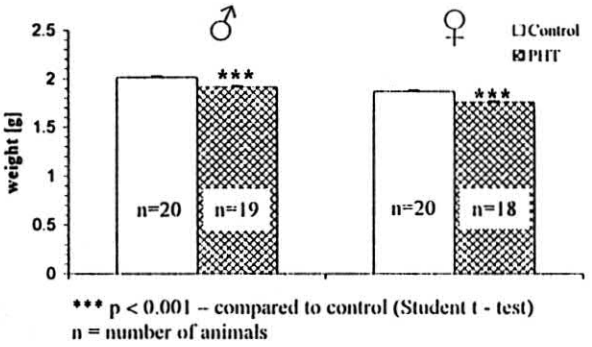

Fig. 5: Brain wet weight of 100-day-old rats

Figure 6, panel A, shows the levels of DA in different regions of the brain of rat females. As it is evident, the only two significantly different levels were disclosed in the cortex and hypothalamus. In both these regions, the mean DA concentrations are lower in the PHT females as compared to those found in the control group. Contrary to this finding, the mean DA levels in the cortex, hypothalamus, hippocampus are slightly higher in the PHT males (Figure 6, panel B), although the differences are not significant. In both sexes the DA levels in the striatum are higher compared to control, yet the differences is significant in PHT males only.

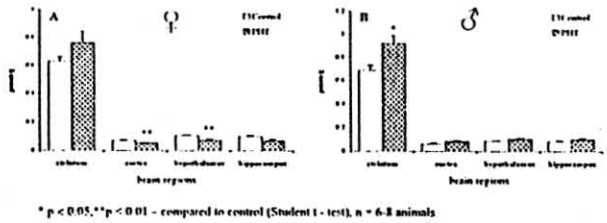

***Fig. 6:** Levels of dopamine in 100-day-old animals*

Importantly, the model presented in this paper may be considered to be relevant for investigating structural, functional and neurochemical changes in the rat offspring in further studies intended to investigate the effect of combined PHT and antioxidant administration.

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