

EFFECT OF MELATONIN ON NEUROBEHAVIORAL DYSFUNCTIONS INDUCED BY INTRAUTERINE HYPOXIA IN RATS

Dubovický M.¹, Ujházy E.¹, Kovačovský P.², Navarová J.¹, Juráni M.³, Šoltés L.¹

¹Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava

²Department of Psychology, Philosophical Faculty, Comenius University, Bratislava

³Institute of Biochemistry and Animal Genetics, Slovak Academy of Sciences, Ivánka pri Dunaji, Slovak Republic

SUMMARY

Intrauterine hypoxia associated with oxidative stress represents an important risk factor for development of neurobehavioral dysfunctions. In the present study, we investigated the potential protective effect of melatonin (MEL) on neurobehavioral dysfunctions induced by chronic intrauterine hypoxia in rats by the anticonvulsant drug phenytoin (PHT), which is known by its teratogenic potential. Pregnant female rats (Wistar/DV) were orally treated by PHT (150 mg/kg) from day 7 to 18 of gestation. MEL was dissolved in drinking water (40 µg/ml) and administered from day 0 to 19 of gestation. Neurobehavioral development of offspring was evaluated from birth up to day 90 of postnatal life. The results of the study confirmed the high behavior-teratogenic potential of PHT. Prenatal administration of PHT resulted in delayed neuromotor and reflex development, decreased exploration in the open field, abnormal "circling" and impaired performances in water maze. Co-administration of MEL failed to have any effect on neurobehavioral dysfunctions induced by PHT treatment. Even administration of MEL alone caused developmental alterations in offspring manifested by accelerated testes descent and delayed onset of negative geotaxis and startle reflex. The results suggest to pay increased attention to MEL concerning its exogenous use during pregnancy.

Key words: intrauterine hypoxia, phenytoin, neurobehavioral development, melatonin, rat

Address for correspondence: M. Dubovický, Institute of Experimental Pharmacology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava. E-mail: exfadubm@savba.sk

INTRODUCTION

Intrauterine hypoxia/ischemia associated with oxidative stress represents an important risk factor for development of neurobehavioral dysfunctions (1, 2). Animal studies showed that medium hypoxia resulted in neuronal death in the basal ganglia and brain stem. Moreover, severe hypoxia caused extra injuries

in the cortex and cerebellum. Oxidative stress can interfere with the brain development resulting in structural and functional changes. These changes can be manifested by neurobehavioral dysfunctions varying from mild cerebral dysfunction, different neuromotor impairments up to severe cerebral palsy (3). It is conceivable that substances with antioxidative properties may protect the brain against oxidative damage. Antioxidant

treatment strategies have been intensively studied using different antioxidants and experimental models (4, 5). Melatonin (MEL), epiphyseal hormone regulating biorhythms, has been found to be highly effective antioxidant (6). On the other hand, anticonvulsant drug phenytoin (PHT) exerts its teratogenicity by affecting embryonal heart in its sensitive period resulting in bradycardia and arrhythmia accompanied by hypoxia/reoxygenation and production of reactive oxygen species (7).

In the present study, we investigated potential protective effect of MEL on neurobehavioral dysfunctions induced by chronic intrauterine hypoxia in rats by means of PHT.

MATERIAL AND METHODS

Animals: Wistar/DV female pregnant rats (n = 60, aged 2 month, weight 200 - 220 g) were used in the study. Rats were housed in plastic cages (1 dam/cage) at 22 ± 2 °C and $55 \pm 5\%$ relative humidity, 12:12 h light cycle (lights off at 17.00 h). Water and food were provided *ad libitum*.

Treatment: MEL (Sigma-Aldrich Chemie GmbH, Germany, batch No: 13961-081) was administered from day 0 to 19 of gestation at the dose of 40 µg/ml dissolved in 0.4% ethylalcohol daily from 17.00 to 07.00 h. PHT (Slovakofarma, J.S.C., Hlohovec Slovakia, batch No. 0080499, 99% purity) was administered orally at the dose 150 mg/kg from day 7 to 18 of gestation.

Neurobehavioral development: Pregnant rats were allowed to deliver the pups. Development of pups from birth up to day 90 post partum (PP) was evaluated. Following variables were observed: *Somatic growth and maturation* (days 0-35 PP): body weight, unfolding of external ear, incisor eruption, opening of ears and eyes, testes descent and vaginal lumen opening. *Neuromotor and reflex development:* *righting reflex* (day 5 PP) - the pup's ability to turn over from supine position, *negative geotaxia* (day 8 PP) - the pup's ability to turn 180° on a 25° incline placed head down, *forelimb grip strength* (day 13 PP) - ability to hold on to a thin wire, performances on *rotating rod* (day 20 PP). *Sensory function:* *startle reflex* (day 15 PP) - the presence or absence of sensorimotor reaction (jerks) to auditory stimulus. *Learning and memory processes* (day 50 PP): *water maze* (diameter 110 cm) - time latency to reach a hidden platform (diameter 10 cm) under surface of the water (total of 4 sessions consisting of 4 consecutive trials). *Activity and emotional reactivity* (day 90 PP): *exploratory*

behavior in an open field, sized 42 x 42 cm (intensity of motor activity and rearings, defecation rate in 4 min session).

Statistical evaluation: Data were analyzed by means of ANOVA followed by Duncan post hoc test ($p \leq 0.05$). Results are presented as means \pm S.E.M.

RESULTS

Prenatal administration of PHT resulted in increased mortality of pups during the first 4 days of postnatal life (mortality 85.5%). MEL administration did not have significant effect on viability of PHT-treated rats (mortality for MEL+PHT group: 79.5%). Except to testes descent, there was no significant effect of treatment on somatic growth and maturation of pups. Appearance of testes descent was accelerated in the group of MEL-treated rats compared to control (mean value for control: 24.31 ± 0.17 and for MEL: 23.20 ± 0.13 , $p < 0.05$). There was a significant increase in reaction time for negative geotaxia in MEL-treated rats compared to control. Percentage of pups with positive startle reflex was significantly decreased in both MEL-treated groups (Fig. 1). Percentage of pups with successful air righting during 6 day testing was markedly declined in the PHT and MEL+PHT group (percentage of air righting on day 6 of testing: control 89.88 ± 2.01 , PHT $44.45 \pm 9.30^{**}$, MEL 99.03 ± 0.96 , MEL+PHT $53.06 \pm 15.73^{**}$, $**p < 0.01$ - compared to control). Intensity

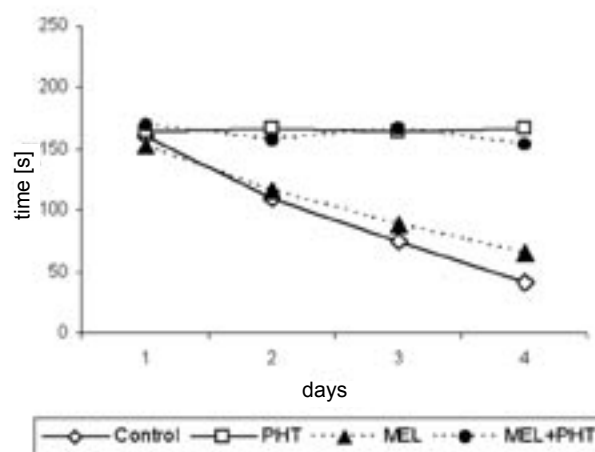


Fig. 2. Effect of PHT and MEL on water maze learning in male rats. Significant main effect of time and treatment ($**p < 0.01$, ANOVA).

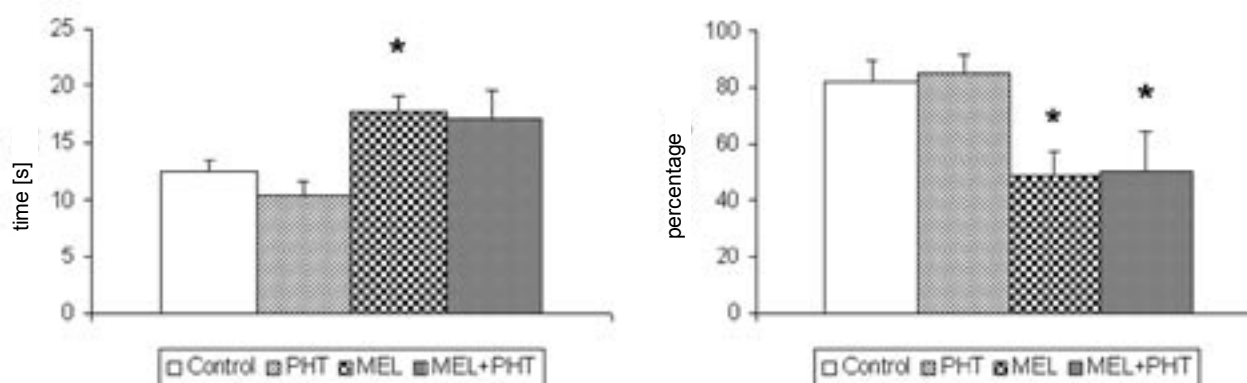


Fig. 1. Effect of PHT and MEL on negative geotaxia (first chart) and startle reflex (second chart). * $p < 0.05$ - significant differences compared to control (ANOVA).

of rearings was significantly decreased in PHT-treated animals in both genders (**p < 0.01, data not shown). Water maze test revealed marked cognitive deficit in PHT and MEL+PHT-treated rats of both genders compared to control as well as MEL+PHT group (Fig. 2).

DISCUSSION

Results of the study confirmed high behavior-teratogenic potential of PHT. Prenatal administration of PHT resulted in delayed neuromotor and reflex development, decreased exploration in the open field, abnormal “circling” and impaired performances in water maze test. Co-administration of MEL failed to have any effect on neurobehavioral dysfunctions induced by PHT treatment. Even administration of MEL alone caused subtle developmental alterations in offspring manifested by accelerated testes descent and delayed onset of negative geotaxia and startle reflex.

Hanson and Smith (8) have described “fetal hydantoin syndrome” in children of mothers treated with PHT during pregnancy. This syndrome manifests by various developmental defects and dysfunctions including craniofacial defects, pre- and postnatal growth retardation, microcephaly, cleft palate, heart defects and cognitive deficit (9). Animal studies showed that PHT administered during pregnancy at the doses 100 - 200 mg/kg induced severe behavioral changes in offspring, such as retarded air righting, increased motor activity, “circling behavior”, decreased performance in rotating rod and impaired learning (10, 11, 12). Our findings are in accordance with results of most behavioral studies performed with PHT. In our study there was no effect of PHT on motor activity which is contradictory to above mentioned studies reporting increase in this activity. However, we found a decreased intensity of rearings indicating an inhibition of exploratory behavior of the animals in a new environment. This discrepancy may be attributed to different methodological approach used in our study, especially related to strain of rats and size of the open field.

Interestingly, our results showed that MEL administered during pregnancy could interfere with neurobehavioral development and in turn, could accelerate and/or inhibit some developmental variables. MEL is considered the “wonder” drug for ailments ranging from sleeplessness to aging without any clinical evidence of efficacy (except to its chronobiotic and resynchronizing effect). Very little attention has been paid to the possible side effects of MEL. Nightmares, hypotension, sleep disorders and abdominal pain have been reported (13). Moreover, MEL may disrupt mothers’ endogenous melatonin rhythm. This in turn could affect prenatal development of the brain. Actually, rare experimental studies showed negative effect of MEL on developing organism. Toxicological studies on the chick embryos revealed

increased mortality after PHT administration (14). Maternal MEL treatment in rats was found to modify open field behavior of the offspring (15).

In conclusion, prenatal administration of MEL failed to protect neurobehavioral development from chronic intrauterine hypoxia induced by PHT. MEL induced subtle developmental alterations in rats offspring. The results suggest to pay increased attention to MEL concerning its exogenous use during pregnancy.

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