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Research Report

Beneficial effects of melatonin on morphological changes in postnatal cerebellar tissue owing to epileptiform activity during pregnancy in rats: Light and immunohistochemical study

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Abstract

Although it has been demonstrated that maternal epilepsy has some harmful effects on newborn individuals, current data concerning the effects of epileptic phenomena in pregnant mothers on newborn pups are still limited. This study was undertaken to investigate the changes in the cerebellum of newborns of pinealectomized rats subjected to experimental epilepsy during pregnancy. In our study, the rats were randomly divided into six groups: intact control group, anesthesia control group, epilepsy group, melatonin-treated epileptic group, surgical pinealectomy group, and group of melatonin treatment following pinealectomy procedure. At 1 month after pinealectomy, an acute grand mal epileptic seizure was induced by 400 IU penicillin-G administration into their intrahippocampal CA3 region during the 13th day of their pregnancy in all animals except intact control group. On the neonatal first day, pups were perfused transcardially and the cerebellums removed were processed for light microscopic and immunohistochemical studies. Normal migration and maturation were determined in the postnatal rat cerebellum in both intact control and anesthesia (ketamine–xylazine) control groups, but the morphological structure of cerebellum in the epilepsy control group corresponded to the early embryonal period. It was found that experimental epilepsy or pinealectomy procedure enhanced nestin immunoreactivity, but exogenous melatonin treatment (30 μg/100 g body weight, i.p.) following pinealectomy inhibited increased nestin expression induced by melatonin deprival in vermis region of newborn rat cerebellum ($P < 0.001$). Our results confirm that epileptic seizures during pregnancy may impair neurogenesis and neuronal maturation in newborns, which are more dramatic in the presence of melatonin deficiency during pregnancy, explaining more harmful effects of epileptic seizures to embryos of aged mothers. To the best of our knowledge, this is the first study reporting the effects of maternal epilepsy during pregnancy in pinealectomized rats on nestin immunoexpression in the newborn rat cerebellum.

Theme: Development and regeneration
Topic: Cell differentiation and migration

Keywords: Cerebellum; Epilepsy; Melatonin; Nestin; Postnatal; Rat

1. Introduction

Although previous studies have reported that epilepsy is one of the most common chronic diseases affecting people, very limited knowledge is available regarding its pathophysiology in the current literature [3,11,37–39]. Some researchers have demonstrated the negative effects of mothers' epileptic seizures on newborn individuals, but the effects of maternal epilepsy on different regions of the brain are not clearly established [37,38]. Nevertheless, little attention has been paid to postnatal morphological alterations induced by epilepsy and ischemia during pregnancy [23,29]. Furthermore, some immunohistochemical markers such as nestin have been used as a specific marker for multipotent neuro-

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epithelial stem cells in both animal models and humans [5,7–9,19,46]. Recently, expression of nestin in the postnatal cerebellum and its potential functional significance have been analyzed and discussed [47]. On the other hand, it is known that melatonin, a secretory product synthesized nocturnally by the pineal gland, has a neuroprotective role against the harmful effects in patients with epilepsy [6,15,20,27,33]. Similarly, it is possible that melatonin may have an effect on morphological alterations in postnatal cerebellar formation induced by maternal epilepsy. In the present study, we therefore investigate the developmental expression of nestin in newborn individuals' cerebellums following epileptic activity in pinealectomized rats and the effect of exogenous melatonin administration following pinealectomy, using light and immunohistochemical techniques.

2. Materials and methods

2.1. Animals and experimental design

In this study, the animals were used for experiment in the following schema after approval of the experimental protocol by the local ethical committee. Adult female Swiss Albino rats, weighing 210 g (±10 g) each, were obtained from Experimental Research Center of Ege University. They were housed with a free access food and water ad libitum. All the animals were kept in light (L)–dark (D) conditions L:D = 12:12 during the current experiment. In this study, all surgical procedures were performed under general anesthesia by intraperitoneal (i.p.) injection of a mixture of ketamine (0.15 mg/100 g body weight, Alfamine®, Ege Vet, İzmir, Turkey) and xylazine (0.02 ml/100 g body weight, Alfazyne®, Ege Vet, İzmir, Turkey). Exogenous melatonin (Sigma Chemical Co., St. Louis, MO, USA) treatment was given at a dose of 30 μg/100 g body weight.

The newborn rats were randomly divided into six groups: intact control group (n = 6), anesthesia (ketamine–xylazine) control (n = 6), epilepsy control group (n = 6), melatonin-treated epileptic group (n = 6), surgical pinealectomy group (n = 6), and group of melatonin treatment following pinealectomy procedure (n = 6). The animals in surgical pinealectomy and melatonin treatment groups underwent a surgical intervention consisting of pineal gland removal. Daily vaginal smears were taken for estrus cycle detection, as described previously [40,41]. At 1 month after surgical pinealectomy, male rats were mated with female rats in proestrus night. Then, an acute grand mal epileptic seizure was induced by penicillin-G administration into their hippocampi during the 13th day of their pregnancy in all animals except intact control and anesthesia control groups.

2.2. Pinealectomy procedure

The animals underwent pinealectomy procedure under deep general anesthesia by intraperitoneal injection of a mixture of ketamine and xylazine at the doses given above, 1 month before induction of experimental epilepsy. Pinealectomy procedure was performed with a modification of the method described previously by Bliss and Bates [4]. Briefly, the skull skin was opened by a longitudinal midline incision and an area of skull was removed between the sagittal and lambdoid sutures using an apparatus for holding the head steady. The dura mater is then cut and the pineal gland is removed by using an iridectomy forceps which is held at an angle of 30° from the lambdoid suture and dissected downward at an angle of 15° from horizontal, towards the midline at the junction of the sagittal and lambdoid sutures. Meticulous care was taken to avoid injuring the venous sinuses adjacent to pineal gland. During the surgical intervention, any bleeding was controlled with cotton wool pledgets. At the end of the surgery, the skin was approximated with 5/0 nylon sutures. All animals were checked at daily control examinations for neurological complications.

2.3. Experimental epilepsy

In 13th day of pregnancy, to get acute grand mal epileptic seizure, 400 IU penicillin-G was injected unilaterally to right intrahippocampal CA3 sector of all rats except intact control and anesthesia control groups with the guidance of stereotaxic tool and rat stereotaxic atlas, which is located in coordinates of 5 mm in caudal, 4.5 mm in lateral, and 7.0 mm in depth with respect to bregma point and has dense afferent and efferent connections, after anesthesia with ketamine plus xylazine [30,45]. All of operated rats were observed with a video camera (Sony 360 × Handycam). Pregnant rats were then put into the separated cages for delivery. Immediately after the delivery, the newborn rats were anesthetized with a lethal dose of ketamine and were fixed with intracardiac perfusion.

2.4. Melatonin treatment

Melatonin at a dose of 30 μg/100 g body weight dissolved in the vehicle containing 2% (v/v) ethyl alcohol in physiological saline was intraperitoneally injected to non-pinealectomized rats in melatonin-treated epileptic group as well as pinealectomized rats in exogenous melatonin treatment group beginning the date of pinealectomy procedure. All the injections were given once daily between 1600 and 1700 h for 2 months. The doses were chosen based on the existing literature data and pilot studies done in our laboratory.

2.5. Immunohistochemical study

At the day of birth (P0), all pups were perfused with intracardiac fixative solution (2% gluteraldehyde and 2% paraformaldehyde buffered by 0.1 M cacodylate) under anesthesia for light and immunohistochemical studies. After