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Postnatal dexamethasone-induced programmed hypertension is related to the regulation of melatonin and its receptors

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ABSTRACT

Adulthood hypertension can be programmed by glucocorticoid exposure in early life. We found that maternal melatonin therapy prevents postnatal dexamethasone (DEX)-induced programmed hypertension. Melatonin acts through specific receptors, including MT1 and MT2 membrane receptors, and retinoid related orphan nuclear receptors of the RZR/ROR family. Thus we tested whether postnatal DEX-induced hypertension is related to changes of melatonin receptors in the kidney and heart, which was preserved by maternal melatonin therapy. Male neonates were assigned to four groups ($n = 6–8$/group): control, DEX, control + melatonin (MEL), and DEX + MEL. Male rat pups were injected i.p. with DEX on d 1 (0.5 mg/kg BW), d 2 (0.3 mg/kg BW), and d 3 (0.1 mg/kg BW) after birth. Melatonin was administered in drinking water (0.01%) during the lactation period. We found DEX group developed hypertension at 16 weeks of age, which melatonin therapy prevented. Postnatal DEX treatment increased mRNA expression of MT1 and MT2, while decreased RORα and RZRβ in the kidney. These changes were prevented by melatonin therapy. Postnatal DEX decreased protein level of MT2 in the kidney, which was attenuated by melatonin therapy. Renal protein level of RORα was higher in DEX + MEL group compared to control and DEX group. Renal melatonin level was higher in the MEL and DEX + MEL groups compared to control. We concluded that melatonin therapy has long-term protection on postnatal DEX-induced programmed hypertension, which is associated with regulation on melatonin receptors in the kidney. Our findings would offer potential therapeutic approaches to prevent programmed hypertension in premature baby receiving glucocorticoids.

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1. Introduction

Postnatal glucocorticoid administered has been commonly used in premature infants suffering from respiratory distress syndrome to prevent chronic lung disease [1]. However, systemic glucocorticoid administration, predominantly dexamethasone (DEX), is associated with serious adverse effects later in life, including hypertension [2–4]. Hypertension, one of the most common diseases all over the world, can be programmed from early life [5]. Programmed hypertension has been observed in a variety of early DEX exposure models [6–9]. Our previous study showed that early melatonin therapy provides protection from postnatal DEX-induced programmed hypertension in male offspring, which is associated with histone deacetylase inhibition [8]. However, whether postnatal DEX exposure can regulate melatonin signaling pathway remains unclear.

Melatonin is an endogenously produced hormone, mainly secreted during the night by the pineal gland. Melatonin has multifaceted biological functions, including antihypertensive effect. Melatonin can interact with two transmembrane melatonin receptors, melatonin receptor-1 (MT1) and -2 (MT2), as well as retinoid related orphan nuclear hormone receptors of the RZR/ROR family for signal transduction [10]. Several antihypertensive mechanisms of melatonin have been proposed, including modulation of central sympathetic tone [11], reduction of oxidative stress [12], improvement of circadian rhythm [13], and activation of MT2 receptor to induce vasodilatation [14]. Although emerging evidence indicates that melatonin is beneficial to reverse DEX-induced adverse programming effects [8,9,15–17], whether melatonin protects programmed hypertension is because of the restoration of the melatonin signaling pathway is unknown.

Abbreviations: DEX, dexamethasone; HDAC, histone deacetylase; MEL, melatonin; MT1, melatonin receptor-1; MT2, melatonin receptor-2; RORα, retinoid acid receptor-α; RZRβ, retinoid Z receptor-β.

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The kidney and heart are two important organs for blood pressure (BP) control. This study was therefore designed to address two specific questions: First, to examine whether postnatal DEX exposure had long-term effects on melatonin receptors expression in the kidney and heart of adult male offspring; Second, to test if maternal melatonin therapy can prevent programmed hypertension via restoration of melatonin signaling pathway.

2. Materials and methods

2.1. Animal models

This study was carried out under the Guidelines for Animal Experiments of Chang Gung Memorial Hospital and Chang Gung University and was in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee of the Kaohsiung Chang Gung Memorial Hospital. Virgin Sprague–Dawley (SD) rats (12–16 weeks old) were obtained from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan). Male SD rats were caged with individual females until mating was confirmed. All rats were housed in a room maintained at 22 ± 1 °C with 12-h light/dark cycles (dark period from 19.00 to 07.00) in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Given that hypertension occurs at a higher rate and at an earlier age in males than females [18], only male offspring was selected from each litter and used in subsequent experiments. After birth, the subjects that came from litters were culled to eight pups to standardize the received quantity of milk and maternal pup care. Pups were not weighed at birth to prevent maternal rejection. Male neonates were assigned to four groups (n = 6–8/group): control, DEX, control + melatonin (MEL), and DEX + MEL. Male rat pups were injected i.p. with DEX on d 1 (0.5 mg/kg BW), d 2 (0.3 mg/kg BW), and d 3 (0.1 mg/kg BW) after birth. This treatment protocol was based on a 21-d tapering treatment given to premature infants to prevent chronic lung disease [19]. Melatonin was administered in drinking water at the dose of 0.01% for a total of 3 weeks during the lactation period. The dose of melatonin used here was based on our previous study conducted in rats [8,9]. Melatonin was prepared two times a week by dissolving the drug (10 mg) in 1 ml of 100% ethanol. This solution was further diluted with tap water to a final concentration of 0.01%. Water bottles were wrapped with aluminum foil to protect from light.

2.2. BP measurement

BP was measured in conscious rats at age 3, 4, 6, 8, 10, 12, 14, and 16 weeks by using an indirect tail-cuff method (BP-2000, Visitech Systems, Inc., Apex, NC, USA) as previously described [9]. To ensure accuracy and reproducibility, the rats were allowed to adapt to restraint and tail-cuff inflation for 1 week prior to the experiment. Rats were placed on specimen platform, and their tails were passed through tail cuffs and secured in place with tape. Following a 10-min warm-up period, 10 preliminary cycles of tail cuff inflation were performed to allow the rats to adjust to the inflating cuff. A total of five cycles were recorded at each time point. Three stable consecutive measures were taken and averaged.

2.3. Tissue sampling

Rats were killed at 10.00 during daytime at 16 weeks of age. Rats were anesthetized using an i.p. injection of ketamine (50 mg/kg) and xylazine (10 mg/kg), then euthanized by an i.p. overdose of pentobarbital. The midline of the abdomen was opened. The aorta was dissected from the adjacent vena cava, connective tissue, and fat. The aorta was cannulated with a 20- to 23- gauge butterfly, heparinized blood samples were collected, the vena cava was cut, and phosphate-buffered saline was perfused until the kidneys were blanched. Kidney and ventricular myocardium were harvested, and stored at −80 °C freezer for further analysis. Renal melatonin level was measured using an ELISA kit (MyBioSource, San Diego, CA, USA) as previously described [17]. In short, 100 mg kidney cortex was homogenized in 500 μL PBS and protein concentration was determined by Bradford assay (Bio-Rad, Hercules, CA, USA). After two freeze–thaw cycles were performed, tissue homogenate samples were centrifuged. Duplicate determinations in 100 μL of supernatant samples were made and the average of two measurements was used in subsequent statistical analysis of the data. The melatonin level was quantified spectrophotometrically at 450 nm. The results were expressed as pg melatonin per mg of protein.

2.4. Quantitative real-time polymerase chain reaction (RT-qPCR)

RNA was extracted as previously described procedures [8,9]. Two-step quantitative real-time PCR was conducted using the QuantiTect SYBR Green PCR Kit (Qiagen, Valencia, CA, USA) and the iCycler iQ Multi-color Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). We analyzed 4 melatonin receptors including *Mtnr1a* (encodes for MT1), *Mtnr1b* (encodes for MT2), *Rora* (encodes for RORα), and *Rorb* (encodes for RZRβ). We used 18S rRNA (r18S) as a housekeeping gene in all analyses as it did not change its threshold cycle (CT) in control and the prenatal DEX model [8,9]. Primers were designed using GeneTool Software (BioTools, Edmonton, Alberta, Canada). For *Mtnr1a*, a forward primer 5′ CAGTACGACCCCC GGATCTA 3′ and a reverse primer 5′ GGCAATCGTGTACGCCG 3′ were designed from rat-specific sequences (NM_053676.2). For *Mtnr1b*, a forward primer 5′ ATGTTCGCAGTGTTTGTGGTTT 3′ and a reverse primer 5′ ACTGCAAGGCCAATACAGTTGA 3′ were designed from rat-specific sequences (NM_001100641.1). For *Rora*, a forward primer 5′ AGAACAACACCGTGTACTTTG 3′ and a reverse primer 5′ CTGTAGGACGTGCTGAAG 3′ were designed from rat-specific sequences (NM_001106834). For *Rorb*, a forward primer 5′ ACCCTT CTTACATCAGCCCTACTG 3′ and a reverse primer 5′ TGTCCAAAACC-TACCCCACATAT 3′ were designed from rat-specific sequences (NM_001270958.1). For *R18s*, a forward primer 5′ GCCGCGGTA ATTCCAGCTCCA 3′ and a reverse primer 5′ CCCGCCCGCTCCCAA GATC 3′ were designed from rat-specific sequences. All samples were run in duplicate (2.5 μl of cDNA/well in a 96-well format). All samples were run in duplicate. To quantify the relative gene expression, the comparative threshold cycle (C_T) method was employed. For each sample, the average C_T value was subtracted from the corresponding average r18S value, calculating the ΔC_T. $\Delta\Delta C_T$ was calculated by subtracting the average control ΔC_T value from the average experimental ΔC_T. The fold-increase of the experimental sample relative to the control was calculated using the formula $2^{-\Delta\Delta CT}$.

2.5. Western blot

Western blot analysis was performed as previously described [17]. Three melatonin receptors, including MT1, MT2, and RORα, were analyzed. We used the following antibodies: a goat anti-rat MT1 antibody (1:1000, overnight incubation; Santa Cruz Biotechnology, Santa Cruz, CA, USA; SC13186) [20]; a rabbit anti-rat MT2 antibody (1:1000, overnight incubation; Biorbyt, AllBio Science Inc., Taichung, Taiwan; ORB11086) [21]; and a rabbit anti-rat RORα antibody (1:2000, overnight incubation; Proteintech Group, Inc., Chicago, IL, USA; 10616-1-AP). We did not analyze the protein level of RZRβ because we were unable to find an antibody passing the validation for specificity and reproducibility. The bands

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