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Melatonin prevents neonatal dexamethasone induced programmed hypertension: Histone deacetylase inhibition

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ABSTRACT

Adulthood hypertension can be programmed by corticosteroid exposure in early life. Oxidative stress, epigenetic regulation by histone deacetylases (HDACs), and alterations of renin–angiotensin system (RAS) are involved in the developmental programming of hypertension. We examined whether melatonin prevented neonatal dexamethasone (DEX)-induced programmed hypertension and how melatonin prevented these processes. We also examined whether HDAC inhibition by trichostatin A (TSA, a HDAC inhibitor) had similar effects. Male offspring were assigned to 5 groups ($n = 6$/group): control, DEX, melatonin, DEX + melatonin, and DEX + TSA. Male rat pups were injected i.p. with DEX on day 1 (0.5 mg/kg BW), day 2 (0.3 mg/kg BW), and day 3 (0.1 mg/kg BW) after birth. Melatonin was administered in drinking water at the dose of 0.01% during the lactation period. The DEX + TSA group received DEX and 0.5 mg/kg TSA subcutaneous injection once daily for 1 week. All rats were killed at 16 weeks of age. Neonatal DEX exposure induced hypertension in male offspring at 16 weeks of age, which melatonin prevented. Neonatal DEX exposure decreased gene expression related to apoptosis, nephrogenesis, RAS, and sodium transporters. Yet DEX treatment increased protein levels of HDAC-1, -2, and -3 in the kidney. Melatonin therapy preserved the decreases of gene expression and decreased HDACs. Similarly, HDAC inhibition prevented DEX-induced programmed hypertension. In conclusion, melatonin therapy exerts a long-term protection against neonatal DEX-induced programmed hypertension. Its beneficial effects include alterations of RAS components and inhibition of class I HDACs. Given that the similar protective effects of melatonin and TSA, melatonin might inhibit HDACs to epigenetic regulation of hypertension-related genes to prevent programmed hypertension.

1. Introduction

Hypertension may origin from early life. A reduction in nephron number, activation of the renin–angiotensin system (RAS), and oxidative stress are potential mediators of adulthood hypertension programmed in response to developmental insults [1]. Melatonin is known to regulate blood pressure (BP) [2,3]. Melatonin and its metabolites function as antioxidants and free radical scavengers [4]. We recently found that melatonin prevents oxidative stress and hypertension concurrently in young spontaneously hypertensive rats (SHR) [5]. Whether melatonin can prevent programmed hypertension and the underlying mechanisms remain undetermined.

Programmed hypertension can be induced by dexamethasone (DEX) exposure [6], which is associated with reduced nephron number, cardiac hypertrophy, and alterations of RAS [1,7,8].

Abbreviation: ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme 2; ADMA, asymmetric dimethylarginine; AGT, angiotensinogen; AT1R, angiotensin II type 1 receptor; AT2R, angiotensin II type 2 receptor; DEX, dexamethasone; GR, glucocorticoid receptor; HAT, histone acetyl transferase; HDAC, histone deacetylases; MEL, melatonin; NO, nitric oxide; NaKATPase, Na^+/K^+ATPase α1 subunit; NCC, Na^+/Cl^-cotransporter; NHE3, type 3 sodium hydrogen exchanger; NKCC2, Na–K–2Cl cotransporter; PRR, prorenin receptor; RAS, renin–angiotensin system; SDMA, symmetric dimethylarginine; SGK1, serum glucocorticoid kinase 1; SHR, spontaneously hypertensive rat; TSA, trichostatin A.

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Although glucocorticoid administration is commonly used in premature infants suffering from respiratory distress syndrome to prevent chronic lung disease [9], major concern has been arisen about its long-term adverse effects [10–13]. Epigenetic regulation, oxidative stress, and alterations of RAS and sodium transporters have been proposed relate to the DEX-induced programmed hypertension [1].

Glucocorticoid signaling mechanisms involve glucocorticoid receptor (GR) binding to glucocorticoid response element in the gene's upstream promoter region. GR requires the interaction with transcriptional cofactors, including histone deacetylases (HDACs). HDACs are known to be associated with epigenetic regulations to silence genes. HDACs can be regulated by oxidative stress, such as nitric oxide (NO) [14]. Class I HDACs play critical roles in nephrogenesis, especially HDAC1–3 are highly expressed in nephron precursors [15]. Next, HDACs can regulate RAS components during nephrogenesis [16]. Furthermore, glucocorticoid can activate serum glucocorticoid kinase 1 (SGK1) gene, which mediates sodium transporters, leading to hypertension [17]. These observations are indicative of close interrelations between these pathways in the development of neonatal DEX-induced hypertension.

In addition to its antioxidant property, emerging evidence indicates novel roles of melatonin in epigenetic modulation involving HDACs [18–20]. Our recent reports demonstrated that prenatal DEX-induced programmed hypertension is associated with reduced nephron numbers, oxidative stress, and alterations of RAS and HDACs in the kidney [21,22]. However, whether neonatal DEX exposure can induce programmed hypertension and whether melatonin can prevent it remain unclear. We therefore examined whether melatonin can prevent programmed hypertension in the rats exposed to DEX postnatally by reduction of oxidative stress, alterations of RAS components, and modulations of HDACs. Moreover, we tested whether HDAC inhibition can prevent programmed hypertension in this model.

2. Material and methods

2.1. Animal study

This study was carried out in strict accordance with the recommendations in 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) (BioLASCO Taiwan Co., Ltd., Taipei, Taiwan) were housed and maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Rats were exposed to a 12-h light/12-h dark photoperiod (dark period from 1900 to 0700). The temperature and the humidity were maintained at $22 \pm 1\,^{\circ}C$ and $40 \pm 5\%$, respectively. Male SD rats were caged with individual females until mating was confirmed by observation of a vaginal plug. After birth, each litter was left with the mother until weaning; pups were not weighed at birth to prevent maternal rejection. Male offspring, selected at random from each litter, were used in all subsequent experiments.

Male neonates were assigned to 5 groups ($n = 6$/group): control, DEX, control + melatonin (MEL), DEX + MEL, and DEX + TSA. Male rat pups were injected i.p. with DEX on day 1 (0.5 mg/kg BW), day 2 (0.3 mg/kg BW), and day 3 (0.1 mg/kg BW) after birth. This treatment protocol was based on a 21-day tapering treatment given to premature infants to prevent chronic lung disease [8]. 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 [5]. Melatonin was prepared two times a week by dissolving the drug (10 mg) in 1 mL of 100% ethanol. This solution was then diluted with water to a final concentration of 0.01%. Water bottles were wrapped with aluminum foil to protect from light. Every 3 days, the water intake was measured and represented as mL/100 g of BW/day. The DEX + TSA group received DEX and trichostatin A (TSA, a HDAC inhibitor) with 0.5 mg/kg TSA subcutaneous injection once daily for 1 week [23].

Blood pressure was measured in conscious rats by an indirect tail-cuff method (BP-2000, Visitech Systems, Inc., Apex, NC, USA). To ensure accuracy and reproducibility, the rats were allowed to adapt to restraint and tail-cuff inflation for 1 week prior to the experiment, and measurements were taken between 1300 and 1700 each day. 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. For each rat, 5 cycles were recorded at each time point. Average of values from three stable measurements was taken. Rats were killed at 16 weeks of age. Heparinized blood samples were collected at sacrifice. Kidney and ventricular myocardium were harvested after perfusion with PBS, and snap frozen for western blot or RT-PCR.

2.2. Detection of L-arginine, L-citrulline, and dimethylarginine by HPLC

Plasma and kidney L-arginine (L-Arg), L-citrulline (L-Cit), ADMA, and symmetric dimethylarginine (SDMA, a stereoisomer of ADMA) levels were measured using HPLC (HP series 1100, Agilent Technologies, Inc., Santa Clara, CA, USA) with the OPA-3MPA derivatization reagent as described in our earlier study [5]. Concentrations of L-Arg L-Cit ADMA, and SDMA in the standards were in the ranges of 1–100 μM, 1–100 μM, 0.5–5 μM, and 0.5–5 μM, respectively. The recovery rate was ~95%. Homogenate was prepared by adding 100 mg of kidney tissue to 250 μL lysis buffer. Protein concentration was determined by Bradford assay. The tissue concentration was factored for protein concentration, which was represented as micromolar per milligram protein.

2.3. Quantitative real-time polymerase chain reaction (PCR) analysis

RNA was extracted using TRI Reagent (Sigma, St. Louis, MO) and treated with DNase I (Ambion, Austin, TX) to remove DNA contamination. 2 μg RNA was reverse-transcribed (SuperScript II RNase H-Reverse Transcriptase, Invitrogen, Bethesda, MD) using random primers (Invitrogen) in a total volume of 40 μL. Control RT reactions were performed by omitting the RT enzyme, and two-step quantitative real-time PCR was carried out using Quantitect SYBR Green PCR Reagents (Qiagen, Valencia, CA) on an iCycler iQ Multi-color Real-Time PCR Detection System (Bio-Rad Hercules, CA). Nephron deficit could result from changes in the expression of genes known to be involved in branching morphogenesis (BMP4, FGF2, and PAX2) and apoptosis (p53 and BAX). Several components of RAS, including renin, prorenin receptor (PRR), angiotensinogen (AGT), angiotensin II type 1 and 2 receptors (AT1R and AT2R), angiotensin (1–7) receptor Mas angiotensin-converting enzyme (ACE), and ACE2 were analyzed in this study. Next, SGK1 and several sodium transporters were analyzed in this study included Na–K–2Cl cotransporter (NKCC2), type 3 sodium hydrogen exchanger (NHE3), Na^+/Cl^- cotransporter (NCC), and Na^+/K^+ATPase α1 subunit (NaKATPase). R18S was used as a reference. Primers were designed using GeneTool Software (Biotools, Edmonton, Alberta, Canada) as we previously published [21,22]. All PCR reactions were run in duplicate. For the relative quantification of gene expression, the comparative threshold cycle (C_T) method was employed. The average C_T for the unknown was subtracted from