Journal of Steroid Biochemistry & Molecular Biology 152 (2015) 8–15

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Mutual effects of melatonin and activin on induction of aldosterone production by human adrenocortical cells

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ARTICLE INFO

Article history:
Received 1 December 2014
Received in revised form 18 March 2015
Accepted 10 April 2015
Available online 15 April 2015

Keywords:
Activin
Adrenocorticotropin
Aldosterone
Melatonin
Steroidogenesis

ABSTRACT

Melatonin has been reported to suppress adrenocorticotropin (ACTH) secretion in the anterior pituitary and cortisol production in the adrenal by different mechanisms. However, the effect of melatonin on aldosterone production has remained unknown. In this study, we investigated the role of melatonin in the regulation of aldosterone production using human adrenocortical H295R cells by focusing on the activin system expressed in the adrenal. Melatonin receptor MT1 mRNA and protein were expressed in H295R cells and the expression levels of MT1 were increased by activin treatment. Activin increased ACTH-induced, but not angiotensin II (Ang II)-induced, aldosterone production. Melatonin alone did not affect basal synthesis of either aldosterone or cortisol. However, melatonin effectively enhanced aldosterone production induced by co-treatment with ACTH and activin, although melatonin had no effect on aldosterone production induced by Ang II in combination with activin. These changes in steroidogenesis became apparent when the steroid production was evaluated by the ratio of aldosterone/cortisol. Melatonin also enhanced dibutyryl-AMP-induced aldosterone/cortisol levels in the presence of activin, suggesting a functional link to the cAMP-PKA pathway for induction of aldosterone production by melatonin and activin. In accordance with the data for steroids, ACTH-induced, but not Ang II-induced, cAMP synthesis was also amplified by co-treatment with melatonin and activin. Furthermore, the ratio of ACTH-induced mRNA level of CYP11B2 compared with that of CYP17 was amplified in the condition of treatment with both melatonin and activin. In addition, melatonin increased expression of the activin type-I receptor ALK-4 but suppressed expression of inhibitory Smads6/7, leading to the enhancement of Smad2 phosphorylation. Collectively, the results showed that melatonin facilitated aldosterone production induced by ACTH and activin via the cAMP-PKA pathway. The results also suggested that mutual enhancement of melatonin and activin receptor signaling is involved in the induction of aldosterone output by adrenocortical cells.

1. Introduction

Aldosterone production in the adrenal glomerulosa is directly stimulated by angiotensin II (Ang II), potassium and adrenocorticotropin (ACTH). The major signal transduction pathway for ACTH stimulation of aldosterone production occurs through cAMP-protein kinase A (PKA), while Ang II action is transduced by diacylglycerol-protein kinase C, inositol 1,4,5-trisphosphate/Ca^{2+} signaling and mitogen-activated protein kinase (MAPK) via the Ang II type-I receptor (AT1R) [1].

In the presence of these major stimulators, adrenocortical steroidogenesis is modulated by local autocrine/paracrine factors

Abbreviations: ACTH, adrenocorticotropin; ACTH-R, adrenocorticotropin receptor; ActRI, activin type-I receptor; ActRII, activin type-II receptor; ALK, activin receptor-like kinase; AngII, angiotensin II; AT1R, Ang II type 1 receptor; BMP, bone morphogenetic protein; BMPRI, BMP type-I receptor; BMPRII, BMP type-II receptor; CYP11B2, P450 aldo gene; CYP17, P450 c17 gene; IBMX, 3-isobutyl-1-methylxanthine; MAPK, mitogen-activated protein kinase; MR, mineralocorticoid receptor; MT, melatonin receptor; PKA, protein kinase A; TGF, transforming growth factor.

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http://dx.doi.org/10.1016/j.jsbmb.2015.04.012
0960-0760/

that reside in adrenal tissues [2]. Basic fibroblast growth factor, insulin-like growth factors, and transforming growth factor (TGF)-β1 have been postulated to play roles in the regulation of adrenal steroidogenesis [2–5]. We previously reported the existence of a bone morphogenetic protein (BMP) and activin system consisting of specific type-I and -II receptors and Smads in adrenocortical cells [6–9]. TGF-β superfamily members including BMPs, growth and differentiation factors, and activins play important roles as autocrine/paracrine factors in the regulation of ovarian steroidogenesis [10,11]. In adrenocortical cells, BMP-6 is involved in the stimulation of Ang II-induced aldosterone production by upregulating the MAPK pathway [7–9], while the activin system is functionally linked to the ACTH-induced cAMP-PKA cascade in adrenocortical cells [6]. In addition, BMP signaling in the adrenal medulla was also found to play a regulatory role in catecholamine synthesis induced by adrenocortical steroids [12,13].

On the other hand, melatonin is involved in the physiological control of circadian and seasonal rhythms as well as in the activities of hormones and cytokines [14–16]. Melatonin actions are elicited via two types of G protein-coupled receptors, MT1 and MT2, which are expressed in the brain and various peripheral tissues. Melatonin receptors have also been detected in adrenal tissues. Regarding the effects of melatonin on adrenocortical hormones, it has been shown that melatonin, acting directly on the adrenal gland, inhibits the glucocorticoid response to ACTH in monkeys, sheep, rats and humans [17–20].

The circadian rhythms of melatonin and ACTH are inversely fluctuated. In humans, melatonin secretion peaks at night and decreases in the daytime. In contrast, circulating ACTH-cortisol peaks in the early morning and declines during the night. Interestingly, in Cushing's syndrome, which exhibits a lack of ACTH-cortisol secretory rhythm, the circadian change of melatonin was shown to be abnormal [21]. This finding implies that the increased melatonin at night plays a physiological role in suppression of ACTH-cortisol secretion or that excessive cortisol may lead to the abolishment of normal melatonin rhythm. It has been revealed that melatonin inhibits ACTH-induced cortisol production via MT1 expressed on the adrenals in various mammals [17,19,20]. Given that melatonin secretion can be abnormally lower at night and higher in the daytime in patients with Cushing's syndrome [21], we assumed that the key circadian factor melatonin is involved in the pathogenesis of disturbed circadian changes in ACTH and cortisol.

Melatonin has been reported to suppress ACTH secretion in the anterior pituitary and cortisol production in the adrenal by different mechanisms. However, the effect of melatonin on aldosterone production, in comparison with cortisol changes, has remained unknown. In order to clarify the interaction between melatonin and adrenal steroidogenesis under the influence of ACTH, we studied undefined roles of melatonin in the regulation of aldosterone production using human adrenocortical cells by focusing on the activin system.

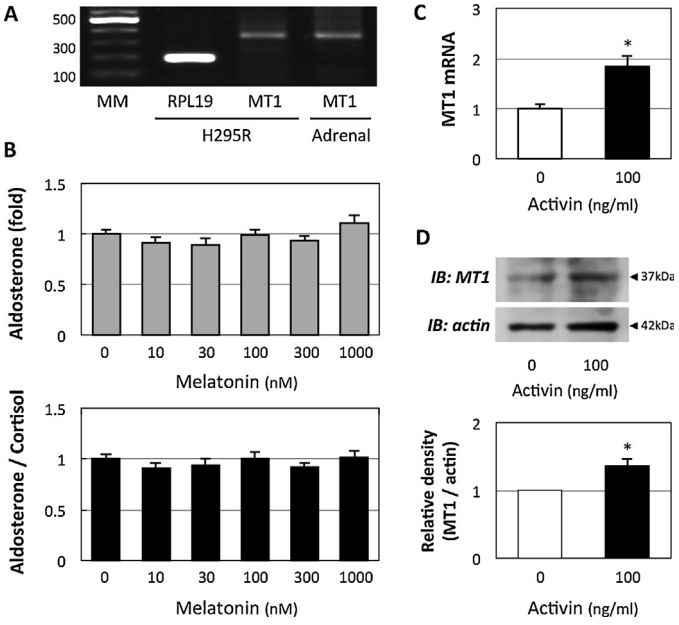

Fig. 1. Expression of melatonin receptors and effect of melatonin on aldosterone synthesis in human adrenocortical cells. (A) Expression of mRNAs encoding MT1 (368 bp) and RPL19 (190 bp) was examined by RT-PCR analysis in H295R cells compared with that in normal human adrenal tissue. MM indicates molecular weight marker. (B) After cells (3×10^5 cells/well) had been precultured in 24-well plates with 10% FCS, the medium was changed to DMEM/F12 containing 1% FCS, and then the cells were treated with indicated concentrations of melatonin. After 24-h culture, aldosterone concentrations and ratios of aldosterone/cortisol production in the culture media were determined. (C) Total cellular RNA was extracted from H295R cells that had been treated with activin in DMEM/F12 containing 1% FCS for 24 h, and mRNA levels of MT1 were determined by quantitative PCR. The mRNA levels of MT1/RPL19 were expressed as fold changes. (D) After preculture in a serum-free condition, cells (1×10^5 viable cells/well) were treated with activin for 24 h. The cell lysates were then subjected to SDS-PAGE/immunoblotting analysis using anti-MT1 and anti-actin antibodies. The integrated signal density of each protein band was digitally analyzed, and the ratios of signal intensities of MT1/actin were calculated. Results are shown as means ± SEM. The results were analyzed by ANOVA (B) and unpaired *t*-test (C, D). *, $P < 0.05$ vs. control group.

2. Materials and methods

2.1. Reagents and supplies

A 1:1 mixture of Dulbecco's Modified Eagle's Medium/Ham's F-12 medium (DMEM/F12), penicillin-streptomycin solution, and Ang II acetate salt adrenocorticotropic hormone human fragment 1-24 (1-24 ACTH), recombinant human activin A, N^6,O^2-dibutyryl adenosine-3′,5′-cyclic monophosphate monosodium salt (BtcAMP), 3-isobutyl-1-methylxanthine (IBMX), melatonin and bovine serum albumin were purchased from Sigma–Aldrich Co., Ltd. (St. Louis, MO). Insulin-transferrin-sodium selenite plus (ITS+) was from BD Falcon (Bedford, MA). Total human adrenal RNAs (Stratagene, San Diego, CA) were used as a control study.

2.2. Cell culture and hormone assays

The NCI-H295R human adrenocortical cell line was obtained from American Type Culture Collection (Manassas, VA). H295R cells were maintained in DMEM/F12 supplemented with 10% FCS. Cells (3×10^5 viable cells/well) were precultured in 24-well plates with 10% FCS for 24 h. The medium was then changed to DMEM/F12 containing 1% FCS and 4 mM potassium, and the cells were treated with indicated reagents. After 24-h culture, aldosterone and cortisol concentrations in the culture media were measured by a radioimmunoassay (SPAC-S aldosterone, TFB Co., Tokyo) and chemiluminescent immunoassay (ACS-E Cortisol II, Siemens Healthcare Diagnostics Co., Tokyo), respectively. Steroid contents were undetectable in the cell-free medium. To assess cellular cAMP synthesis, cells (3×10^5 viable cells/well) were cultured in DMEM/F12 containing 1% FCS and 0.1 mM of a phosphodiesterase inhibitor, IBMX. After 24-h culture, the conditioned medium was collected and the extracellular contents of cAMP were determined by EIA (Cyclic AMP EIA Kit Cayman Co., Ann Arbor, MI) with assay sensitivity of 0.3 nM.