

Modulation of bone morphogenetic protein activity by melatonin in ovarian steroidogenesis

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Abstract

Background: Melatonin regulates circadian and seasonal rhythms and the activities of hormones and cytokines that are expressed in various tissues, including the ovary, in which melatonin receptors are expressed. In the ovary, follicular growth occurs as a result of complex interactions between pituitary gonadotropins and autocrine and paracrine factors, including bone morphogenetic proteins (BMPs) that are expressed in the ovary.

Methods: The effects of melatonin and BMPs on steroidogenesis were examined by using the primary cultures of rat granulosa cells.

Main findings (Results): It was shown that melatonin has antagonistic effects on BMP-6 actions in the granulosa cells, suggesting that melatonin is likely to contribute to balancing the biological activity of endogenous BMPs that maintain progesterone production and luteinization in the growing follicles. Similar interactions between melatonin and BMP–smad signaling also were shown in the mechanism of controlling ovarian steroidogenesis by other ligands.

Conclusion: A new role of melatonin in the regulation of endocrine homeostasis in relation to BMP activity is introduced in this review.

KEYWORDS

bone morphogenetic protein, follicle-stimulating hormone, granulosa cells, melatonin, smad, steroidogenesis

1 | INTRODUCTION

The various processes of follicular growth and development are regulated by complex interactions between gonadotropins that are secreted from the anterior pituitary and autocrine and paracrine factors that are expressed in the ovary. Melatonin, which is predominantly derived from the pineal gland, is closely linked to the physiologic regulation of biological rhythms and related hormonal activities.^{1,2}

Local growth factors and cytokines that are expressed in the ovary, including bone morphogenetic proteins (BMPs), elicit various activities for regulating ovarian steroid production and the proliferation of granulosa

cells.^{3–5} The expression of BMP system molecules has been shown in the cellular components of growing ovarian follicles⁶ and the regulatory mechanism of reproductive function has been gradually revealed.^{4,7,8}

Recently, the authors reported an interesting activity of melatonin in the control of BMP receptor (BMPR) signaling and ovarian steroidogenesis by regulating inhibitory smad expression.⁹ The existence of a molecular interaction between melatonin and BMPR-to-smad signaling also seems to be applicable to various ligands other than melatonin. This functional interrelationship could be linked to therapeutic approaches for various ovarian dysfunctions, such as polycystic ovary syndrome (PCOS).

2 | BONE MORPHOGENETIC PROTEIN ACTIONS AND OVARIAN STEROIDOGENESIS

The fine-tuning of follicle-stimulating hormone (FSH) responsiveness in the follicles is critical for dominant follicle selection and subsequent ovulation. The finding suggesting that defects of oocyte-specific growth factors, such as growth differentiation factor (GDF)-9 and BMP-15, cause infertility became a breakthrough in the field of mammalian reproduction.^{10,11} The expression of BMP-15 is localized to oocytes,¹² in which BMP-15 induces granulosa cell mitosis but inhibits FSH actions by suppressing FSH receptor (FSHR) expression.^{13,14}

The BMP system in the ovary plays key roles in the maintenance of female fertility in mammals.^{4,5} It is interesting that BMPs commonly suppress FSH-induced progesterone synthesis by granulosa cells.^{4,7,8,15-17} The BMP-15 that is secreted from oocytes suppresses FSH actions by inhibiting FSHR expression in the granulosa cells.¹³ The BMP-2 from the granulosa cells, BMP-6 from the oocytes and granulosa cells, BMP-4 and -7 from the theca cells, and BMP-9 in the serum and granulosa cells act to suppress the FSH-induced production of progesterone.¹⁵⁻¹⁹ Hence, the major action of BMPs was revealed to be to control the sensitivity of the FSHR in relation to the granulosa cells in the process of folliculogenesis.^{4,7,8}

In a clinical aspect regarding BMP expression in the ovary, it was shown that the expression of GDF-9 messenger (m)RNA was delayed and reduced during the growth and differentiation phase, in comparison to BMP-15 expression, in human PCOS ovarian tissues.²⁰ It also was reported that GDF-9 protein expression was decreased in the cumulus granulosa cells, whereas the level of the GDF-9 and BMP-15 proteins was not different in the oocytes between the PCOS cases and the control cases.²¹ Of interest, it was further demonstrated that the expression level of BMP-6 was enhanced in the granulosa cells that had been isolated from the PCOS ovaries.²²⁻²⁴

3 | MELATONIN'S ACTIONS IN THE OVARY

Melatonin is functionally involved in the formation of the reproductive rhythm through its effect on the pars tuberalis in the pituitary gland, in which melatonin receptors are highly expressed, for seasonal animals.^{25,26} It has been shown that melatonin has various effects on follicle-component cells, such as granulosa cells and oocytes, in the ovary.¹ The bioactivity of melatonin is induced via G protein-coupled receptors, including MT1 and MT2, that are expressed not only in the brain but also in the peripheral tissues.²⁷ MT1 and MT2 are expressed in endocrine tissues, including gonadotropin-releasing hormone neurons in the hypothalamus and also in the ovarian follicles, indicating the involvement of melatonin's actions in the female reproductive system composed of the hypothalamic-pituitary-ovary (HPO) axis.

MT1 and MT2 expression was shown in the rat ovary, in which the binding capacity to melatonin was altered by the cycling phases.²⁸ The expressional modulation of the melatonin receptors was likely to have been caused by estrogen in the follicles. The differential expression of the MT1 and MT2 receptors, regulated by melatonin, also was shown

in the cell membrane, cytoplasm, and nuclear membrane of the granulosa cells in the bovine ovary.²⁹ The expression of a synthetic enzyme of melatonin, acetylserotonin O-methyltransferase, as well as MT1, was detected in the bovine cumulus-oocyte complex, suggesting the possibility of the local production of melatonin in the ovary, though MT2 expression was detected only in the oocytes.³⁰

The melatonin concentrations in the fluid that was collected from human ovaries were shown to be high, compared with the concentrations in the blood.^{2,31} The melatonin concentrations in follicles fluctuate³² and are increased by follicular enlargement and ovulation induction.³³ In this regard, the whole ovary,³⁴ granulosa cells,³⁰ and oocytes³⁵ seem to have the capacity to synthesize melatonin *in situ*. As melatonin that is synthesized in the ovary is not secreted elsewhere, melatonin seems to act for the ovarian cells themselves, possibly as an anti-oxidant and/or an autocrine or paracrine factor.³⁶

4 | INTERACTION OF MELATONIN AND BONE MORPHOGENETIC PROTEINS IN OVARIAN STEROIDOGENESIS

Given that both melatonin³⁷ and BMP-6¹⁸ are involved in progesterone synthesis and the luteinization process in the ovary, a functional interaction between melatonin and BMP signaling is thought to exist in the granulosa cells (Figure 1). The results of experiments

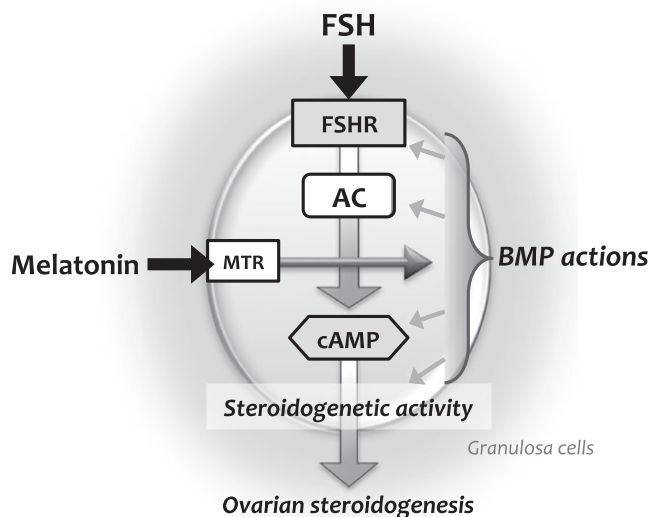


FIGURE 1 Interaction of melatonin and bone morphogenetic proteins (BMPs) in ovarian steroidogenesis. The BMPs commonly inhibit follicle-stimulating hormone (FSH) actions by suppressing the cascade of follicle-stimulating hormone receptor (FSHR) to adenylate cyclase (AC) activity, resulting in the suppression of progesterone production and the luteinization of granulosa cells. The inhibitory effect of the BMPs on FSH-induced progesterone production is impaired by melatonin action. The FSH-induced steroidogenesis in the granulosa cells is regulated by the balanced interaction of the BMP signaling and melatonin activity. cAMP, cyclic adenosine monophosphate; MTR, melatonin receptor

using rat granulosa cells⁹ showed that the melatonin treatment did not affect steroidogenic activities, such as estradiol or progesterone production by granulosa cells. However, of note, the inhibitory effect of BMP-6 on FSH-induced progesterone production was reversed in the presence of melatonin actions (Figure 1).⁹ In accordance with its effect on progesterone synthesis by granulosa cells, melatonin reversed the inhibitory effects of BMP-6 on cyclic adenosine monophosphate synthesis, as well as the mRNA expression of steroidogenic factors and enzymes, including steroidogenic acute regulatory protein, P450 steroid side-chain cleavage enzyme, and 3 β -hydroxysteroid dehydrogenase, induced by FSH stimulation (Figure 2).⁹

As a mechanism by which melatonin antagonizes BMP-induced progesterone suppression, it was found that BMP-6-induced smad and Id-1 signaling were impaired by melatonin.⁹ The expression levels of the BMP type-I and type-II receptors, such as activin receptor-like kinase (ALK)-2, ALK-6, activin type-II receptor, and BMP type-II receptor, on the granulosa cells were not changed by melatonin treatment.⁹ On the contrary, MT1 expression in the granulosa cells was not affected by BMP-6 and BMPR expression remained stable under the condition of melatonin treatment. Of interest, the mRNA and protein levels of inhibitory smad6, but not those of smad7, were significantly augmented by melatonin supplementation, indicating a new regulatory mechanism of melatonin in such BMP-6 actions on progesterone suppression in granulosa cells (Figure 2).

The effects of various factors that can affect FSH-induced steroidogenesis were investigated further (Figure 2). Among the

examined factors, androgens,³⁸ growth hormone, and insulin-like growth factor-I³⁹ were found to be key molecules that induce smad6/7 expression. In contrast, prolactin (PRL),⁴⁰ somatostatins,⁴¹ and incretins⁴² were found to be suppressors of inhibitory smad6/7 expression in the granulosa cells (Figure 2). Thus, the modulatory effects on BMP activity in granulosa cells via the expression of smad6/7 molecules could be critical for integrating steroidogenesis through controlling endogenous BMP signaling.

5 | MELATONIN AND BONE MORPHOGENETIC PROTEINS IN OTHER ENDOCRINE TISSUES

As mentioned above, the mechanism by which melatonin suppresses BMP activity in granulosa cells was found to be the induction of inhibitory smad-6 expression in the granulosa cells.⁹ As an antagonistic effect of melatonin on BMP action was shown in ovarian steroidogenesis, similar regulatory interactions between melatonin and BMPs are seen in adrenocortical steroidogenesis (Figure 3). The expression of melatonin receptors, mainly the MT1 receptor, has been reported in adrenal tissues.⁴³ As for the effects of melatonin on adrenocortical functions, it has been shown that melatonin inhibits glucocorticoid synthesis by the zona fasciculata in response to adrenocorticotropin (ACTH).⁴³ It was of note that melatonin suppressed ACTH secretion via the action of BMP-4 that was expressed in the corticotrope cells⁴⁴ (Figure 3). In addition to the suppression of ACTH secretion, melatonin

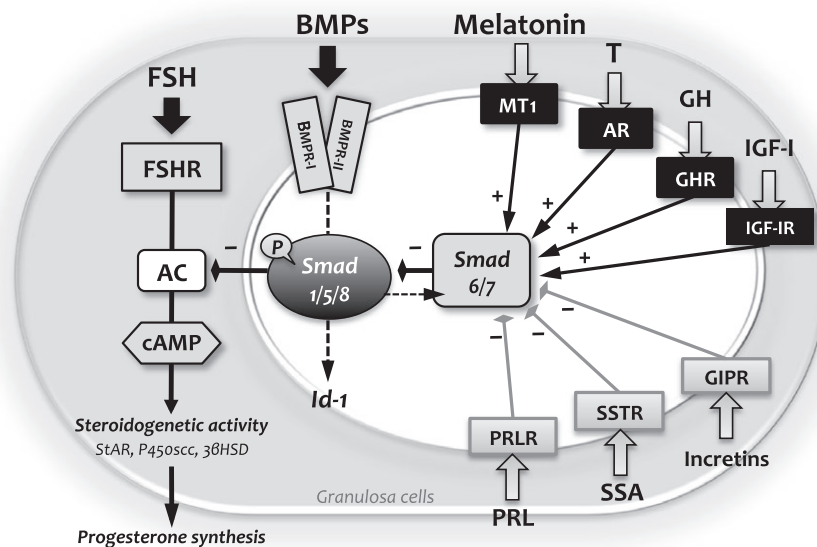
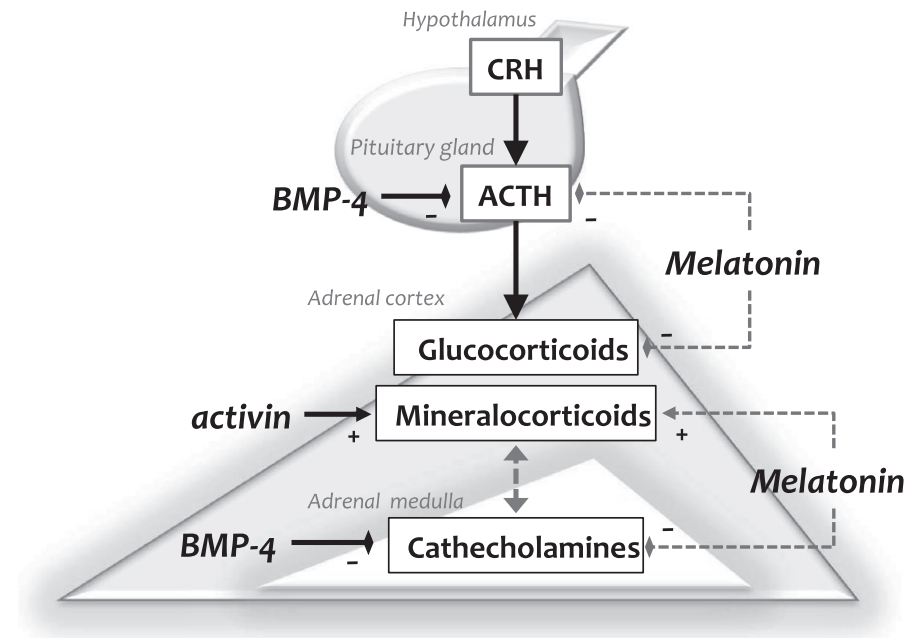


FIGURE 2 Regulation of bone morphogenetic proteins (BMPs)–smad signaling in ovarian granulosa cells. The mechanism by which melatonin suppresses BMP activity in the granulosa cells was found to be in the reduction of inhibitory smad6/7 expression in the granulosa cells. Androgen (T), growth hormone (GH), and insulin-like growth factor (IGF)-I were found to be the key molecules that can induce smad6/7 expression. On the contrary, prolactin (PRL), somatostatin (SST), and incretins were found to be suppressors of the expression of inhibitory smad6/7 in the granulosa cells. The modulatory effects on BMP activity in the granulosa cells via smad6/7 functions are likely to be critical for controlling steroidogenesis via endogenous BMP signaling. AC, adenylate cyclase; AR, androgen receptor; BMPRI and -II, BMP type-1 and -2 receptors; cAMP, cyclic adenosine monophosphate; FSH, follicle-stimulating hormone; FSHR, follicle-stimulating hormone receptor; GHR, growth hormone receptor; GIPR, GIP receptor; HSD, 3 β -hydroxysteroid dehydrogenase; IGF-IR, IGF-1 receptor; MT1, melatonin type-1 receptor; P, progesterone; P450scc, P450 steroid side-chain cleavage enzyme; PRLR, PRL receptor; SSA, single strand annealing; SSTR, SST receptor; StAR, steroidogenic acute regulatory protein

FIGURE 3 Functional interaction of melatonin and bone morphogenetic proteins (BMPs) in the hypothalamic–pituitary–adrenal (HPA) axis. In addition to its regulatory effect on ovarian steroidogenesis, melatonin is involved in the regulation of the HPA axis, including the suppression of adrenocorticotropin (ACTH) production, in cooperation with BMP-4 action, in the pituitary corticotrope cells, a reduction of adrenal cortisol production, activation of aldosterone production that is induced by ACTH and activin in the adrenocortical cells, and the suppression of catecholamine production by cooperating with BMP-4 in the adrenomedullary cells. CRH, corticotropin-releasing hormone



also reduced the secretion of another pituitary hormone, PRL, in the lactotrope cells,⁴⁵ in which melatonin acts as a functional modulator of pituitary BMP-4 action that can enhance PRL secretion.⁴⁶

In contrast to its effect on cortisol secretion, in the zona glomerulosa of the adrenal cortex, melatonin facilitates aldosterone synthesis in the presence of ACTH and activin in the adrenocortical cells (Figure 3).⁴⁷ In the adrenomedullary cells, it was revealed that melatonin suppresses catecholamine synthesis in cooperation with the effects of BMP-4 and glucocorticoids.⁴⁸ Hence, melatonin is functionally involved not only in the HPO but also in the hypothalamic–pituitary–adrenal axis, which regulates adrenal steroidogenesis and the mutual interaction between the adrenal cortex and medulla.

6 | CLINICAL IMPLICATIONS OF MELATONIN AND BONE MORPHOGENETIC PROTEINS IN OVARIAN DISORDERS

Recently, the clinical application of the effect of melatonin in relation to female infertility-related PCOS has been proposed.⁴⁹ Infertility that is caused by PCOS is associated with a lowered quality of oocytes, granulosa cells, and embryos and with anovulation. Melatonin acts as a direct free-radical scavenger to reduce oxidative stress without binding to the ovarian receptors.⁵⁰ Melatonin passes through the physiological barriers and elicits anti-oxidant activities⁵¹ by scavenging reactive oxygen species (ROS) and reactive nitrogen species.⁵¹ The ROS suppress progesterone synthesis by inhibiting the actions of the steroidogenic enzymes and transport of cholesterol to the mitochondria. Melatonin restores the progesterone reduction that is caused by the ROS in luteinized granulosa cells.⁵⁰ The usefulness of melatonin as a therapeutic tool in the reduction of ovarian graft rejection also has been reported by virtue of its anti-oxidative and anti-apoptotic properties.⁵² Given that ROS-induced oxidative stress is likely to be

responsible for the poor quality of oocytes and granulosa cell apoptosis in PCOS, the maintenance of the melatonin level in the follicular fluid would be important for healthy follicular growth and successful ovulation.^{1,2,49,50,53} Melatonin could be effective in ameliorating ovarian dysfunction and poor oocyte quality in women with PCOS.^{49,50}

It also has been reported that BMP-6 mRNA expression was increased in granulosa cells that were isolated from the ovaries of patients with PCOS, in comparison with its expression in control granulosa cells for in vitro fertilization.²²⁻²⁴ The BMP-2, -4, and -6 were not detected in the serum from patients with PCOS, while BMP-7 was weakly detected in some cases.⁵⁴ The BMP-6 has been reported to be expressed in the granulosa cells of healthy follicles but not in atretic follicles in the human ovary.⁵⁵ The enhancement of BMP-6 expression in the granulosa cells from patients with PCOS may imply a certain disturbance of folliculogenesis. The counteracting effect of melatonin on BMP-6 activity in granulosa cells might compensate the progesterone reduction and improve the arrested follicular growth in the PCOS ovaries. Considering that BMP-6 acts as a luteinization inhibitor for normal folliculogenesis, melatonin supplementation might contribute to a reduction of the biological activity of endogenous BMP-6 in order to maintain the progesterone level and the luteinizing process.

7 | CONCLUSION

Melatonin is likely to exert a regulatory effect on BMPR signaling in granulosa cells. As the ovarian BMP system plays a physiological role as a luteinization inhibitor in the growing follicles, melatonin can be an effective modulator to control the progesterone balance and luteinization. Given that the expression of BMP-6 in granulosa cells is enhanced in patients with PCOS, melatonin might play a critical role in the restoration of folliculogenesis and the ovulation process.

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DISCLOSURES

Conflict of interest: The author declares no conflict of interest. **Human Rights Statement and Informed Consent:** This article does not contain any study with human participants that was performed by the author. **Animal Studies:** The animal protocols regarding the study's experimental results were approved by Okayama University Institutional Animal Care and Use Committee, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan.

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