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What is the role for melatonin in plants?
- Review on the current status of phytomelatonin research -

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ABSTRACT

Animal neurohormone melatonin, *N*-acetyl-5-methoxytryptamine, has been found in diverse plants. Melatonin is widely distributed in the plant kingdom and in some plants, e.g. St. John's wort, exists in high concentrations. However, the synthesis and action of melatonin in plants are still largely unknown. In this review we will discuss the proposed biosynthetic pathways and probable physiological roles of phytomelatonin.

Key words : melatonin, biosynthesis, physiological function, plant

Melatonin, *N*-acetyl-5-methoxytryptmine, has been studied and focused mainly as an indole amine neurohormone that is synthesized and secreted from the pineal gland in animals, since it was found by Lerner and his colleagues in 1958 (1). Melatonin is also found in diverse organisms including prokaryotes, unicellular eukaryotes, fungi, algae and higher plants (2). Based on the ubiquitous distribution of melatonin in all kingdoms, melatonin was even suggested as the nature's most versatile biological signal (3). The synthesis and function of melatonin in other organisms except animals and humans has been very poorly understood so far. In this paper, we review the status of contemporary understanding of the biochemistry and physiology of melatonin especially in higher plants.

Melatonin in animals

1. Function of melatonin in animals

Melatonin is involved in the regulation of biological rhythm that is related to "the time of the day" and "the time of the year" (for review see (3, 4, 5)). Melatonin adjusts the periods of the daily oscillations of the main biological clock and therefore can be defined as chronobiotic (6). For example, exogenously applied melatonin advances the body clock and stimulates early sleeping (7). Therefore, melatonin is applied for curing circadian rhythm sleep disorders (8)

At high concentrations, melatonin exerts anti-oxidant functions (9) that are also observed with other indole compounds. Melatonin scavenges hydroxyl radicals with a rate constant of 10^{10} $M^{-1} \cdot S^{-1}$ range (10, 11). In addition, melatonin indirectly induces the expression of anti-oxidative genes and represses the pro-oxidative genes. For example, melatonin acts as the negative regulator of 12-lipoxygenase (12) and nitric oxide synthase (13, 14).

Melatonin modulates the antibody response and antagonizes the corticosterone that acts as an immunesuppressor (15). Cancer promotion may be prevented by melatonin that increases during night (16). Inhibition of tumor growth by melatonin was reported in brest- (17) and other types of cancers (18). Melatonin also has diverse roles in cardiovascular regulation (19), the formation of bone (20) en-

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ergy expenditure (21) and reproduction (22).

2. Biosynthetic pathway of melatonin in animal

Melatonin originates from the amino acid tryptophan (Trp) via four steps of biochemical reactions in animals (for review see (4), **Fig 1**). Tryptophan is converted to 5-hydroxytryptophan by the enzyme tryptophan monooxygenase (T5M; EC 1.14.16.4). Then, 5-hydroxytryptophan is changed to 5-hydroxytryptamine by the aromatic-L-amino acid decarboxylase (AAAD; EC 4.1.1.28). The next step is the conversion of 5-hydroxytryptamine to N-acetylserotonin by the serotonin-N-acetyltransferase (AANAT); EC 2.3.1.87. This step works as the rate-limiting step of the whole biosynthetic pathway of melatonin. Finally, melatonin is synthesized from N-acetylserotonin by hydroxyindole-O-methyltransferase (HIOMT; EC 2.1.1.4). Alternatively, melatonin can be derived from N-acetyl-5-hydroxytryptamine by O-methylation that is catalyzed by hydroxyindole-O-methyl transferase (23, 24).

Because the regulation of melatonin action is essentially

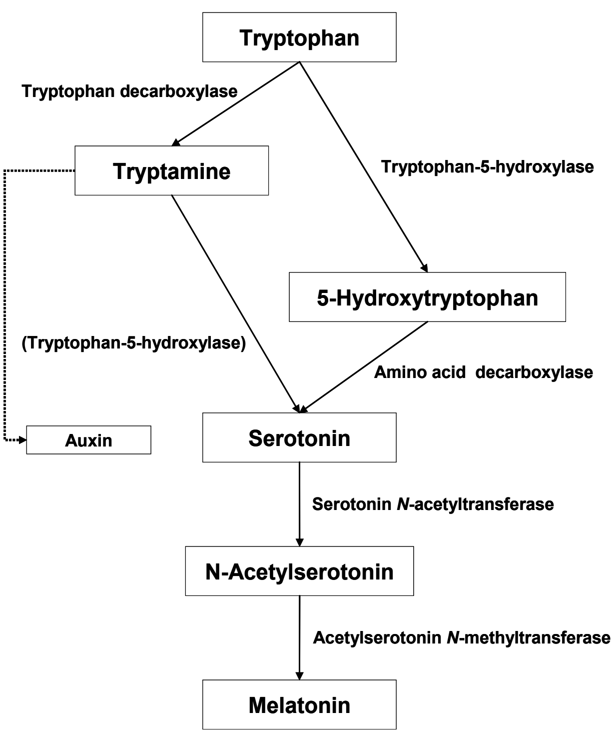

Fig 1. The biosynthesis of melatonin from tryptophan via four-steps in biochemical pathways.

based on the modulation of the biosynthesis, although the degradation and conjugation contributes to the regulation of melatonin level (4), the rate-limiting step that is catalyzed by the enzyme serotonin-N-acetyltransferase bears ample importance for the physiological function of melatonin in animal system. Therefore, the studies on the biosynthesis of melatonin would be the starting point for the elucidation of physiological roles for melatonin also in plants.

Current status of melatonin research in plants

1. Qualitative and quantitative analysis of melatonin in plants

Diverse extraction media have been applied to prepare melatonin from plant tissues: simple solvent, e.g. ethanol (25), buffers, e.g., phosphate buffer (26) and phosphate-buffered saline (27), and also alkaline solution, e.g., Na_2CO_3 solution (28). To prevent degradation of melatonin by photooxidation or free radical reactions, use of 0.4 M $HClO_4$ was recommended (29). Light condition was also carefully controlled to prevent photooxidation (29). After extraction, the extract is generally pre-purified by solid-phase extraction using C_{18} cartridges (30) or by partitioning (31). For analysis, diverse modern methods are available including HPLC, LC-MS, GC-MS, LC-MS/MS, radioimmunoassay. For mass spectrometry, duterium-labled melatonin is commercially available for internal standard. However, stable isotope-labeled melatonin is inadequate for HPLC to use as the internal standard, because HPLC generally equips with one of UV-monitor, diode array monitor, electrochemical (EC) detector and fluorescence detector, or with combination of the devices that cannot sense molecular weight. To solve this problem, a detection method resolving chemically modified melatonin together with highly fluorescent melatonin analogues was developed (32). The modified melatonin (N-((6-methoxy-4-oxo-1,4-dihydroquinolin-3-yl)methyl)acetamide,6-MOQMA) was excellently traced by the flurescence emission at 392 nm with excitation at 247 nm (32). The linearity of the calibration curve was established over the range of 5 - 500 fmol, which is sensitive enough to detect endoge-

Table 1. Examples of plants containing high amount of melatonin.

Scientific name	source	content ng/g tissue	References
Coptis chinensis Franch	plant	1,008	(35)
Epimedium brevicornum Maxim	plant	1,105	(35)
Hypericum perforatum	Leaf	1,750	(36)
Hypericum perforatum	flower	2,400-4,000	(36, 41)
Morus alba L	leaf	1,510	(35)
Phellodendron amurense	plant	1,235	(35)
Rheum palmatum L.	plant	1,078	(35)
Scutellaria baicalensis	plant	2,000-7,000	(29, 36, 41)
Tanacetum parthenium	leaf (fresh/dried)	1,300-7,000	(29, 36, 41)
Uncaria rhynchophylla	plant	2,460	(27)
Viola philipica Cav.	plant	2,368	(27)

nous melatonin. Although this method was originally established for mammalian tissues, it can be applied also for plant melatonin analysis. When facilities for handling radio-active substances, radio-labeled melatonin also could be used for HPLC as the internal standard.

Melatonin was detected in diverse angiosperms including both monocot and dicot plants (Table 1). Melatonin exists in ornamental-, wild- (33, 34), medicinal- (35, 36), and even in edible plants (26, 37). Some plants contain high concentrations of melatonin, about 10 pg - 2800 ng/g tissue (25), and evoke physiological and pharmacological effects on animals when the plants are fed. The level of melatonin in human blood can be increased by eating walnut, which contain 3.5 ± 1.0 ng melatonin/ g walnut fruits (37). This suggests the necessity of measuring the level of melatonin in the plants that are used for daily human diet. Some plants containing high amount of melatonin are listed in Table 1.

2. Biosynthesis of melatonin in plants

Tryptophan is a precursor for melatonin in St John's wort (*Hypericum perforatum* L. cv. Anthos) like in animals. (38) Radio-labeled tryptophan, ^{14}C-Trp, was fed and the products were monitored. The applied ^{14}C-Trp to the basal growth medium was converted to various indole compounds that include melatonin and serotonin and their precursors as reported in animals. This indicates the major backbone of the synthetic pathway may be similar both in plants and animals. However, the location of biosynthetic enzymes in plants is still undiscovered. The rice genomic data suggest the existence of three putative genes of tryptophan decarboxylases (www.gramene.org). Recently plant aromatic-L-amino acid decarboxylase (39) and tryptamine-5-hydroxylase were characterized (40).

The function of the putative genes warrants to be investigated in terms of the biosynthesis of melatonin in plants.

3. Proposed function of melatonin in plants

3.1 Functions similar to that in animals

Regulator of circadian rhythm

In *Chenopodium rubrum*, the level of melatonin shows diurnal fluctuation with increase during the night and decrease during the day (34, 41). Such a circadian rhythm is photoperiod-dependent. As in animals, the level of melatonin is high at night and low during the light period. The elevated period of melatonin is related to the length of the night (42). Kolar *et al*. (34) reported that both the nocturnal increase and the range of concentration are similar to the changes that have been reported in animals. However, the duration of the elevated melatonin was unaffected by the photoperiod (42). Not only in higher plants but also in algae and dinoflagellates, circadian changes of melatonin contents was reported (29). In animals melatonin treatment under long-day conditions evokes short-day responses (43). However, melatonin treatment under short-day conditions to short-day plants causes decrease of flowering, which is a typical short-day response of short-day plants (44). In a long day *Arabidopsis*, melatonin also delays flowering. Therefore, the regulation mechanism of circadian rhythm in plants seems to be different with that of animals. In addition, it should be noted that the concentrations of melatonin that induces plant responses are higher than the concentrations found in nature. In Arabidopsis, the active concentration for exogenous application is around 100 μM. The endogenous concentration of melatonin in *Hypericum perforatum* maximally reaches 4000 ngl/g.f.w. (36, 41). In animals, it is known that the effect of melatonin is saturated in nM range. Therefore, it should be carefully investigated to check whether melatonin is really involved in the regulation of circadian rhythm in plants.

Anti-oxidant as radical scavenger

Melatonin is a scavenger of reactive oxygen- and nitrogen species. Hydroxyl radical can be neutralized by melatonin, too. Because the radical scavenging activity is purely chemical, the same scavenging activity of melatonin could be equally expected also in plant bodies. However, the correlations between melatonin and plant enzymes, which act against oxidative stresses, is still unknown (45). Therefore, studies on the effect of melatonin on plant enzymes, e.g., superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GRd), glucose-6-phosphate dehydrogenase, seem to be necessary. Melatonin induces the gene expression of animal superoxide dismutase. Although the anti-oxidative action of melatonin was reported in plants like in animals, it should be noted that the effect could be just a result from chemical reactions without involvement of anti-oxidative enzymes.

3.2 Unique functions in plants

Auxin activity

Promotion of plant growth in lupin (46) and some monocot plants, (47) and root formation (48, 49) was reported in some monocot plants, indicating that melatonin could have auxin activity. However, melatonin does not always reveal auxin activities (45). Because auxin activity needs strict structural requests, e.g., aromatic ring should be connected to an acidic side chain, and the distance from the carboxyl group to the aromatic ring should be 5.4 Å, the melatonin, which is a substituted tryptamine lacking carboxyl group, does not fulfil the structural requirements of auxin. Because tryptamine derivatives could be converted to indole-3-acetic acid both enzymatically and non-enzymatically, auxin activities of melatonin could be evoked by spontaneous formation of indole-3-acetic acid, when melatonin is applied in high concentrations. In this case, the observed effects of melatonin cannot be defined as physiological, but as pharmacological.

Defense against herbivores

Melatonin in plants can disturb the physiology of herbivores when melatonin is accumulated in the animal body. Walnut contains high amount of melatonin (3.5 ± 1.0 ng/g of fruit), and its feeding increases blood melatonin level from 11.5 ± 1.9 pg/ml to 38.0 ± 4.3 pg/ml in rats (37). This phenomenon could be interpreted in terms of plant defense against herbivores. When tryptophan decarboxylase, which can convert 5-hydroxytryptophan to 5-hydroxytryptamine, was over expressed in tobacco, the reproduction of white fly was decreased (50), However, whether the inhibitory effect on white fly is really due to the produced melatonin is uncertain. Plant-animal interaction mediated by melatonin still remains to be systematically investigated. Besides melatonin, plants synthesize and accumulate diverse neurotransmitters, neuroregulators and neurotoxins, e.g., acetylcholine, dopamine, epinephrine, levodopa, gamma-aminobutyric acid, caffeine, hyperforin, △-9-tetrahydrocannbinol, nicotine, ß-oxalaminoalanine, and ß-methylaminoalanine (for review, see (51)).

3.3 Other effects of melatonin in plants

Exogenously applied melatonin affects cytoskeleton. In african blood lily, melatonin increases the birefringence and the number of mitotic spindle (52). Melatonin even disrupts mitosis in the roots of onion (53).

Binding of melatonin to calmodulin in plants is still unobserved. In rat cerebellum, melatonin binds to calmodulin and inhibits nitric oxide synthase (54).

3.4 Recognition of melatonin, the receptor problem

Melatonin is sensed by MT_1 and MT_2 receptors that are located on the plasma membrane in animals (55). If melatonin is really perceived by plants, there must be a receptor for melatonin. However, blast search with amino acid sequences of the animal receptors did not find any plant proteins, indicating that the plant melatonin receptor is different with that of animals. Given that anti-oxidation is the main function of melatonin, the receptor proteins could be absent, because the anti-oxidative activity of melatonin seems to be purely chemical. In this case signal transduction cannot play special roles for the melatonin action. There is currently no clues on the perception mechanism of melatonin in plants.

Conclusions

Plant melatonin derives from tryptophan probably via the biosynthetic pathway that is similar with that of animals. Roles of melatonin have been suggested as the regulator of circadian rhythm, anti-oxidant, auxin, and defense molecule. Although the physiological roles for melatonin in plants are still uncertain, wide distribution of melatonin in plant kingdom suggests the importance of melatonin also in plants. Research of phytomelatonin is certainly interesting and important new field of plant physiology.

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