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Melatonin: Another Phytohormone?

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Short communication

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ABSTRACT

Melatonin is known to be produced by plants and to exert numerous effects, which overlap with actions of known phytohormones, including auxins, ethylene, abscisic, jasmonic and salicylic acids. It exhibits growth effects, alleviates stress by heat, cold, drought, and toxic chemicals, counteracts infections by bacterial or fungal pathogens, favours wound healing, delays senescence and acts as an antioxidant and photoprotectant. Stressors and intense radiation frequently induce substantial increases in melatonin. High levels are particularly found in oily seeds. The criteria are discussed which have to be fulfilled before melatonin might be classified as a phytohormone. These include, in particular, the identification of high-affinity binding sites, of components of signal transduction pathways, the determination of freely movable melatonin and its movements within the organism.

INTRODUCTION

Since melatonin (N-acetyl-5-methoxytryptamine) had been discovered in phototrophic organisms, first in a dinoflagellate and later in plants, this compound has been detected in numerous algae and plant species^[1-4]. Notably, the concentrations measured in plants were highly divergent, frequently in the nanomolar range, sometimes at the borderline of detection, but in other cases, above 20 or even 30 µg/g^[4,5]. Even levels in the range of 200 µg/g have been reported to be present in kernels of some Iranian *Pistacia vera* cultivars^[6]. Early research in this field had focused on two aspects, the presence of high melatonin levels in medicinal plants, and the possibility of functions similar to those known from vertebrate animals, especially the transmission of the signal 'darkness' in photoperiodic responses^[7,8]. Although there was evidence for such a role in a dinoflagellate, this function was not clearly demonstrated in plants^[5,9,10]. A circadian rhythm with a prominent nocturnal peak, as in vertebrates, was found in a short-day ecotype of *Chenopodium rubrum*, but flower induction by melatonin could not be shown^[11,12]. The absence of any florigenic activity became obvious in several other short-day plants, and a nocturnally peaking circadian rhythm was also not generally found in other species^[5,10].

Meanwhile, a number of functions have been ascribed to melatonin in various plants, both dicots and monocots, and have been repeatedly reviewed^[5,10,12-18]. The aim of this short communication is not to summarize another time the findings on melatonin in plants, but rather to focus on a crucial point, namely the questions of whether melatonin may be a phytohormone and which criteria have to be fulfilled to justify such a conclusion. The actions of melatonin are remarkably diverse and not entirely free of some contradictions. A frequently made observation concerns auxin-like actions, as discussed in the reviews mentioned. Although melatonin is an indolic compound like indole 3-acetic acid (IAA), the structural differences are too large for assuming actions via the same binding protein. Occasionally, melatonin was reported to increase IAA levels, such as in roots of *Brassica juncea*^[19]. However, elevations of melatonin by a transgene in tomato plants caused decreases in IAA^[20]. It might be assumed that melatonin and auxins act via different signal transduction pathways, which finally converge at some but not necessarily all regulatory checkpoints, a concept that would still require elaboration of mechanistic details. On the other hand, findings exist that are not easily compatible with auxin-related actions or may be even perceived as conflicting. Although melatonin as well as two auxins stimulated lateral root formation in *Arabidopsis thaliana*, melatonin failed to enhance the expression of an auxin-dependent GUS reporter^[21]. A transcriptomic study in *A. thaliana* revealed further details that seem to be poorly compatible with

auxin-like actions, since genes of auxin signaling were mostly downregulated by melatonin, whereas genes related to signaling of ethylene, abscisic, jasmonic and salicylic acids were upregulated [22]. These divergencies may still be conditional, and melatonin may behave differently in a context of growth. Nevertheless, these findings strongly support roles of melatonin in stress resistance, defense, wound healing and senescence, which have been well documented in various functional studies, too. In fact, melatonin exhibited a broad spectrum of actions in alleviating stress by heat, cold, drought, and toxic chemicals, counteracting infections by bacterial or fungal pathogens, in photoprotection, favoring wound healing and delaying senescence, as summarized elsewhere [5,10,18]. Notably, melatonin formation is, sometimes strongly, upregulated by several of these stress-related factors. In particular, exposure to high light intensities and, especially, UV radiation causes considerable increases in melatonin, which become also evident when comparing same or closely related species from different habitats. In many of these cases, alpine or Mediterranean samples contained much higher melatonin levels than those from less-exposed sites [4].

In an attempt of approximating towards a classification of melatonin in its functional role, the attractive idea of considering melatonin as a new phytohormone has to be distinguished from (i) roles as a local regulator of defense responses or growth and (ii) that of a directly protective agent. This latter possibility would include the multiple antioxidant actions, which have meanwhile been demonstrated also in plants and which comprise upregulation of antioxidant enzymes, reduction of free-radical formation, direct scavenging of free radicals and singlet oxygen, as well as possible contributions of oxidatively formed melatonin metabolites, such as cyclic 3-hydroxymelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK) [5,10,23-25]. These properties may be especially relevant to photoprotection [4,5]. In this context, a study in *Eichhornia crassipes* (Pontederiaceae) should be mentioned, which showed (i) a correlation of melatonin levels with radiation intensity, (ii) an increase of melatonin over the photophase and (iii) a corresponding increase of the oxidation product, AFMK [26]. On the one hand, the distinction between possible roles as a phytohormone, local regulator and protective agent seems to be necessary for precisely judging the functional significance of the compound, but, on the other hand, these three facets do not at all exclude each other. In vertebrates, melatonin is known to not only act as a hormone, but also as a paracrine and autocrine regulator in many tissues, and as a both locally and systemically acting antioxidant [27].

In some plants containing particularly high levels of melatonin, the role as a product of secondary metabolism with antioxidant properties may prevail. This has already been assumed decades ago for oily seeds [28]. The uptake of melatonin into oil bodies has been recently demonstrated in sunflower seedlings and may likewise take place during seed development [29]. The antioxidant actions of melatonin appear to be of particular value, since neither antioxidant enzymes nor water-soluble low-molecular-weight antioxidants can provide efficient protection in dry seeds [4,28]. Under conditions of dormancy, a role as a phytohormone is excluded. However, melatonin may contribute to preservation, survival and maintenance of germination capacity of seeds. Whether it may additionally contribute to the maintenance of dormancy has not been directly studied. In both seeds and other plant tissues containing high amounts of melatonin, a further fundamental problem exists those conflicts with the role as a signaling molecule such as a phytohormone. If low levels of melatonin act in other organs or species, or under different conditions, by regulating gene expression and enzyme activities, this requires high-affinity binding sites. At concentrations elevated by, sometimes, orders of magnitude, such primary binding sites would be either completely saturated or, as observed with many receptors, desensitized or internalized. Therefore, signaling via high-affinity binding sites would no longer work at strongly elevated levels. If one would assume the existence of additional low-affinity binding sites, which would take over the function of signaling, this might appear as an escape from the problem, but there is no single evidence for this.

Generally, important information is still missing concerning the role of melatonin in tissues enriched with this compound. In particular, the distribution of melatonin between cytoplasm, vacuole and apoplast awaits thorough determination. If melatonin, which can cross membranes because of its amphiphilicity, should accumulate in vacuoles, cytoplasmic and/or nuclear concentrations suitable for signaling purposes might be considerably lower than determined by overall tissue measurements. In principle, the same problems might arise if melatonin is retained in subcellular structures such as oil bodies or organelles, as known from vertebrate mitochondria [23]. The same problem should also exist in plant tissues containing lower amounts of melatonin. Therefore, the determination of the soluble, non-retained fraction that is suitable for signaling purposes should be seen as a prerequisite for the possible classification of melatonin as a phytohormone.

Nevertheless, the numerous effects of melatonin in plants, as far as they are similar to or are influencing actions of known phytohormones, seem worth the effort of clarifying the question of whether this agent may itself be another phytohormone. The fact that it modulates genes known to be controlled by classic phytohormones does not speak against such a role, even not when melatonin is shown to directly modulate the expression or release of these phytohormones. In animals, hormonal cascades are not uncommon, and the modulation of other hormones by melatonin is established knowledge [30]. Therefore, the same should not be excluded in plants from the beginning.

However, several additional requirements remain to be fulfilled before melatonin could be classified as a phytohormone. Some of these would be even necessary for attributing a role as a local regulator. First, it is not sufficient to demonstrate changes in gene expression or specific protein levels. It should be a matter of priority to identify binding sites of melatonin, apart from calmodulin, and especially those with high ligand affinity and selectivity [10]. Next, it would be necessary to clarify the signal transduction pathways, something that should bring light into the puzzling details of interference with known phytohormones.

Importantly, it should not be seen as being sufficient to state changes in cytosolic calcium, which is influenced by numerous treatments and agents. Instead, it will be necessary to identify the proteins that sequentially transmit the information from the binding site to the intracellular messenger, whether this may be calcium or another molecule. It will be a matter of experimental strategy to identify components of signal transduction pathways, whether mutant screening followed by map-based cloning may be most promising, or whether investigators should start by first identifying high-affinity binding sites by direct binding studies with radiolabeled melatonin, a procedure that has been conducted with this hormone in animals and, correspondingly, with numerous other vertebrate hormones. Finally, movements of melatonin within the plant should be studied, in order to describe the changing distribution of the signaling molecule melatonin and, thereby, the spreading of communication within the organism. To date, it is not yet possible to classify melatonin as a new phytohormone. However, this decision can be made, after the open points mentioned here have been investigated.

REFERENCES

1. Poeggeler B, et al. Pineal hormone melatonin oscillates also in the dinoflagellate *Gonyaulax polyedra*. *Naturwissenschaften*. 1991;78:268-269.
2. Dubbels R, et al. Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. *J Pineal Res*. 1995;18:28-31.
3. Hardeland R. Melatonin and 5-methoxytryptamine in non-metazoans. *Reprod Nutr Dev*. 39:399-408.
4. Hardeland R, et al. Melatonin in plants – Focus on a vertebrate night hormone with cytoprotective properties. *Funct Plant Sci Biotechnol*. 2007;1:32-45.
5. Hardeland R. Melatonin in Plants - Diversity of Levels and Multiplicity of Functions. *Front Plant Sci*. 2016;7:198.
6. Oladi E, et al. Spectrofluorimetric determination of melatonin in kernels of four different *Pistacia* varieties after ultrasound-assisted solid-liquid extraction. *Spectrochim Acta A Mol Biomol Spectrosc*. 2014;132:326-329.
7. Hardeland R. Plants – Sources of melatonin. In: *Botanical Medicine in Clinical Practice* (Watson RR, Preedy VR, eds.), CABI, Wallingford. 2008;752-760.
8. Chen G, et al. Melatonin in Chinese medicinal herbs. *Life Sci*. 2003;73:19-26.
9. Balzer I and Hardeland R. Photoperiodism and effects of indoleamines in a unicellular alga, *Gonyaulax polyedra*. *Science*. 1991;253:795-797.
10. Hardeland R. Melatonin in plants and other phototrophs: advances and gaps concerning the diversity of functions. *J Exp Bot*. 2015;66:627-646.
11. Kolár J, et al. Melatonin: occurrence and daily rhythm in *Chenopodium rubrum*. *Phytochemistry*. 1997;44:1407-1413.
12. Kolár J and Machácková I. Melatonin in higher plants: occurrence and possible functions. *J Pineal Res*. 2005;39:333-341.
13. Arnao MB and Hernández-Ruiz J. The physiological function of melatonin in plants. *Plant Signal Behav*. 2006;1:89-95.
14. Paredes SD, et al. Phytomelatonin: a review. *J Exp Bot*. 2009;60:57-69.
15. Tan DX et al. Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. *J Exp Bot*. 2012;63:577-597.
16. Arnao MB and Hernández-Ruiz J. Melatonin: plant growth regulator and/or biostimulator during stress? *Trends Plant Sci*. 2014;19:789-797.
17. Arnao MB and Hernández-Ruiz J. Functions of melatonin in plants: a review. *J Pineal Res*. 2015;59:133-150.
18. Zhang N, et al. Roles of melatonin in abiotic stress resistance in plants. *J Exp Bot*. 2015;66:647-656.
19. Chen Q, et al. Exogenously applied melatonin stimulates root growth and raises endogenous indoleacetic acid in roots of etiolated seedlings of *Brassica juncea*. *J Plant Physiol*. 2009;166:324-328.
20. Wang L, et al. Changes in melatonin levels in transgenic 'Micro-Tom' tomato overexpressing ovine AANAT and ovine HIOMT genes. *J Pineal Res*. 2014;56:134-142.
21. Koyama FC, et al. The structurally related auxin and melatonin tryptophan-derivatives and their roles in *Arabidopsis thaliana* and in the human malaria parasite *Plasmodium falciparum*. *J Eukaryot Microbiol*. 2013;60:646-651.
22. Weeda S, et al. *Arabidopsis* transcriptome analysis reveals key roles of melatonin in plant defense systems. *PLoS One*. 2014;9:e93462.
23. Hardeland R. Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. *Endocrine*. 2005;27:119-130.
24. Tan DX, et al. Cyclic-3-hydroxymelatonin (C3HOM), a potent antioxidant, scavenges free radicals and suppresses oxidative reactions. *Curr Med Chem*. 2014;21:1557-1565.

25. Hardeland R, et al. Kynuramines, metabolites of melatonin and other indoles: the resurrection of an almost forgotten class of biogenic amines. *J Pineal Res.* 2009;47:109-126.
26. Tan DX, et al. Novel rhythms of N1-acetyl-N2-formyl-5-methoxykynuramine and its precursor melatonin in water hyacinth: importance for phytoremediation. *FASEB J.* 2007;21:724-1729.
27. Tan DX, et al. Melatonin: a hormone, a tissue factor, an autocoid, a paracoid, and an antioxidant vitamin. *J Pineal Res.* 2003;34:75-78.
28. Balzer I and Hardeland R. Melatonin in algae and higher plants - Possible new roles as a phytohormone and antioxidant. *Bot Acta.* 1996;09:180-183.
29. Mukherjee S, et al. Salt stress-induced growth inhibition coincides with differential distribution of serotonin and melatonin in subflower seedling roots and cotyledons. *Physiol Plant.* 2014;152:714-728.
30. Hardeland R, et al. Melatonin--a pleiotropic, orchestrating regulator molecule. *Prog Neurobiol.* 2011;93:350-384.