Mini-Reviews in Recent Melatonin Research

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Preface

During the last two years, I had been repeatedly invited to submit mini-reviews to mostly new online journals, sometimes to inaugural volumes. I had accepted several invitations, in order to contribute to a good start of these journals. However, as a result of their new appearance, the visibility of these papers to a normal reader remained limited, also because they did not appear in frequently consulted databases. In a few cases, even a doi number was missing. Moreover, I was not generally satisfied with the layout, including losses of formatting concerning italics, super- and subscripts.

More importantly, it was, in my impression, difficult for a reader interested in melatonin research to obtain a complete overview about the various mini-reviews distributed over a spectrum of different new journals that are not always perceived to exist.

Therefore, I decided to combine a number of selected papers in this collection. I also added some further newly written manuscripts to this volume, to incorporate bridging information between the other chapters.

This volume may help to disseminate the information on various recent developments in melatonin research, which exceed the traditional view of this important, pleiotropic and orchestrating regulator molecule. Notably, melatonin is present in bacteria and all major eukaryotic clades and exerts numerous, partially taxon-specific actions, a remarkably large field for new discoveries. I hope that this collection will be helpful to interested readers.

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Prologue

Recent Developments in Melatonin Research

Rüdiger Hardeland

Melatonin, originally only perceived as the hormone of the pineal gland, is now known to be a ubiquitous compound present in all eukaryotic clades studied and in bacteria as well [1]. Moreover, its formation in mammals has been demonstrated in numerous extrapineal sites, and the quantities of extrapineal melatonin exceed those in the pineal gland and in the circulation by orders of magnitude [2,3]. The knowledge of the numerous additional sources of melatonin within a mammalian body and in organisms beyond the animals has, of course, led to studies on functions that had never been considered before. This augmentation of information is, however, not restricted to the recently investigated melatonin-producing organisms and organs, but has also been expanded because of advancements in cell biology and new insights in regulation mechanisms.

One of the fields in which remarkable advances have been made is that of melatonin in plants. This concerns the identification of deviating routes of synthesis, new functions concerning morphogenetic modulation, protection of photosystems, gene expression, interactions with phytohormones, protection against abiotic and biotic stress [4-10], and remarkable quantitative differences in metabolism [11]. Melatonin synthesis was also demonstrated in chloroplasts [10] and mitochondria of plants [12] and animals [13], findings that are interpretable in terms of the bacterial origin of melatonin [1].

Another important and recently expanding field concerns the regulation by non-coding RNAs, such as microRNAs and lncRNAs as well as other epigenetically relevant factors, which are either modulating or mediating melatonin effects and also influence melatonin synthesis in mammals [14]. Other new aspects of regulation have emerged by the finding that the melatonin receptor MT_1 is localized in mammalian mitochondria [15-17]. In conjunction with the mitochondrial formation of melatonin, this automitocrine [17] action changes our view on the role of this substance considerably.

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Melatonin – Another Phytohormone?

Rüdiger Hardeland

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, favors 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.

Keywords: Abiotic stress, Auxin-like effects, Biotic stress, Melatonin, Photoprotection, Seeds.

Since melatonin (*N*-acetyl-5-methoxytryptamine) had been discovered in phototrophic organisms, first in a dinoflagellate [1] and later in plants [2], this compound has been detected in numerous algae [3] and plant species [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 [7,8], and the possibility of functions similar to those known from vertebrate animals, especially the transmission of the signal 'darkness' in photoperiodic responses. Although there was evidence for such a role in a dinoflagellate [9], this function was not clearly demonstrated in plants [5,10]. A circadian rhythm with a prominent nocturnal peak, as in vertebrates, was found in a short-day ecotype of *Chenopodium rubrum* [11], but flower induction by melatonin could not be shown [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].

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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 transgenes 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 phyto-

hormone 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 [5,10] and which comprise upregulation of antioxidant enzymes, reduction of free-radical formation, direct scavenging of free radicals and singlet oxygen [23], as well as possible contributions of oxidatively formed melatonin metabolites, such as cyclic 3-hydroxymelatonin [24], N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) and N^1-acetyl-5-methoxykynuramine (AMK) [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 [29] and may likewise take place during seed development. 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 that 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 is assumed to 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 [10], and especially those with high ligand affinity and selectivity. 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,