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Melatonin in Plants – Focus on a Vertebrate Night Hormone with Cytoprotective Properties

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ABSTRACT

Melatonin, first described as a vertebrate hormone produced by the pineal gland, is, in fact, a ubiquitous substance found in the majority of taxa studied, from bacteria and eukaryotic unicells to fungi, pheophyceans, rhodophyceans, chlorophyceans and angiosperms. Since one of the first physiological functions discovered was that of mediation of the signal "darkness", several studies in photoautotrophs were also focussed on this aspect, with particular attention to photoperiodism. In some species, e.g., the dinoflagellate *Lingulodinium* and the dicot *Chenopodium*, melatonin exhibits a robust circadian rhythm peaking at night. The precise role of this rhythmicity is not fully understood and may serve functions other than photoperiodic control. Circadian variations are not always detected or can be differently phased. They should be absent in dry oily seeds, which are particularly rich in melatonin. High quantities are also reported for several medicinal plants and plants exposed to intense natural UV radiation. Not all reports on melatonin levels in photoautotrophs are equally reliable, for methodological reasons. Nevertheless, high amounts found in a number of well-designed studies, also in relation to UV, indicate that cytoprotective properties known from animals may play a role in plants, too. In dry seeds, free radicals cannot be detoxified enzymatically. Therefore, melatonin's scavenging properties could be of biological value and contribute to antioxidative protection. Elevated levels in some juicy fruits may indicate a role during fruit ripening and a function in maintenance of developmental stages that may extend to the persistence of dormancy.

Keywords: 5-methoxyindoles, circadian rhythms, free radicals, medicinal plants, photoprotection, seeds, UV radiation

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INTRODUCTION

Melatonin is an indoleamine (**Fig. 1**) formed by *N*-acetylation and *O*-methylation of serotonin, i.e., a precursor that is widely abundant in numerous taxa including plants (Smith 1971; West 1984; Badria 2002; Murch and Saxena 2002; Cao *et al*. 2006). After the discovery of melatonin as a hormone of the pineal gland of vertebrates, it took quite some time until researchers perceived that this molecule is present in other organisms, too, although it should have been obvious from the beginning that *N*-acetylation and *O*-methylation of suitable aromates by specific or unspecific enzymes are processes relatively likely to occur, so that the presence of serotonin could easily lead to melatonin. After the discovery of melatonin in various invertebrate animals (summarized in: Hardeland and Fuhrberg 1996), its presence in a photoautotroph was for the first time demonstrated in a marine dinoflagellate, *Lingulodinium polyedrum* (syn. *Gonyaulax polyedra*) (Poeggeler *et al*. 1989, 1991). Since then, numerous groups have searched for the presence of melatonin outside the animals and, in fact, the compound was detected in almost any organism thoroughly investigated, from bacteria and various eukaryotic unicells to fungi, pheophyceans, rhodophyceans, chlorophyceans, and angiosperms (reviews covering different aspects: Hardeland and Fuhrberg 1996; Hardeland 1999; Hardeland and Poeggeler 2003; Hardeland and Pandi-Perumal 2005). Thus, melatonin nowadays appears as a practically ubiquitous substance.

However, the mere presence of a compound is *per se* not very meaningful. Functional roles had to be sought. Several of the earlier concepts were oriented at the functions known at that time for melatonin in vertebrates. Since this

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Invited Review

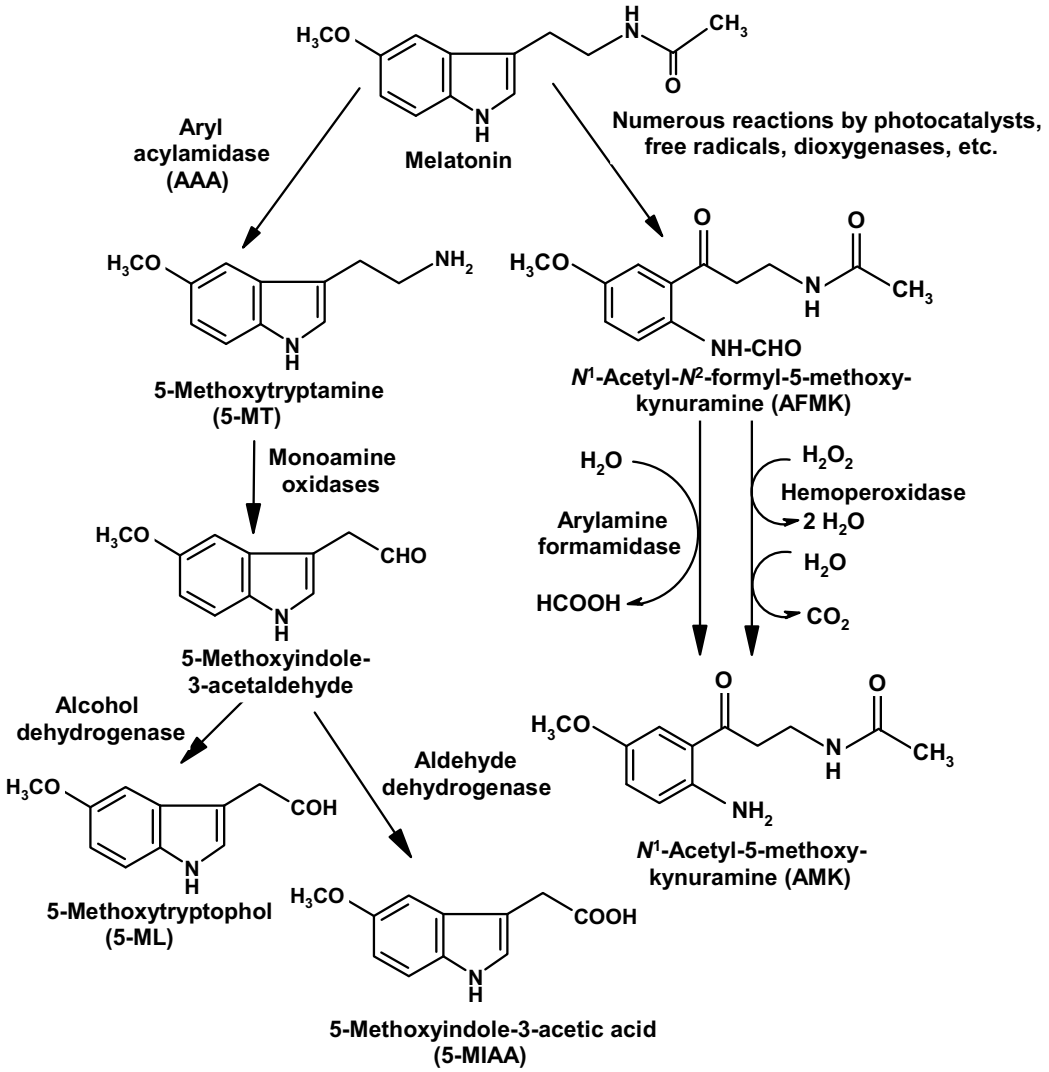

Fig. 1 Two pathways of melatonin catabolism with relevance to photoautotrophs.

indoleamine was shown to act as a transducer of the information darkness, when released preferentially at night from the pineal gland (Reiter 1991, 1993), investigators intended to find such a function also in other organisms including photoautotrophs (Balzer and Hardeland 1991a; Kolář *et al*. 1997; Tilden 1997; Kolář *et al*. 1999a). As will be discussed, such actions still appear to be possible, but can neither cover nor explain the entire spectrum of findings in plants and other non-animal species studied. Even in animals, melatonin is not generally associated with darkness (Hardeland and Fuhrberg 1996), and so it is neither in other organisms. A relatively clear example for this is yeast (*Saccharomyces cerevisiae*), which is capable of producing high amounts of melatonin, however, independently of darkness. Instead, it is formed in proportion to the availability of precursors (Sprenger *et al*. 1999; Hardeland and Poeggeler 2003). In this case, the reason for why yeast does not exhibit a circadian, nocturnally peaking rhythm of melatonin may have been identified: In *Saccharomyces*, the rate-limiting enzyme of melatonin biosynthesis, arylalkylamine *N*-acetyl-transferase (AA-NAT), lacks N- and C-terminal control regions which are reportedly essential for the circadian control of this enzyme (Ganguly *et al*. 2001).

The expansion of knowledge on the presence of melatonin in numerous different taxa coincided historically with another considerable expansion of findings in mammals. Melatonin was shown to be formed in numerous extrapineal tissues and cells and to exhibit various actions that were not foreseen nor covered by the chronobiological aspects of a pineal hormone. Among these different effects was one that was neither species- nor cell-specific, but rather the consequence of a chemical property: melatonin turned out to be a very potent electron donor and radical scavenger (Hardeland *et al*. 1993; Tan *et al*. 1993; Hardeland *et al*. 1995; Poeggeler *et al*. 1996; Tan *et al*. 2002; Hardeland 2005). Melatonin was, therefore, regarded as an antioxidant (Reiter *et al*. 1995; Reiter 1998; Reiter *et al*. 2003) and, later, also as a compound undergoing electron exchange reactions with electron transport chains, e.g., in mitochondria (Hardeland *et al*. 2003; Hardeland and Pandi-Perumal 2005; Hardeland 2005). Reactions of that type could, of course, also take place in plant tissues, and the question arose as to whether this might be of relevance or not.

With the exception of some dinoflagellates, the functions of melatonin in photoautotrophs are uncertain, although numerous suggestions have been made. However, regardless of its respective physiological functions, the occurrence of high melatonin concentrations in several medicinal herbs, in plants exposed in their habitat to intense UV radiation, and in various seeds, especially oily nuts (Hardeland and Pandi-Perumal 2005) makes these sources interesting in terms of their medicinal and nutritional value. Biotechnological applications seem possible, including the enhancement of melatonin production in suitable species by interventions such as adaptation to rising intensities of UV light and/or enhanced precursor availability by metabolic engineering.

UBIQUITY OF MELATONIN IN PHOTOAUTOTROPHS

Many data on melatonin are available for unicellular and multicellular algae and for angiosperms, whereas pertinent investigations in photosynthetic bacteria and in other plant

taxa have been rarely conducted so that they do not allow general conclusions. Therefore, our considerations will be restricted to the first groups mentioned. Although dinoflagellates are, in phylogenetical terms, not closely related to plants in a strict sense, they will be discussed in some detail, because of (a) their relatively high contents of melatonin and (b) functional aspects which have not been studied in the other groups. Moreover, this was the taxon in which melatonin was not only detected for the first time outside the animals (Poeggeler *et al*. 1989), but also in which methodological procedures were developed for efficient extraction and reliable determination of this compound in photoautotrophs (Poeggeler *et al*. 1991; Poeggeler and Hardeland 1994). The presence of melatonin in an angiosperm was first reported by Van Tassel *et al*. (1993).

Lists of photosynthetic organisms that contain melatonin have been published and reviewed several times (Hattori *et al*. 1995; Balzer and Hardeland 1996; Hardeland and Fuhrberg 1996; Manchester *et al*. 2000; Reiter *et al*. 2001; Reiter and Tan 2002; Conti *et al*. 2002; Chen *et al*. 2003; Hardeland and Pandi-Perumal 2005), so that this shall not be repeated here *in toto*. Moreover, not any of the published data on melatonin levels reported seem equally reliable. Therefore, the focus will be laid on findings which appear suitable for generalizations or, at least, indicate possible functions.

To date, melatonin has been detected in 5 photoautotroph dinoflagellates, in euglenoids, in several species of pheophysicans, rhodophysicans and chlorophyceans (summarized in Hardeland 1999) and in more than 30 dicot and monocot families, a number which is continually rising. Despite this widespread occurrence, the levels reported for melatonin are extremely divergent, in remarkable contrast to that what is known from animals. In some species, the concentrations determined are relatively low, i.e., a few pg per g fresh weight, or even at the borderline of detection. This holds particularly true for those macroalgae which have been thoroughly investigated under methodological aspects (Pape 2004; Pape and Lüning 2006), data which partially contrast with earlier data. Small levels in a similar range were also reported, e.g., for strawberry and kiwi fruits, asparagus shoots (Hattori *et al*. 1995), and beetroot, whereas the indoleamine remained undetectable in potato tubers (Dubbels *et al*. 1995). However, these few examples are already affected by considerable problems of variability. In strawberries from Egypt, melatonin attained levels in the range of 200 pg/g (Badria 2002), i.e., approximately 20-fold more than that reported by Hattori *et al*. (1995). This does not necessarily imply false determinations in one case, since melatonin concentrations seem to be enhanced in several angiosperms by UV radiation (Conti *et al*. 2002). Moreover, cultivars can largely differ in their melatonin content, which has been observed in tomatoes (166 vs. 506 pg/g: Dubbels *et al*. 1995; two other cultivars were reported to contain only about 3 or 15 pg/g: Van Tassel *et al*. 2001) or in tart cherries (2 vs. 15 ng/g: Burkhardt *et al*. 2001c). In these cherries, levels were only moderately affected by the time of harvest and location of the orchard, but strongly different between individual trees (Burkhardt *et al*. 2001c).

Contrary to the low concentrations of melatonin detected in some of the above-mentioned species, extremely high amounts were reported in other cases. While several ten or hundred ng/g were obtained in numerous species (Manchester *et al*. 2000; Reiter and Tan 2002; Conti *et al*. 2002), the "record holders" were in the range of several µg/g and sometimes exceeded even 20 µg/g (Conti *et al*. 2002; Chen *et al*. 2003; **Table 1**). Such extremely high concentrations were obtained in green parts of the respective plants (total young plants, fresh or dried leaves), but, exceptionally, also in a root (peregrine bibernelle, *Pimpinella peregrina*: Conti *et al*. 2002). It is noteworthy that many of these plants are known as officinal herbs, although a possible relationship between high melatonin and medicinal value remains entirely open. Seeds were frequently found to be another source of high melatonin, but the concentrations were not that extreme and mostly remained between 1 and 100 ng/g (**Table 2**). The more or less consistently elevated amounts in seeds, especially those which are rich in oily contents, may be indicative of a specific function in these dormant stages, as will be discussed next. Elevated levels, which were above the average of other plant material, but not as high as in medicinal herbs or in seeds, were also found in some juicy fruits (Hattori *et al*. 1995; Burkhardt *et al*. 2001c) and changes occurred during fruit ripening (Van Tassel *et al*. 2001), findings which demand further extension.

Differences in melatonin content between plant organs can be considerable. In a convolvulacean, the Japanese morning glory *Pharbitis nil*, cotyledons and leaves of seedlings did not contain detectable amounts of the indoleamine, whereas it was clearly present in stems and roots (Van Tassel *et al*. 2001). In seeds of the same species, much higher amounts were present. These findings do not imply that leaves are generally devoid of melatonin, since very high amounts were found in other cases (**Table 1**).

The extreme divergence of melatonin levels between species and between plant organs may, on the one hand, appear puzzling, but, on the other hand, it is clearly indicative for the fact that melatonin cannot have a single function in plants. This does not exclude that one of the possible functions is present in all of them, e.g., a cytoprotective role, but mediation of the signal darkness is highly unlikely to occur in the whole ensemble of species containing amounts of melatonin differing by several orders of magnitude. It should also be clear that dark signals cannot be transmitted, because of absent melatonin biosynthesis and rhythmicity, in dormant seeds.

In part, a certain lack of plant physiological information is the consequence of a search aimed at detecting melatonin in edible or medicinal plants or their parts, with the intention of identifying materials of nutritional or nutraceutical value. In these cases, plant organs were not systematically analyzed, but only their edible parts. These different objectives are also responsible for another information gap, namely concerning the enzymes of melatonin biosynthesis and catabolism. These pathways are known in dinoflagellates, as will be discussed later, but, in the case of angiosperms, data are usually restricted to serotonin biosynthesis and do not comprise the subsequent enzymes. This would, however, be required for understanding the dynamics of melatonin concentration, the sources and the sinks of the compound.

With regard to sources, the decisive differences between the high-melatonin species among angiosperms and dinoflagellates on the one hand and animals on the other hand are obvious. Endocrinologists have frequently asked how such enormous amounts of hundreds of ng or even several µg per g tissue can be attained. This appeared utmost surprising when comparisons were made with melatonin in the vertebrate blood plasma. However, such a comparison does not consider (a) that melatonin-producing vertebrate cells, in particular, pinealocytes can also contain melatonin in the upper ng/g range, and (b) that certain limits of biosynthetic rates which exist in the vertebrate are not valid for plants or dinoflagellates. In animals, the availability of tryptophan would become rate-limiting even when biosynthetic enzyme activities are sufficiently high, whereas such a limit should not exist in organisms that possess the shikimic acid pathway. Another limit seems to exist in the vertebrate blood because of hemoglobin: the combination of oxygenated hemoglobin, NADH and melatonin causes formation of free radicals (Tan *et al*. 2005), so that the levels of melatonin have to be restricted in the circulation for avoiding such unfavorable effects, again a limit that is inexistent in the photoautotrophs.

SAFE EXTRACTION, SAFE DETERMINATION AND THE PROBLEM OF RELIABILITY

As already mentioned, not all published values of melatonin levels are equally reliable. Although deviations between dif-

Table 1 A selection of plants reported to contain particularly high levels of melatonin.

Species	Family	Material	Melatonin [ng/g]	References
Coptis chinensis (huanglian)	Ranunculaceae	Powder	1,000	Ch
Epimedium brevicornum (yinyanghuo)	Berberidaceae	Powder	1,105	Ch
Rheum palmatum (dahuang)	Polygonaceae	Powder	1,078	Ch
Adinandra nitida (Shiya tea)	Theaceae	Dry leaves	2,120	Ch
Hypericum perforatum (St. John's wort)	Clusiaceae	Leaf	1,750	Mu
		Flower	>2,400->4,000	Mu, R
Morus alba (mulberry)	Moraceae	Leaf powder	1,510	Ch
		Cortex	1,110	Ch
Ziziphus jujuba (suanzhaoren)	Rhamnaceae	Powder	256	Ch
Viola philippica (diding)	Violaceae	Powder	2,368	Ch
Pyrola decorata (luxiancao)	Pyrolaceae	Powder	750	Ch
Cornus officinalis (shanyurou)	Cornaceae	Powder	821	Ch
Eucommia ulmoides (duzhong)	Eucommiaceae	Powder	500	Ch
Pimpinella peregrina (peregrine bibernelle)	Apiaceae	Dried root	38,000	Co
Angelica sinensis (danghui)	Apiaceae	Powder	700	Ch
Uncaria rhynchophylla (gouteng)	Rubiaceae	Powder	2,460	Ch
Rubus chingii (fupenzi)	Rosaceae	Powder	387	Ch
Syzygium aromaticum (dingxiang)	Myrtaceae	Powder	450	Ch
Taxillus chinensis (parasitic loranthus, sangjisheng)	Loranthaceae	Powder	650	Ch
Polygala tenuifolia (yuanzhi)	Polygalaceae	Powder	850	Ch
Phellodendron amurense (huangbo)	Rutaceae	Powder	1,235	Ch
Gentiana scabra (longdacao)	Gentianaceae	Powder	780	Ch
Lycium barbarum (gouqi, wolf berry)	Solanaceae	Powder	530	Ch
Aloysia triphylla (lemon verbena)	Verbenaceae	Young plant	22,000	Co
Melissa officinalis (balm mint)	Lamiaceae	Young plant	16,000	Co
Mentha piperita (peppermint)	Lamiaceae	Young plant	19,500	Co
Salvia officinalis (sage)	Lamiaceae	Young plant	29,000	Co
Thymus vulgaris (thyme)	Lamiaceae	Young plant	38,000	Co
		Dried plant	13,000	Co
Scutellaria laterifolia (scullcap)	Lamiaceae	Plant	1,600	Co, Ma
Scutellaria baicalensis (huang-qin)	Lamiaceae	Plant	>2,000->7,000	Co, Mu, R
Agastache rugosa (huoxiang)	*Lamiaceae*	Powder	300	Ch
Scrophularia ningpoensis (xuanshen)	Scrophulariaceae	Powder	342	Ch
Andrographis paniculata (chuanxinlian)	Acanthaceae	Powder	500	Ch
Achillea millefolium (yarrow)	Asteraceae	Young plant	43,000	Co
Tanacetum parthenium (feverfew)	Asteraceae	Fresh leaf	>1,300->1,700	Mu, Co
		Dried leaf	90->7,000	Mu, Co
Lobelia chinensis (banzhilian)	Lobeliaceae	Powder	257	Ch
Ophiopogon japonicus (maidong)	Convallariaceae	Powder	200	Ch
Aloe vera (officinal aloe)	Asphodelaceae	Powder	516	Ch

Ch: Chen *et al*. 2003 (dried material analyzed as used in Chinese medicine); Co: Conti *et al*. 2002 (materials from alpine or Mediterranean habitats); Ma: Manchester *et al*. 2000; Mu: Murch *et al*. 1997; R: Reiter and Tan 2002

Table 2 Elevated levels of melatonin in various seeds.

Species	Family	Melatonin [ng/g]	References
Brassica nigra (black mustard)	Brassicaceae	129	Ma, R1
Brassica hirta (white mustard)	Brassicaceae	189	Ma, R1
Pimpinella anisum (anise)	Apiaceae	7	Ma, R1
Foeniculum vulgare (fennel)	Apiaceae	28	Ma, R1
Juglans regia (walnut)	Juglandaceae	3.5	R2
Prunus amygdalus (almond)	Rosaceae	39	Ma
Trigonella foenum-graecum (fenugreek)	Fabaceae	43	Ma
Lycium barbarum (gouqi, wolf berry)	Solanaceae	103	Ma
Helianthus annuus (sunflower)	Asteraceae	29	Ma, R1
Elettaria cardamomum (green cardamom)	Zingiberaceae	15	Ma, R1
Oryza sativa (rice)	Poaceae	1	H
Zea mays (Indian corn)	Poaceae	1.3	H
Avena sativa (oat)	Poaceae	1.8	H
Festuca arundinacea (tall fescue)	Poaceae	5	H

H: Hattori *et al*. 1995; Ma: Manchester *et al*. 2000; R1: Reiter and Tan 2002; R2: Reiter *et al*. 2005

ferent studies may be also related to external influences, such as intensities of UV or visible light, temperature, composition of the substrate, as well as to cultivar, age and season, the suspicion frequently remains that methodological reasons could be responsible for the sometimes considerable differences, especially when melatonin has been determined by a single method only, or if the percentage of recovery after extraction has been disregarded. It may be up to the reader to judge when scrutinizing the original publications under these aspects.

Extraction, determination of extraction efficiency, and reproducible measurements of melatonin with careful avoidance of false-positive or false-negative results are sometimes not easy in materials from photoautotrophs. Without any doubt, it is insufficient to simply apply a procedure established for mammalian or avian melatonin without modification. The first problem is the destruction of melatonin during extraction, and this is the premier source of false-negative results. Levels of endogenous oxidants are much higher in photoautotrophs than in vertebrates and the additional presence of Fenton-reactive metals may cause a rapid and substantial decay of the indoleamine present in the

material. In dinoflagellates, for which we first developed the respective procedures, inter-assay variability was strongly reduced when we froze buffered suspensions of collected cells in liquid nitrogen and produced a powder (Poeggeler *et al*. 1991), which was extracted either in an acidic environment or in suitable organic solvents which did not easily form, e.g., halogenated organic radicals (such as chloroform) or contain peroxides. For details of possible variants see Poeggeler and Hardeland (1994). Other modifications are summarized by Kolář and Macháčková (2005). All procedures have to be carried out in darkness or under dim red light, because melatonin can be easily oxidized in the presence of various photocatalytically active compounds present in plant material, including chlorophyll (Hardeland *et al*. 1995, 2004; Hardeland 1996). The problem of oxidative destruction is, of course, more severe in the photosynthetic tissues of a plant than in subterranean parts or in mature, dormant seeds, which contain less of oxidants and of photo-excitable compounds. In suspensions of unicells, extraction efficiency can be more easily determined than in massive parts of a plant. Cutting of plants may already cause changes, and the pieces obtained cannot be mixed homogeneously with external melatonin added as a standard. Another point frequently neglected is the fact that extraction efficiency varies with the amount of endogenous melatonin. One has to be aware that any biological material has a certain, specific destruction capacity for the oxidizable compound. If the intrinsic concentration of melatonin is low, the sum of endogenous and exogenous (standard) melatonin can be substantially diminished, so that the recovery may be as low as 30%. If intrinsic melatonin is high in the same material, for reasons of a physiological response, the sum is higher and the fraction destroyed relatively smaller, while the absolute amount of oxidized melatonin may be virtually the same. In such a case, about 80% may be recovered. In reliable investigations, data on extraction efficiencies are presented.

The methods of determination are equally important. False-positive results or overestimations are almost certain with materials from photosynthetic parts, if procedures are not controlled for such pitfalls. Any chromatographic procedure is potentially affected by coelution. A peak at the appropriate retention time may contain other compounds, too. Therefore, conditions of separation (eluent, flow rates, etc.) should be varied and several detection procedures may be applied, in HPLC, e.g., electrochemical and fluorescence detection. All this has been done in the original studies on the first photoautotrophic organism investigated, the dinoflagellate *Lingulodinium polyedrum* (syn. *Gonyaulax polydra*) (Poeggeler *et al*. 1991; Poeggeler and Hardeland 1994). Favorable techniques are those of liquid chromatography with tandem mass spectrometry (LC-MS) or, alternately, GC-MS. Mass spectrometry allows to clearly distinguish melatonin from coeluting compounds, and it is highly unlikely that another, coeluting substance has, by chance, the same mass as melatonin. Moreover, deuterated melatonin can be used as a reference standard. Eventually, underestimations can be caused by so-called matrix effects leading to insufficient ionization (Kolář and Macháčková 2005), a problem which can, however, be overcome (Cao *et al*. 2006).

Frequently, immunological procedures have been applied, too, such as RIA or ELISA. The advantage is usually a higher sensitivity, but the severe problem is that of false-positive results, due to compounds interacting with the antibody used. This can be cross-reactivity in the strict sense, i.e., interference with the antigen-binding site, but also any other interaction with the antibody at whatever site, if this leads to a decrease of antigen binding. However, false-positive determinations can be frequently uncovered, if serial dilution and parallel inhibition experiments are carried out, which should be done anyway with unknown material. Usually, interfering compounds have a different affinity to the antibody than melatonin, which is seen by the lack of parallelism. Highly instructive examples for considerably differing determinations of melatonin by GC-MS vs. RIA have been presented by Van Tassel *et al*. (2001), data which strongly speak for substantial immunological overestimations. In the ideal case, chromatographic and immunological procedures should result in (approximately) identical values. Again, this was achieved in *Lingulodinium* (Poeggeler *et al*. 1991; Poeggeler and Hardeland 1994), but many later publications by others have disregarded this necessity.

If melatonin concentrations are very low, normal chromatographic procedures may be too insensitive for carrying out such a comparison. In this case, derivatization of melatonin may provide the solution for safe determinations by HPLC (Pape 2004; Pape and Lüning 2006), as carried out in several macroalgae (pheophyceans, rhodophyceans, and chorophyceans), and in *Zingiber*, but the procedure was also applicable to a source containing much more melatonin, the tomato fruit (in this case, both HPLC and ELISA gave similar results, with concentrations even above the earlier data by Dubbels *et al*. 1995).

In summary, overestimations of melatonin can be and should be avoided by using the appropriate techniques. Underestimations are much more difficult to circumvent, especially when the extraction efficiency cannot be easily determined, in cases in which plant material cannot be thoroughly mixed with the standard compound.

Another difficulty for judging the significance of melatonin, even if determined appropriately, concerns the localization of the compound within cells or the plant tissue. In some unicells which are lacking cell walls and contain small vacuoles only, the presence of substantial amounts of cytoplasmic melatonin can be inferred. However, for an angiosperm or a kelp, several uncertainties remain, which are related to the question of how much melatonin is located in the large vacuoles and how much is in the apoplast (Hardeland 1997). As the amphiphilic molecule melatonin can easily cross membranes, it may enter the apoplast and move there. Owing to its slightly alkaline properties as a biogenic amine, melatonin may also accumulate in the acidic vacuolar compartment. This question, which may be important also with regard to eventual signaling mechanisms, has been addressed (Hardeland 1997, 1999; Hardeland and Poeggeler 2002), but not yet solved. Moreover, with regard to assumed photoprotective roles of melatonin (Balzer and Hardeland 1996; Behrmann *et al*. 1997), its percentage entering the chloroplasts would be of high interest. These problems will be difficult to solve, for the simple reason that the amphiphilic molecule melatonin penetrates membranes and is readily translocated or lost during isolation procedures of subcellular and extracellular compartments.

SECONDARY ACCUMULATION OF MELATONIN IN AQUATIC ORGANISMS

In land plants, an appropriately measured level of melatonin can presumably be attributed to formation by the plant investigated. This is not necessarily so in aquatic species. Uptake of the indoleamine released from other, high-melatonin organisms to the water represents a possibility which should be considered in the future. Uptake and strong accumulation of exogenous melatonin from water were demonstrated in organisms as different as the dinoflagellate *Lingulodinium polyedrum* (Mueller and Hardeland 1999; Mueller *et al*. 2000) and a floating angiosperm, the pontederiacean water hyacinth *Eichhornia crassipes* (Tan *et al*. 2007). Differences in melatonin levels between earlier and later determinations in macroalgae may have been caused by uptake of melatonin of, e.g., bacterial or microalgal origin from the seawater.

Whether or not absorption and accumulation of melatonin are only laboratory artifacts or biologically meaningful, remains to be investigated. Marine unicells, in particular, dinoflagellates can produce enormous amounts of melatonin, especially, under adverse conditions, which lead to intracellular concentrations sometimes approaching the millimolar range (Fuhrberg and Hardeland 1997; Fuhrberg *et al*. 1997). During mass abundance of dinoflagellates in the so-called

red tides, high numbers of cells are dying or undergo encystment, a process associated with dramatic rises in melatonin formation (Fuhrberg and Hardeland 1997; Fuhrberg *et al*. 1997). Therefore, substantial amounts of the indoleamine should be released to the seawater. One might speculate whether the compound will be taken up by other marine organisms, such as kelps.

Dinoflagellates can accumulate exogenous melatonin by orders of magnitude (Mueller and Hardeland 1999). In *Lingulodinium*, administration of 1 μM exogenous melatonin added to the medium led frequently to intracellular levels between 10 and 50 μM, values which depended on the circadian time of administration. Since these high concentrations were only transiently observed, with peaks around 3 hours after addition, while the extracellular concentration did not substantially change because of the much higher, about 2,000-fold extracellular volume, it was concluded that melatonin binding sites are responsible, which are progressively downregulated upon saturation. These binding sites should be different from classical melatonin membrane receptors, which were not detected in *Lingulodinium* (Masson-Pévet *et al*. 1997).

HIGH LEVELS OF MELATONIN IN UV-EXPOSED AND MEDICINAL PLANTS

While high concentrations of melatonin were first detected in dinoflagellates, especially under adverse temperature conditions (Fuhrberg and Hardeland 1997; Fuhrberg *et al*. 1997), extremely elevated levels were also reported for several angiosperms (cf. **Table 1**). When trying to identify common properties of these organisms, two facts became apparent: Many of them are exposed to high UV radiation in their natural habitat, either in an alpine or in a Mediterranean environment (Conti *et al*. 2002; Caniato *et al*. 2003). Comparisons with data of the same species from other habitats revealed a correlation with radiation, a finding which, however, does not immediately imply a causal relationship, since other factors such as temperature, water stress, etc. may be also of significant influence. Several high-melatonin species are well-known as medicinal herbs (Murch *et al*. 1997; Conti *et al*. 2002; Murch and Saxena 2002; Chen *et al*. 2003; Afreen *et al*. 2006), and in a number of cases they are identical with those of the aforementioned category. The medicinal value of melatonin as a plant constituent is still uncertain, although its properties as an antioxidant (Reiter *et al*. 1995, 2003; Reiter 1998; Reiter and Tan 2002; Tan *et al*. 2003a; Hardeland 2005), immune regulator (Guerrero and Reiter 2002; Carillo-Vico *et al*. 2005), sleep inducer (Monti *et al*. 1999; Pandi-Perumal *et al*. 2005; Niles 2006), antiexcitotoxin (Hardeland *et al*. 2003; Srinivasan *et al*. 2005, 2006) and multiply acting cell protectant (Hardeland *et al*. 2003; Hardeland 2005; Pandi-Perumal *et al*. 2006) are highly suggestive for assuming such actions. Any such consideration should, however, take into account that these medicinal plants contain numerous other compounds, frequently of demonstrated medicinal or nutritional utility (Hardeland and Poeggeler 2003; Murch *et al*. 2004; Afreen *et al*. 2006; Iriti and Faoro 2006). Nevertheless, the possibility remains that melatonin interacts with other therapeutical compounds, either in plant physiological or medicinal terms, as has been discussed for the Chinese officinal herb huang-qin (*Scutellaria baicalensis*, Lamiaceae), which contains numerous substances – flavonoids and others – with antioxidant, antiinflammatory or sedating properties (Hardeland and Poeggeler 2003). Melatonin, which displays these same activities, could act synergistically or may protect, in its capacity as a highly efficient radical scavenger, the other compounds from oxidation. In this context, one should take notice of the known interactions of melatonin with other antioxidants such as ascorbate or tocopherols, which are clearly more than additive but rather potentiating *in vitro* (Poeggeler *et al*. 1995; Gitto *et al*. 2001; Tan *et al*. 2003a) and presumably also *in vivo* (Balkan *et al*. 2004).

Apart from the applied aspect, the high levels of melatonin measured in UV-exposed angiosperms are also of considerable plant physiological interest. In a recent publication (Afreen *et al*. 2006), the papilionacean *Glycyrrhiza uralensis* was shown to directly respond to UV-B radiation by accumulating melatonin in its roots. Again, melatonin was also detected in the leaves, though at lower concentrations, but not in the stem. Since the rise in melatonin took place in the roots, which are not directly exposed to UV, either translocation of melatonin or a humoral message towards the roots should be implied. Interestingly, also red light (by a lamp emitting preferentially between 600 and 700 nm) caused rises of melatonin in the roots. Unfortunately, the eventual involvement of the phytochrome system and a possible reversion by far-red were not tested in that work, which might have also given hints for a role in seasonality.

Another case in which UV might have been implicated in a rise of melatonin has been described more recently (Tan *et al*. 2007): In leaves of *Eichhornia crassipes*, melatonin levels were considerably higher (up to 300 ng/g fresh weight) in plants directly collected from a pond (irradiation during the day 10,000-15,000 $\mu W/cm^2$) than those (ca. 3 ng/g) from laboratory conditions (400-450 $\mu W/cm^2$). However, plants from natural conditions were also subjected to higher and cyclically varying temperature (29.5-31.5°C during the day and 24.5-26.5°C at night), whereas laboratory plants were kept at 22°C, so that an exclusive effect of sunlight is not certain. In the plants exposed to natural light and temperature conditions, melatonin underwent a diurnal cycle with highest values at the end of the photophase. This rhythm was paralleled by another one detected in the concentration of an oxidation product from melatonin, N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK).

Such findings might be interpreted in terms of photoprotection by melatonin, as suggested earlier (Balzer and Hardeland 1996; Behrmann *et al*. 1997). Also in other photoautotrophs (dinoflagellates, kelps), short-wave visible light and UV lead to AFMK formation (Behrmann *et al*. 1997; Hardeland *et al*. 2004). Generally, melatonin is oxidized by various photooxidants, including several organic cation radicals, by singlet oxygen and UV-induced hydroxyl radicals (Hardeland *et al*. 1995; Hardeland and Fuhrberg 1996). Scavenging of such reactive species can also be seen as an aspect of protection from these oxidants. The phasing of the melatonin and AFMK rhythms, as observed by Tan *et al*. (2007), may be indicative of such a function. With increasing exposure to light during the photophase, plastidial photoprotection becomes deteriorated, including the impairment of the violaxanthin cycle, and also light-harvesting complexes and photosystems are progressively damaged, so that more and more superoxide is formed, which is largely converted to H_2O_2. In aquatic photoautotrophs, H_2O_2 is light-dependently released in measurable quantities to the water (Hardeland and Coto-Montes 2000). In *Lingulodinium*, a rhythm of H_2O_2 was described, which exhibited a pronounced peak at the end of the photophase and was elevated to higher average levels with increasing light intensity (Pape and Hardeland 1999a). When the cells were transferred to constant light, a progressively rising oxidative pressure, presumably due to insufficient recovery from photooxidative destruction of plastidial components, became apparent by further increases in H_2O_2 released to the medium (Pape and Hardeland 1999b). From our point of view, photoprotection by melatonin, in plants and other photoautotrophs, supporting these organisms to cope with enhanced radical formation under intense or prolonged irradiation deserves future attention.

MELATONIN IN PLANTS AS A COMPONENT IN NORMAL FOOD

As the majority of studies on melatonin in plants has been conducted under nutritional or nutraceutical aspects, and since this may become an applied or even biotechnological topic in the future, the content of melatonin in normal food will be briefly discussed, too. Melatonin is present in most

edible plants or parts thereof, however, at highly variable concentrations. As mentioned, relatively high amounts were found particularly in seeds, including cereal grains, oily nuts and spices (**Table 2**), but also in some fruits, e.g., tart cherries (Burkhardt *et al*. 2001c), and vegetables such as purslane (*Portulaca oleracea*; 19 ng/g: Simopoulos *et al*. 2005). Although much higher concentrations are present in the above-mentioned medicinal herbs, the amount of melatonin taken in with a respective tea or extract may not be so high as to substantially change blood levels of melatonin. In fact, no increase of salivary melatonin was demonstrable after treatment of humans with an extract of St. Johns's wort, which is particularly rich in the indoleamine (Franklin *et al*. 2006). However, this does not mean that the melatonin was not taken up by the body. The vertebrate gastrointestinal tract is not only another site of melatonin synthesis, but also an important sink which can be loaded from both the circulation and intestinal lumen (Poeggeler *et al*. 2005). On the other hand, rises in circulating melatonin in response to a high-melatonin diet have been reported in experimental animals (Hattori *et al*. 1995), and enhanced excretion of the urinary metabolite 6-sulfatoxymelatonin was observed in humans after intake of vegetables (Nagata *et al*. 2005). However, these findings are not yet fully conclusive because melatonin can be released from the gut after a meal, as a post-prandial response also to other compounds (Bubenik 2001, 2002; Hardeland and Pandi-Perumal 2005). A more convincing result was obtained by reducing the melatonin content in chicken feed, which caused decreases in its blood level (Tan *et al*. 2003b).

After the recent discovery of the melatonin metabolite AFMK in plants (Tan *et al*. 2007), products of the kynuramine pathway of melatonin catabolism may also come into the focus of interest (cf. **Fig. 1**). Since AFMK was the main product in numerous photocatalytic oxidation reactions (Hardeland *et al*. 1995, 1996b; Hardeland 1996; Behrmann *et al*. 1997; Burkhardt *et al*. 2001a; Hardeland *et al*. 2004), this metabolite is likely to be found in plant material exposed to intense visible or UV light. Because of its cytoprotective and antioxidant properties (Burkhardt *et al*. 2001b; Tan *et al*. 2001) the nutritional value of AFMK may be of interest, too. This holds even more for its deformylated product, N^1-ace-tyl-5-methoxykynuramine (AMK), which is easily formed by arylamine formamidases and hemoperoxidases (Tan *et al*. 2000; Hardeland *et al*. 2004; Hardeland 2005; Hardeland and Pandi-Perumal 2005; Srinivasan *et al*. 2005). AMK is a potent antioxidant (Ressmeyer *et al*. 2003; Hardeland *et al*. 2003, 2004), scavenger of reactive nitrogen species (Guenther *et al*. 2005), inhibitor of neuronal NO synthase (León *et al*. 2006), and antiinflammatory agent (Kelly *et al*. 1984; Mayo *et al*. 2005). To date, AMK has never been determined in angiosperms. With regard to the high reactivity of this compound, its concentrations may remain low, even if it is produced from AFMK at substantial rates.

MELATONIN – A MEDIATOR OF THE SIGNAL DARKNESS IN PLANTS?

The idea that melatonin might have a function similar to that in animals was in the beginning attractive, but there was no logical necessity to assume that this had to refer to the mediation of the signal darkness, since melatonin is highly pleiotropic also in animals (Hardeland *et al*. 2006; Pandi-Perumal *et al*. 2006). After the discovery of melatonin in plants, it was, nevertheless, important to direct botanists to the possibility of photoperiodic effects of melatonin in angiosperms (Balzer and Hardeland 1996), especially as something similar was found in the photoautotrophic dinoflagellate *Lingulodinium* (Balzer and Hardeland 1991a, 1992, 1993a). *Lingulodinium* fulfills a requirement for a dark-mediating role of melatonin, namely, a robust circadian rhythm of the indoleamine with a nocturnal peak (Poeggeler *et al*. 1991). Moreover, this dinoflagellate showed a short-day response of cyst formation, which was inhibited by night-breaks with same duration of total light (photoperiod plus nocturnal light pulse) (Balzer and Hardeland 1991a, 1993a). Cyst induction was, however, only found at a decreased temperature (15 instead of 20°C), a change which causes substantial rises in the melatonin content, as will be discussed next.

A possible induction of short-day responses by conveying dark signals was, therefore, at least worth of being tested. Alternately, the possibility existed that a melatonin metabolite might be involved. In particular, AFMK, which is formed (a) by photocatalytical mechanisms and (b) by interactions with free radicals, which are produced in photoautotrophs predominantly under light exposure, was thought to be a candidate for transmitting light signals (Hardeland 1993). This latter possibility has never been tested in angiosperms, mainly because of commercial unavailability of the substance until recently.

Phase shifting of circadian rhythms is one of the important actions of melatonin in vertebrates. In the photoautotroph dinoflagellate *Pyrocystis acuta*, which can be maintained as a dim-light organism – contrary to many other dinoflagellates – in constant darkness for up to 5 cycles, melatonin induced phase shifts under such conditions, and a phase response curve was determined showing a dominant advance section (Poeggeler *et al*. 1989; Fischer and Hardeland 1994). In *Lingulodinium*, no phase shifts by melatonin were observed in constant dim light (Balzer *et al*. 1999), whereas phase delays were repeatedly detected in constant darkness, which could, however, not be followed over a sufficient number of cycles in this less dark-tolerating bright-light species (Antolín *et al*.1997; Hardeland and Coto-Montes 2000). Phase shifting by melatonin has not yet been studied in angiosperms.

A circadian rhythmicity of melatonin has been demonstrated in a very few angiosperms only. A well-established case is that of *Chenopodium rubrum* (Kolář *et al*. 1997). In this species, melatonin peaks in the middle of the night, resembling the situation in vertebrates. However, in a recent study on *Eichhornia* (Tan *et al*. 2007), the melatonin maximum was detected at the end of the photophase. Earlier assumptions of circadian rhythmicity in the convolvulacian *Pharbitis nil* and in tomato were not confirmed when applying appropriate technology for safe determinations (Van Tassel and O'Neill 2001; Van Tassel *et al*. 2001).

Especially the short-day ecotypes of *Chenopodium*, one of which responds by flower induction to a single overcritical long night, seemed to be highly suitable for testing the involvement of melatonin in photoperiodism. However, flower induction by melatonin was not observed (Kolář and Macháčková 2005). Instead, flowering was partially suppressed when given late at night, and a similar effect was obtained with a putative phamacological agonist of the mammalian nuclear melatonin receptor, CGP 52608 (Kolář *et al*. 1999b). This is in line with other observations. In another short-day plant, the crassulacean *Kalanchoë tubiflora*, no flower induction was observed (Hardeland and Poeggeler 2003), and neither with its metabolite 5-methoxytryptamine (Hardeland, unpublished data), a powerful agonist in *Lingulodinium* (Balzer and Hardeland 1991a, 1992, 1993a; Hardeland 1993; Hardeland *et al*. 1995; Hardeland 1999). The indoleamines were applied either through the water or, in a gel, to the vegetation point, but the problem of sufficient uptake remained. In order to circumvent this, aquatic – floating or submersed – organisms were used, *Lemna minor*, *Lemna trisulca* and *Spirodela polyrhiza*, but neither melatonin nor 5-methoxytryptamine (5-MT) induced flowering (Hardeland 1994a). In the long-day plant *Arabidopsis thaliana*, melatonin caused a moderate delay of flowering, along with some other minor morphogenetic changes (Kolář and Macháčková 2005). In summary, there is to date no indication for a photoperiodic role of melatonin in angiosperms, and actually available data are not encouraging for a continuation of such investigations.

SURVIVAL AS A CYST: ACTIONS OF MELATONIN IN THE DINOFLAGELLATE *LINGULODINIUM* – ROLE OF THE METABOLITE 5-METHOXYTRYPTAMINE

Many more details are known in the dinoflagellate *Lingulodinium polyedrum*. This unicell is more easily accessible to pharmacological treatments and readily takes up biogenic amines. Also standardization is more easily achieved. In this species, the enzymes of melatonin biosynthesis have been measured, and the pathway was found to be identical with that in vertebrates (Balzer and Hardeland 1993b; Burkhardt and Hardeland 1996; Hardeland and Poeggeler 2003). Moreover, two parameters can be easily followed, the emission of light by this bioluminescent organism, and formation of asexual cysts as a response for escaping adverse conditions, also in the context of seasonality. Under basal conditions, melatonin exhibits a circadian rhythm (which persists in constant darkness) with a sharp peak of about 1 μM, which appears shortly after onset of scotophase (Poeggeler *et al*. 1991; Hardeland *et al*. 1995). Melatonin concentration declines in this species throughout the night, at the expense of its metabolite 5-MT, a finding explained by a rhythm of the melatonin-deacetylating enzyme aryl acylamidase (AAA) (Hardeland *et al*. 1997, 2000). The metabolite 5-MT turned out to be crucial for understanding the downstream effects. The maximum of 5-MT coincides with the peak of spontaneous bioluminescence of the glow modality (intensity of the modality flashing is mainly a matter of luminescence capacity and differently phased), a fact which has to be seen on the background of strong stimulations of light emission by 5-MT (Balzer and Hardeland 1991b). Blockade of the indoleamine biosynthetic pathway by tryptophan hydroxylase inhibitors suppressed the glow peak, which was, however, restored by addition of any of the 5-MT precursors, from 5-hydroxytryptophan, serotonin, and *N*-acetylserotonin to melatonin (Burkhardt and Hardeland 1996; Burkhardt *et al*. 1998; Hardeland *et al*. 1999a, 2000). Similar results were obtained when the indoleamines were destroyed by oxidotoxins (Hardeland *et al*. 2000). Therefore, the glow peak was concluded to require 5-MT. In this context, melatonin appears as a source of the active metabolite rather than a regulator. Additionally, melatonin may directly control other physiological functions. During photophase, it was shown to downregulate two diurnally peaking enzymes, tryptophan hydroxylase (Hardeland *et al*. 1997; Hardeland 1999) and superoxide dismutase (Antolín *et al*. 1997), and, when attaining high concentrations, to upregulate AAA, thereby initiating its own catabolism (Hardeland *et al*. 1997; Fuhrberg *et al*. 1997). The full spectrum of direct melatonin effects in these dinoflagellates is still widely unknown.

However, melatonin as a source of 5-MT turned out to be relevant in the control of cyst formation. Asexual cysts represent a dormant stage formed under adverse conditions. This can happen, e.g., in response to strong mechanical agitation, to decreases in environmental pH as occurs when cells are dying in algal blooms, to very low temperatures (Hardeland 1994b), and to short-days in conjunction with moderately decreased temperatures (Balzer and Hardeland 1991a). Although encystment could be induced on a photoperiodic basis, and although this response was mimicked by appropriately timed melatonin given prior to the end of photophase, it was clear already from the initial experiments that 5-MT should be involved, because the deacetylated metabolite was capable of inducing cysts independently of temperature and lighting conditions, whereas encystment was elicited by either short-day or melatonin only at a lower temperature of 15°C (Balzer and Hardeland 1991a, 1992). Surprisingly, melatonin turned out to rise dramatically in response to the decrease in temperature. Sometimes concentrations approaching the millimolar range were measured. These rises were soon followed by a rapid drop, during which high, cyst-inducing amounts of 5-MT were formed, because of an induction of the melatonin-deacetylating enzyme AAA (Fuhrberg and Hardeland 1997; Fuhrberg *et al*. 1997; Hardeland *et al*. 1997). The melatonin effect in otherwise subcritical photoperiods would, therefore, be explained by subcritical rises in melatonin in response to the temperature drop, which do not yet lead to cyst-inducing 5-MT levels, but which are elevated by the addition of melatonin to surpass the 5-MT threshold required for encystment. From this point of view, the melatonin effect does not seem to be a photoperiodic one in the strict sense, in terms of time measurement.

The mechanism of cyst induction by 5-MT appears to be in line with its effects on bioluminescence. These two functions, seemingly unrelated at first glance, are connected to each other by the cytoplasmic pH (Hardeland and Balzer 1993; Hardeland *et al*. 1995). In dinoflagellates, light emission is controlled by proton transfer from an acidic vacuole system to the bioluminescent microsources at the cytoplasmic side. In the excitation process, a conducted proton action potential is spread along the vacuolar membrane, so that protons are also released to the cytoplasmic side outside the microsources. Any stimulation of bioluminescence indicates proton transfer. In fact, the 5-MT effects can be mimicked by protonophores (Balzer and Hardeland 1993a; Hardeland and Balzer 1993; Hardeland *et al*. 1995) and, if these are given at an appropriate dosage not leading to cell death, they elicit not only light emission, but also encystment. The same holds for any other non-lethal agent that stimulates luminescence, such as inhibitors of monoamine oxidase (MAO), which cause rises in 5-MT, for epinephrine, kynuramine and, also, for mechanical agitation (Hardeland and Balzer 1993; Hardeland *et al*. 1995, 1996c). A decrease in cytoplasmic pH was identified as an early event in the encystment response (Hardeland *et al*. 1995; Hardeland and Fuhrberg 1996). This may be seen on the background of a common phenomon observed in resting stages, from bacterial and fungal spores to unfertilized eggs of animals, namely the maintenance of a lower cytoplasmic pH during dormancy. It might be of interest to investigate whether melatonin or one of its metabolites such as 5-MT could act in a similar way during induction of dormancy in plants.

The mechanism as outlined, which starts in *Lingulodinium* with a temperature-dependent rise in melatonin, is subject to an additional control mechanism by nitrate. *L. polyedrum* shows vertical migrations of many meters to access at night high nitrate concentrations available in deeper water layers (Roenneberg and Rehman 1996). High nitrate exerts effects on the circadian system of this dinoflagellate (Roenneberg and Rehman 1996), but additionally strongly downregulates melatonin formation and, consequently, causes declines in 5-MT levels (Fuhrberg and Hardeland 1996; Fuhrberg 1997; Hardeland *et al*. 2001). As a result, cyst formation is inhibited. Thus, cells can "decide" by integrating temperature, photoperiodic and nutritional informations whether it is "worthy" of remaining in the state of active life or whether transition to a dormant stage is preferable (**Fig. 2**). At a favorable temperature such as 20°C, they decide for active life (except they are exposed to low pH or strong agitation, which causes 5-MT-independent cytoplasmic acidification). At a lower temperature (15°C), melatonin is increased and long nights, which extend the period of high nocturnal melatonin, result in overcritical 5-MT levels and, thus, encystment. If, however, nitrate is high, this counteracts the rises in melatonin and 5-MT and, consequently, cyst formation, even at 15°C and in short-day: consequently, cells remain in the active state for reasons of favorable nutritional conditions, although temperature and photoperiod are not optimal.

In other dinoflagellates, these mechanisms have not been analyzed in such detail. Nevertheless, cyst induction by 5-MT or, sometimes, by its precursor melatonin has been repeatedly observed in numerous species (Wong and Wong 1994; Hardeland 1999). In some genera, no typical cysts were formed, but cells became immobile in response to 5-MT and seem to represent resting stages, too. In other bioluminescent species of the genera *Lingulodinium* and *Ale-*

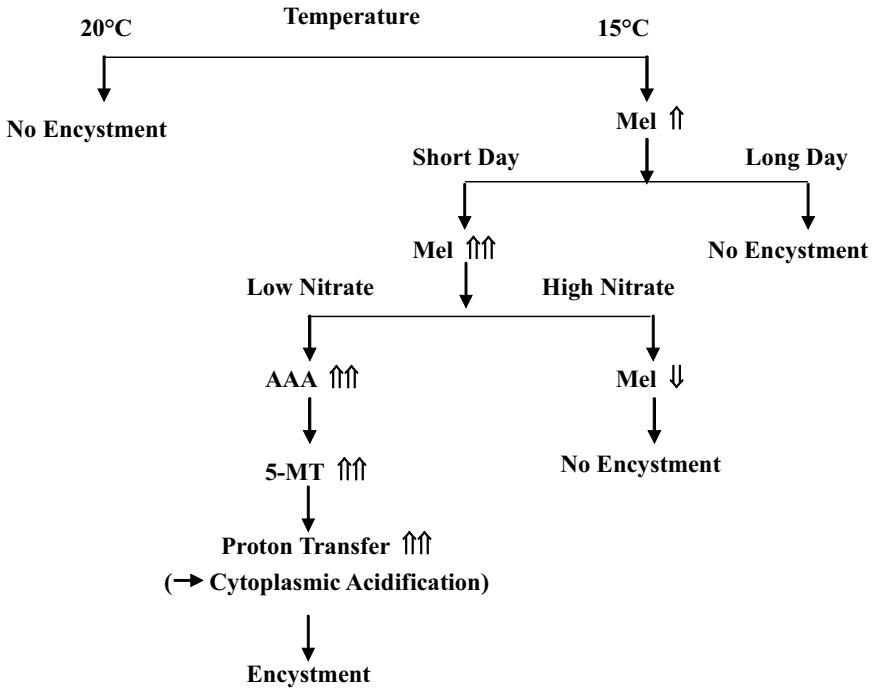

Fig. 2 In the dinoflagellate *Lingulodinium polyedrum*, the decision for or against encystment depends on ambient temperature, photoperiod and nitrate availability. ⇑ rise; ⇑⇑ strong rise; ⇓ decrease.

xandrium, light emission was also stimulated by 5-MT (Mbachu and Hardeland 1997; Hardeland 1999; Hardeland *et al*. 1999b). Attempts to identify conventional signal transduction pathways in the encystment response (Tsim *et al*. 1996a, 1996b, 1996c, 1997, 1998) were interpreted by those authors in terms of an involvement of G proteins, of phospholipase C activation, Ca^{2+} mobilization, but also elevation of cyclic AMP and actions via nuclear melatonin receptors. However, those studies were conducted at very high concentrations of the indoleamines, which are known to be toxic in the case of 5-MT (Hardeland and Balzer 1993), so that secondary effects of cell damage may not have been excluded.

Effects of temperature on melatonin and 5-MT, which were by orders of magnitude stronger than anything similar previously reported for animals, were also found in other dinoflagellates. In *Alexandrium lusitanicum*, melatonin, 5-MT and also its metabolite 5-methoxytryptophol (5-ML) were considerably increased, comparable to findings in *Lingulodinium*, whereas, in a tropical strain of *Amphidinium carterae* collected in the Indonesian sea, the opposite occurred: all three methoxyindoles were decreased at a lower temperature (20°C instead of rearing temperature 24°C), but exposure to 30°C caused strong rises (Fuhrberg 1997). Higher temperatures may be regarded as being more adverse to tropical species, while unfavorable low temperatures have to be survived by species at higher latitudes. The metabolite 5-ML was detected in *Lingulodinium* usually only in conjunction with strong rises of 5-MT. When following the metabolic fate of 5-MT, it turned out that 5-ML formation is a minor pathway which becomes relevant only when 5-MT is high, in terms of an overflow. After oxidation by MAO, the main fraction of the resulting 5-methoxyindole-3-acetaldehyde is converted (cf. **Fig. 1**) to 5-methoxyindole-3-acetic acid (5-MIAA), a compound which has no detectable effect in the dinoflagellate, but is released to the medium (Mueller *et al*. 2001; Hardeland and Poeggeler 2003). At least in the case of this photoautotroph, the formation of 5-MIAA from melatonin is demonstrated. This may be relevant for angiosperms as well, especially with regard to auxin-like growth effects seen after administration of melatonin, as will be discussed next.

OTHER ACTIONS OF MELATONIN IN PLANTS – WHY IS IT RICH IN DORMANT SEEDS?

As the role of melatonin in the photoperiodic seasonal control of angiosperms has not received strong experimental support and, on the contrary, flower induction was not demonstrated in several short-day plants, the identification of its functions is the more urgent, especially with regard to the high levels found in numerous species. One should distinguish between plant-specific and general, ubiquitous actions. While the information on plant-specific effects is scarce and only based on hints, two – entirely different – actions can be identified that are presumably present in all eukaryotes.

The first one is the effect of melatonin on the cytoskeleton, which was the earliest demonstration of an action outside the animals. In the easily accessible endosperm cells of the amaryllidacean *Scadoxus multiflorus* (syn. *Haemanthus katherinae*), melatonin influenced the mitotic spindle (Jackson 1969). Microtubular changes leading to mitotic arrest were observed in *Allium cepa* cells (Banerjee and Margulis 1973). Growth inhibition by melatonin in the pheophycean kelp *Pterygophora californica* (Fuhrberg *et al*. 1996) may be interpreted on that basis. Melatonin effects on the cytoskeleton represent a more generally observed phenomenon, which has been demonstrated from eukaryotic unicells to vertebrates (Banerjee *et al*. 1972; Benítez-King and Antón-Tay 1993; Srinivasan *et al*. 2006). In animals, this could be attributed to direct binding of melatonin at upper physiological concentrations (10^{-9} M) to calmodulin (Benítez-King *et al*. 1993) and to modulations of enzyme activities, e.g., inhibition of CaM kinase II (Benítez-King *et al*. 1996) and stimulation of protein kinase C (Antón-Tay *et al*. 1998). In plants, effects on PK C may not be directly deduced from the animal data, but binding to calmodulin and corresponding inhibitions of Ca^{2+}/CaM-dependent enzymes have to be expected.

A second area of common effects is that of antioxidative protection. The radical-scavenging properties of melatonin (Hardeland *et al*. 1991, 1992, 1993, 1995, 1996b, 1996c, 2003; Tan *et al*. 1993, 2000, 2002, 2003a; Poeggeler *et al*. 1995, 1996; Reiter *et al*. 1995, 2003; Hardeland and Fuhrberg 1996; Reiter 1998; Burkhardt *et al*. 2001a, 2001b; Hardeland 2005; Pandi-Perumal *et al*. 2006) and its kynuric metabolites (Burkhardt *et al*. 2001b; Tan *et al*. 2001; Behrends *et al*. 2004; Hardeland *et al*. 2004; Ressmeyer *et al*. 2004; Guenther *et al*. 2005; Rosen *et al*. 2006; Than *et al*. 2006) should lead to reactions in any organism. The question to be answered in plants would be whether this is relevant in quantitative terms of radical detoxification or rather of product formation. In animals, the antioxidant actions are not restricted to direct radical scavenging, but include upregulation of antioxidant and downregulation of prooxidant enzymes (Reiter *et al*. 1995, 2003; Hardeland 2005; Hardeland

and Pandi-Perumal 2005). However, enzyme regulation may be entirely different in plants, and effects of melatonin have not yet been studied in angiosperms. In the photoautotrophic dinoflagellate *Lingulodinium*, superoxide dismutase and glutathione peroxidase, enzymes induced by melatonin in vertebrates, were not upregulated by melatonin, nor were robust effects detected in hemoperoxidase/catalase and haloperoxidase (Antolín *et al*. 1997). Antioxidative protection may, however, not be limited to radical scavenging and regulation of redox enzymes. The novel concept of radical avoidance by interaction with electron transport, as delineated for the attenuation of mitochondrial electron leakage (Hardeland *et al*. 2003; Hardeland 2005; Srinivasan *et al*. 2005) may correspondingly apply to plants. Whether or not this may be also relevant for plastidial electron leakage remains to be investigated.

Antioxidative protection of photoautotrophs by melatonin has only been studied in a few cases, but additional hints do exist. In *Lingulodinium*, elevated but physiologically possible concentrations of melatonin protected from damage by oxidotoxins such as H_2O_2, paraquat, and buthionine sulfoximine (Antolín *et al*. 1997; Hardeland and Coto-Montes 2000; Hardeland *et al*. 2000). In angiosperms, protection against paraquat was addressed by Fletcher and Sopher (1997), but, to the best of our knowledge, data were never published in detail. In a monocot (*Zea mays*) and two dicots (*Cucumis sativus*, *Nicotiana tabacum*), melatonin was also reported to delay senescence, in a concentration range overlapping with that of cytokinins (Fletcher and Sopher 1997). Other hints for an antioxidant role in plants are indirect. Comparisons of tomato cultivars differing in sensitivity to ozone revealed a positive correlation between resistance and melatonin content (Dubbels *et al*. 1995).

A special aspect of the antioxidative defense is that of photoprotection, which can be regarded as particularly relevant to plants. This was already addressed on the basis of findings on melatonin oxidation with radical- and singlet oxygen-generating photocatalysts and assumed to represent a phylogenetically early role of melatonin (Hardeland *et al*. 1991, 1995, 1996b; Balzer and Hardeland 1996; Hardeland 1997; Behrmann *et al*. 1997). Such reactions were studied with extracts from dinoflagellates, pheophyceans, and photooxidant-containing animal tissues as well, and the resulting main product was usually AFMK. The conjunct presence of high concentrations of both photocatalysts and melatonin was taken as an indication for a photoprotective role, an argument which may still be weak in plants, but which is convincing in an animal organ, the rodent Harderian gland, in which a species- and gender-dependent correlation between melatonin and the photooxidant protoporphyrin IX exists, in which protoporphyrin IX catalyzes a light-dependent formation of AFMK from melatonin, and in which melatonin protects from oxidative stress (Hardeland and Uría 1995; Coto-Montes *et al*. 2001; Tomás-Zapico *et al*. 2002). As mentioned, plants exposed to high natural UV radiation contained much higher melatonin levels than their counterparts from other habitats (Conti *et al*. 2002), and direct elevations of melatonin by UV exposure were measured in *Glycyrrhiza* (Afreen *et al*. 2006). The recent findings in *Eichhornia* (Tan *et al*. 2007) might be interpreted in the same way, but the discrimination between UV and temperature effects is still missing. The phasing of the diurnal rhythms of melatonin and its oxidation product AFMK in *Eichhornia*, both reaching their maxima at the end of photophase (Tan *et al*. 2007), may well be in accordance with a photoprotective role, since free radicals and H_2O_2 are formed with progressively higher rates at the end of photophase, as discussed above for *Lingulodinium*. What is required to substantiate the assumption of photoprotection in angiosperms would be the direct experiment demonstrating the efficacy of externally administered melatonin, at a physiologically possible level, in attenuating damage by UV or high intensities of visible light.

In a different experimental design, protection by melatonin was studied in cell suspensions from *Daucus carota*, which were subjected to cold stress (Lei *et al*. 2004): the indoleamine was reported to prevent cold-induced apoptosis, however, independently of radical detoxification. This effect was attributed to a rise in polyamines (putrescine, spermidine), which represents, at least, the first metabolic action of melatonin in angiosperms after the discovery of cytoskeletal changes.

Another action has been ascribed to melatonin, namely, an auxin-like growth stimulation found in both a dicot (*Lupinus*: Hernández-Ruiz *et al*. 2004) and monocots (coleoptiles of several poaceans: Hernández-Ruiz *et al*. 2005), along with an auxin-like growth inhibition of roots in the poaceans. However, this should be re-analyzed with regard to the formation of 5-MIAA from melatonin, which could take place via 5-MT in the same pathway as in *Lingulodinium* (cf. Mueller *et al*. 2001; Hardeland and Poeggeler 2003). The effects might, therefore, turn out as secondary, caused by a metabolite with structural homology to indole-3-acetic acid (IAA).

The fact that melatonin concentrations were found to be particularly high in seeds (**Table 2**) prompted us to assume a role related to dormancy also in angiosperms, perhaps in conjunction with additional roles in fruit ripening (Balzer and Hardeland 1996; Hardeland *et al*. 2001). In tomatoes, a rise of melatonin in the course of ripening may indicate such a function (Van Tassel *et al*. 2001). Higher levels of melatonin were also detected in flower buds of *Hypericum*, along with an increase in serotonin (Murch and Saxena 2002). Whether or not this is related to morphogenesis and, particularly, flower development, is an intriguing question worth of further investigation. However, the intracellular distribution of melatonin should be determined in the future, so that variations in vacuolar volume can be considered to judge the relevance of quantitative changes. The elevated concentrations in dormant seeds can be seen under two aspects, (a) that of maintenance of the dormant state, and (b) that of antioxidative protection (Balzer and Hardeland 1996; Hardeland *et al*. 1996a; Manchester *et al*. 2000; Hardeland and Pandi-Perumal 2005). A dry, resting seed is incapable of coping with oxidants on the basis of protective enzymes, which can neither be newly synthesized nor be sufficiently active, if at all, nor provide on-site protection, e.g., in lipid stores. Therefore, seeds have to be protected by low molecular-weight antioxidants, such as tocopherols and, sometimes, carotenoids. The additional presence of the amphiphilic potent antioxidant melatonin, especially in oily seeds, may contribute to antioxidative defense and preservation for an extended period of dormancy.

PERSPECTIVES OF APPLICATION AND BIOTECHNOLOGY

The high concentrations of melatonin in several plants has attracted considerable interest in the fields of nutrition and natural medicine. Since melatonin concentration in natural populations of plants cannot be easily standardized, comparable to similar problems with contents of numerous medicinal plants, strategies should be developed for obtaining controlled and sufficiently high levels in the material to be harvested. Several solutions to the problem seem to exist. One is the selection of germ-lines which are particularly rich in melatonin, as demonstrated by Murch and Saxena (2006). A second possibility may come from an appropriate choice of the environment. If high light intensities or UV exposure are required, as discussed, for obtaining enhanced levels of melatonin, high natural radiation should be preferred to cultivation in greenhouses. Adaptation to rising UV intensities during growth might also be experimentally explored. However, the eventual role of ambient temperature should be investigated as well and, if found to be relevant, considered. Moreover, gene technological possibilities may be used to overexpress enzymes of melatonin formation. Overproduction of the precursor serotonin by metabolic engineering has been successfully achieved (Facchini *et al*. 2000).

Finally, one should be aware that not only melatonin

may be of future interest, but also its metabolites, in particular, AFMK. The high concentrations of this metabolite recently discovered in *Eichhornia* (Tan *et al*. 2007), a compound displaying protective properties and representing a source of other pharmacologically interesting compounds (cf. Hardeland and Pandi-Perumal 2005), are encouraging for such considerations.

CONCLUSION

Numerous photoautotrophic organisms contain melatonin, however, in amounts differing considerably. Detailed physiological investigations are still very scarce, and the elucidation of mechanisms is largely restricted to a single dinoflagellate species. In angiosperms, melatonin research is still in its infancy. Some disappointments by researchers who aimed to see in plants what was known from vertebrates, namely, control of circadian rhythms and seasonality, are, from our point of view, somehow the consequence of selective perception, notwithstanding the fact that testing this possibility was justified. We can clearly state that, e.g., cytoskeletal effects based on ubiquitous proteins are common among animals and plants. Also antioxidative protectiton may be similarly relevant to these different organisms, although the unambiguous demonstration for this remains to be presented in angiosperms. In the dinoflagellate *Lingulodinium*, this was shown (Antolín *et al*. 1997; Hardeland and Coto-Montes 2000; Hardeland *et al*. 2000). In angiosperms, only correlations exist between melatonin levels and resistance to oxidants (Dubbels *et al*. 1995) or to toxicity by heavy metals involved in oxidant formation (Tan *et al*. 2007). Although the presence of melatonin in plants may be attractive from a nutritional, nutraceutical or medicinal point of view, the potential roles of this compound in plant physiology are, perhaps, equally or even more exciting. However, melatonin should no longer be exclusively seen as a molecule mediating dark signals, but its other recently discovered broad actions as a metabolic modulator, e.g., of mitochondrial functions should come into focus. Possible actions in differentiation, morphogenesis, ripening, dormancy, interference with other phytohormones, and generation of other bioactive metabolites such as MIAA or methoxylated kynuramines seem to be worth much effort.

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