

Melatonin and 5-methoxytryptamine in non-metazoans

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Abstract — Melatonin seems to be an almost ubiquitous substance, which has been detected not only in metazoans, but also in all major non-metazoan taxa investigated, including bacteria, dinoflagellates, euglenoids, trypanosomids, fungi, rhodophyceans, pheophyceans, chlorophyceans and angiosperms. Despite its vast abundance, little is known to date about its functions. Its presence is not necessarily associated with circadian rhythmicity, which is evident in yeast. Circadian rhythms of melatonin have been reported in non-metazoans only for several unicellular organisms and in one angiosperm. In dinoflagellates, which have been studied in the most detail, the effects on enzyme activities and on phase shifting are known, but the most spectacular actions concerning the stimulation of bioluminescence, changes in cytoplasmic pH and induction of resting stages, can be related to a metabolite of melatonin, the 5-methoxytryptamine; therefore, melatonin should also be considered as a source of other agonists. © Inra/Elsevier, Paris

algae / angiosperms / melatonin / 5-methoxytryptamine / protozoa

Résumé — **Mélatonine et 5-méthoxytryptamine chez les non-métazoaires.** La mélatonine semble être une substance très répandue qui a été détectée non seulement chez les métazoaires, mais aussi dans la majorité des taxa de non-métazoaires, ce qui inclut les bactéries, les dinoflagellés, les eugléniens, les trypanosomes, les champignons, les rhodophycées, les phéophycées, les chlorophycées et les angiospermes. Malgré cette grande abondance, ses fonctions sont très peu connues. Sa présence n'est pas nécessairement associée à une rythmicité circadienne, comme cela devient évident chez les levures. Des rythmes circadiens de mélatonine ont été décrits chez les non-métazoaires, mais uniquement chez quelques unicellulaires et chez une angiosperme. Chez les dinoflagellés, qui ont été étudiés plus en détail, les effets sur des activités enzymatiques et des décalages de phase sont connus, mais les actions les plus spectaculaires qui concernent la stimulation de la bioluminescence, les changements de pH cytoplasmique et l'induction des états de repos, peuvent être reliées au métabolite de la mélatonine, le 5-méthoxytryptamine ; la mélatonine doit donc être considérée comme une source d'autres agonistes. © Inra/Elsevier, Paris

algue / angiospermes / mélatonine / 5-méthoxytryptamine / protozoaire

1. INTRODUCTION

Since the first demonstration of melatonin (= MLT) outside of the metazoa [32, 33], this indoleamine has been detected in numerous organisms belonging to various major taxa (*tables I and II*), including prokaryotes, evolutionarily different lines of eucaryotic unicellular organisms and macroalgae, as well as higher plants and fungi. This large body of evidence permits the statement that melatonin is an (almost) ubiquitous, or highly conserved, substance. Ubiquity frequently indicates fundamental biological significance. This may be so in the case of melatonin, although the answers to the question of what these fundamental properties are can only be given tentatively at the present state of our knowledge.

In the majority of non-metazoan organisms, investigations have been restricted to the mere demonstration of the presence of melatonin. Moreover, in terms of function, a general, common theme is, at least, not clearly evident from a comparison of the many different species. By projecting the findings obtained in mammals or other vertebrates, investigators frequently tend to assume its function as a mediator of dark signals, with possible implications in the control of circadian rhythmicity and seasonality. Although some parallels to mammalian systems can indeed be found, for example in dinoflagellates, such a generalization seems to be currently unjustified. In this context, one should be aware that, even in mammals, melatonin does not always uniformly serve the function of conveying dark signals: in the rodent Harderian gland, high levels of melatonin, associated with the presence of a photocatalyst, protoporphyrin IX, do not exhibit circadian amplitudes large enough for such a role [29, 30]. In various invertebrate animals, melatonins even exhibit diurnal peaks (summarized in [15, 41]).

2. MELATONIN AND CIRCADIAN RHYTHMICITY IN NON-METAZOANS

Although the presence of melatonin has been demonstrated in a comparably large number of non-metazoan species, there are only very few organisms in which this indoleamine has been shown to oscillate in a circadian fashion. This is the case in the dinoflagellate *Gonyaulax polyedra*, in which melatonin rises strongly directly after the onset of the dark phase, and in which rhythmicity persists in constant darkness (= DD) [5, 17, 33]. Nocturnally peaking diurnal rhythms of melatonin were also found in *Euglena gracilis* and in several unicellular chlorophyceans, such as *Dunaliella tertiolecta* and various *Chlamydomonas* species (Balzer I. et al., unpubl. data). In an aerobic photosynthetic bacterium, *Erythrobacter longus*, melatonin levels were shown to be much higher in darkness than in light [37]; however, circadian rhythmicity was not demonstrated in that paper. The difference between melatonin levels in light and darkness may already suffice for an action as a dark mediator regardless of whether melatonin may be produced at a higher rate during darkness, may be photocatalytically destroyed in the light or whether a circadian oscillator may be present in this prokaryote. This possibility remains, however, to be further substantiated on the basis of physiological responses to the indoleamine.

The only higher plant in which rhythmicity of melatonin has been thoroughly investigated is the dicot *Chenopodium rubrum*, a well-known short-day plant appearing to be particularly suitable for studying the possibility of a role in photoperiodism. Again, the indoleamine exhibited a high-amplitude rhythm with a nocturnal peak [23]. Other data from that group showing changes in phase position after transfer to a different photoperiod and persistence in DD have been presented at recent

Table I. Melatonin and other methoxyindoles in various non-metazoan taxa.

Taxon/Organism	Compound	Detection	References
Procaryonta			
<i>Rhodospirillum rubrum</i>	MLT	RIA	[28]
<i>Erythrobacter longus</i>	MLT	RIA,TLC	[37]
Euglenoidea			
<i>Euglena gracilis</i>	MLT,5MT,5ML	RIA,HPLC-ECD	(B*)
Trypanosomida			
<i>Trypanosoma cruzi</i>	MLT	RIA	(A*)
Dinoflagellata			
<i>Gonyaulax polyedra</i>	MLT,5MT,5ML	RIA,HPLC-ECD, HPLC-FD	[5, 12, 13, 15, 17, 31, 32, 33]
<i>Alexandrium lusitanicum</i>	MLT,5MT,5ML	RIA,HPLC-ECD	(F*)
<i>Ceratium horridum</i>	5MT,5ML	HPLC-ECD	(F*)
<i>Amphidinium carterae</i>	MLT,5MT,5ML	HPLC-ECD	(F*)
<i>Pyrocystis lunula</i>	MLT	ICC	(B*)
<i>Noctiluca scintillans</i>	MLT	RIA	(B*)
Ciliata			
<i>Tetrahymena thermophila</i>	5MT	HPLC-ECD	(B*)
Rhodophyceae			
<i>Gracilaria tenustipitata</i>	MLT	ICC,HPLC-ECD	(B*), (L*)
<i>Palmaria palmata</i>	MLT	ELISA,ICC	[26], (B*)
<i>Porphyra umbilicalis</i>	MLT,5MT,5ML	ELISA,HPLC-ECD, HPLC-FD	[8, 26]
<i>Chondrus crispus</i>	MLT,5MT,5ML	HPLC-ECD	[27]
Phaeophyceae			
<i>Pterygophora californica</i>	MLT,5ML	RIA,ELISA,HPLC-ECD	[11, 42]
<i>Laminaria digitata</i>	MLT	HPLC-ECD	(L*)
<i>Petalonia fascia</i>	MLT,5MT,5ML	HPLC-ECD	[27]
Chlorophyceae			
<i>Acetabularia acetabulum</i>	MLT	RIA,ICC,HPLC-UVD	(B*)
<i>Dunaliella tertiolecta</i>	MLT	RIA	(B*)
<i>Chlamydomonas</i> spp.	MLT	RIA	(B*)
Angiospermae species of ten dicot and six monocot families; for list of species see ref. [4]	MLT	RIA,HPLC-ECD, HPLC-FD,HPLC-MS, LC-MS	[10, 20, 22, 23, 24, 40]
Fungi			
<i>Saccharomyces cerevisiae</i>	MLT,5MT,5ML	HPLC-ECD	[36]
<i>Neurospora crassa</i>	MLT	HPLC-ECD	(B*)

* Unpubl. data (except abstracts, theses, lab. reports) by A = Acuña-Castroviejo D.; B = Balzer I. et al.; F = Fuhrberg B., Hardeland R.; L = Lüning K. et al.

Table II. Melatonin levels in several non-metazoans.

Organisms	Levels/Conditions	References
<i>Erythrobacter longus</i>	in light: ca. 0.03–0.13, in darkness up to 10 ng·mg ⁻¹ protein	[37]
<i>Euglena gracilis</i>	< 1 ng–3 µg·mg ⁻¹ protein; circadian	(B*)
<i>Trypanosoma cruzi</i>	up to 200 pg·mg ⁻¹ protein in epimastigotes, up to 500 pg·mg ⁻¹ in trypomastigotes	(A*)
<i>Gonyaulax polyedra</i>	20 °C: 0.1–2.5 ng·mg ⁻¹ protein; 15 °C: transient rises to > 1 µg·mg ⁻¹ protein	[5, 33] [12, 15]
<i>Alexandrium lusitanicum</i>	20 °C: > 30 ng·mg ⁻¹ protein	(F*)
<i>Amphidinium carterae</i>	30 °C: > 800 ng·mg ⁻¹ protein	(F*)
<i>Palmaria palmata</i>	0.1–0.4 ng·mg ⁻¹ fresh weight	[26]
<i>Porphyra umbilicalis</i>	lab.: 0.1–0.4 ng·mg ⁻¹ fresh weight field: 2–5 ng·mg ⁻¹ fresh weight	[26]
<i>Chondrus crispus</i>	up to > 3 ng·mg ⁻¹ protein	[8]
<i>Pterygophora californica</i>	1.5 ng·mg ⁻¹ protein	[27]
Dicotyledonae (summarized in [15])	leaves, shoot, root, fruits, seeds; levels vary from undetectable to 0.9 ng·mg ⁻¹ protein	[11]
Monocotyledonae (summarized in [15])	leaves, shoots, roots, fruits, seeds; levels vary between 10 pg·g ⁻¹ fresh weight (<i>Asparagus</i> shoot), 500 pg·g ⁻¹ fresh weight (<i>Zingiber</i> tuber) and > 0.3–> 5 ng·mg ⁻¹ dry weight (poacean seeds)	[10, 20]
<i>Saccharomyces cerevisiae</i>	level depends on tryptophan availability: < 1–> 100 ng·mg ⁻¹ protein	[36]

* Unpubl. data (except abstracts, theses, lab reports) by A = Acuña-Castroviejo D.; B = Balzer I. et al.; F = Fuhrberg B., Hardeland R.

meetings, but this information was not included in the respective abstracts, presumably because the data were cautiously regarded as being preliminary. Nevertheless, it seems likely that a true circadian rhythm exists in this species. A nocturnally peaking rhythm was also found in another dicot, the tomato, *Lycopersicon esculentum* (Balzer I. et al., unpubl. data).

Elevated levels in darkness are, of course, an essential condition for a role as a mediator of dark signals. Another aspect is its capability of inducing phase shifts in circadian rhythms. This has been demonstrated under DD conditions for bioluminescence rhythms in a few dinoflagellates. A complete phase-response curve was only obtained in *Pyrocystis acuta*, in which the advanced part was more pronounced than the delayed portion (Fischer J., Hardeland R., unpubl. data). Phase-shifting was also observed in *Gonyaulax polyedra*, but owing to other intentions of the study [1], this aspect was not followed systematically; when given in the middle of the day, melatonin caused phase delays of a few hours. Similar data were obtained for *Gonyaulax spinifera* (Hardeland R., Mbachu E.M., unpubl. data).

Although circadian rhythmicity of melatonin exists in non-metazoans, this does not allow for generalizations. The clearest counter-example is presumably *Saccharomyces cerevisiae*, which contains remarkably high amounts of melatonin and other methoxyindoles [36], but for which no indication of circadian rhythmicity exists, neither for these indoleamines nor for other functions. In a brown alga, *Pterygophora californica*, the expression of a circadian rhythm in melatonin concentration seems to be conditional and, during long days, a bimodal pattern with both nocturnal and diurnal peaks is observed [42].

3. PROBLEMS OF MELATONIN DETECTION IN NON-METAZOANS

Some of the reasons for the imbalance between the demonstration of the presence of melatonin in very many species and hard data on circadian and seasonal patterns in only a few of them are of a technical nature. In many plants and unicellular organisms, melatonin is readily destroyed during its extraction from the cell or tissue material, and elaborate preservative procedures have to be applied in order to obtain acceptable rates of recovery [31, 33]. This seems to be particularly difficult in material from some eucaryotic photoautotrophs, even under strict occlusion from light. On the contrary, quantitative extraction is easily possible in a heterotroph such as yeast [36] and also in *Erythrobacter longus* [37], an aerobic photosynthetic bacterium which is not fully photoautotroph. Since melatonin easily undergoes reactions with several free radicals [15–17, 35], instability during extraction may reflect the electron exchange with radicals and with components of electron transport chains and may, therefore, be largely caused by chloroplasts. Moreover, the problem of instability has to be solved again for all organisms that are to be studied, and this can only be achieved on the basis of thorough determinations of recovery. This has been performed in detail in only two photoautotrophs, namely in the dinoflagellate *Gonyaulax polyedra* [31, 33], and in the angiosperm *Chenopodium rubrum* [23].

Measurements of recovery are not easily performed in massive plant tissues, because the externally added melatonin cannot be mixed thoroughly with the cell material before homogenization. For this reason, many determinations of endogenous melatonin in parts from higher plants or macroalgae, in which the yield is unknown, may still be largely underestimated. An additional problem in angiosperms, macroalgae and also in some fungi (but not in yeast)

results from the distribution within the tissue or even the cell, and more precisely from the partition of melatonin between the cytoplasm, the large central vacuole, and cell wall/apoplast. Usually, data are presented on the basis of fresh weight or protein as reference values. This does not, however, provide any information on the effective cytoplasmic concentration of the indoleamine. As long as we do not know how much of the melatonin detected is present extracellularly in the apoplast and how much is present in the vacuole, making up by far the largest part of the plant cell, any considerations of physiological levels remain impossible.

4. RESPONSES TO MELATONIN

Our current knowledge on the effects of melatonin in non-metazoans is still scarce (cf. *table III*). In dinoflagellates the effects are often found only at very high concentrations (0.1 mM and higher; in *Cryptecodinium*, even 10 mM have been used [38, 39]). Moreover, the effects can be conditional, as in *Gonyaulax polyedra*, in which the encystment response occurs only at a lower temperature [2, 13, 14]; such a treatment leads to enormous accumulations of melatonin anyway (transiently more than 1 mM) and finally to high concentrations of 5-methoxytryptamine (= 5MT) [12, 15]. Since 5MT is much more efficient as a regulator of various functions, for example as an inducer of asexual cysts, as a stimulator of bioluminescence and cytoplasmic acidification [2, 3, 13, 14, 17], the question remains as to whether all the other actions are not also due to 5MT. Recent findings in *Cryptecodinium cohnii* [38, 39], showing increases in calcium influx and in the formation of inositol phosphates, are affected by the problem that, apart from the extremely high concentrations required, the dose-responses to melatonin and 5MT are relatively similar, despite the strong difference in cyst-inducing efficiency.

The effects of melatonin in other non-metazoans are only partially interpretable. There is, at least, one common effect discernible from the comparison of the ciliate, *Stentor*, and the two monocots, *Haemanthus* and *Allium*. This is namely an influence on the cytoskeleton, which is also found in mammalian cells and which may relate to the binding of melatonin to calmodulin [9]. This may be, in fact, a wide-spread action, which has been much neglected in the past. It is possible that the effects on growth, or on the release of gametes, as found in several algae (*table III*), or perhaps even the suppressive effects on flower induction in *Chenopodium* can be interpreted on this basis. The expectancy for a short-day plant would have been that melatonin, if involved in photoperiodism, should favour flowering. The contrary was also observed, with a synthetic melatonin analogue, CGP 52608 [24]. There is, however, a promising finding in the brown alga *Pterygophora*, in which melatonin induced the formation of a new blade, as otherwise found during the annual cycle [42], which is a possible hint for melatonin-controlled seasonality.

5. OCCURRENCE OF AND RESPONSES TO 5-METHOXYTRYPTAMINE

In the majority of the investigations, methoxyindoles other than melatonin were not determined. However, when chromatographic techniques were applied, it turned out that most non-metazoans tested also contain high amounts of 5MT and of 5-methoxytryptophol (= 5ML) (cf. *table I*). This is the case in phylogenetically different lines of protozoa and macroalgae as well as in yeast. While strong effects of 5ML have not been reported in non-metazoans, except for extreme concentrations (10 mM) in *Cryptecodinium cohnii* [39], 5MT has proven to be a very efficient agonist in various dinoflagellates (*table IV*). At concentrations below 10 μ M (levels which are frequently

Table III. Effects of melatonin in non-metazoans.

Taxon/Organism	Effects/References
Dinoflagellata	
<i>Gonyaulax polyedra</i>	induction of asexual cysts, at decreased temperature only [2]; stimulation of aryl acylamidase [18]; suppression of tryptophan hydroxylase [18]; decreases of superoxide dismutase and, moderately, of glutathione S-transferase, when given at photophase [1]; protection from lethal oxidative damage [1]; restoration of glow peak after inhibition of tryptophan hydroxylase [19] or oxidative destruction of indoleamines (Burkhardt S., Hardeland R., unpubl. data)
<i>Alexandrium catenella</i>	induction of asexual cysts [38]
<i>Aureodinium pigmentosum</i>	induction of asexual cysts [43]
<i>Cryptocodinium colnii</i>	induction of asexual cysts [38, 39]; stimulation of calcium influx [39]; stimulation of inositol phosphate formation [38, 39] (effects required very high concentrations)
Ciliata	
<i>Stentor coeruleus</i>	interference with cytoskeleton of oral band [7]
Rhodophyceae	
<i>Palmaria palmata</i> ,	inhibition of growth, but not in LL [26]
<i>Porphyra umbilicalis</i>	inhibition of growth, but not in LL [26]
Phaeophyceae	
<i>Pterygophora californica</i>	inhibition of growth, similar to acute dark effect, also in LL [11, 42]; induction of new seasonal blade [42]
Chlorophyceae	
<i>Chlamydomonas gigantea</i>	delay of gametogenesis (Balzer I. et al., unpubl. data)
Angiospermae	
<i>Chenopodium rubrum</i>	reduction of flowering [24]
<i>Allium cepa</i>	changes in mitotic spindle [6]
<i>Haemanthus katherinae</i>	changes in mitotic spindle [21]
Fungi	
<i>Neurospora crassa</i>	decrease in NAD ⁺ kinase activity [25]

For additional effects on phase shifting see section 2.

Table IV. Effects of 5-methoxytryptamine in various dinoflagellates.

Species	Effects/References
<i>Gonyaulax polyedra</i> *	c [2, 14, 17], a [17], b [3, 13, 14]
<i>Gonyaulax spinifera</i> *	b (Hardeland R., Mbachu E.M., unpubl. data)
<i>Alexandrium tamarense</i> *	c [43], a, b (Hardeland R., unpubl. data)
<i>Alexandrium</i> spec. 146#*	c, a, b (Hardeland R., Mbachu E.M., unpubl. data)
<i>Alexandrium lusitanicum</i>	c, a (Hardeland R., unpubl. data)
<i>Alexandrium</i> spec. 155#	c, a (Hardeland R., unpubl. data)
<i>Alexandrium catenella</i>	c [38]
<i>Aureodinium pigmentosum</i>	c [43]
<i>Gymnodinium simplex</i>	c [43]
<i>Gymnodinium catenatum</i>	i (Hardeland R., unpubl. data)
<i>Cryptothecodinium cohnii</i>	c [38, 39, 43]
<i>Scropsiella trochoidea</i>	c [43]
<i>Prorocentrum</i> , many species	i (Hardeland R., unpubl. data)
<i>Symbiodinium</i> spec.	formation of coccoid, cyst-like stage, as formed in coelenterate hosts [34]

Effects: immobilization without encystment (= i); induction of asexual cysts (= c); cytoplasmic acidification (= a); stimulation of bioluminescence (= b). * Bioluminescent species; # BAH-ME collection numbers.

attained in dinoflagellates) 5MT stimulates the formation of asexual cysts, cellular immobilization, cytoplasmic acidification and light emission in bioluminescent species. 5MT was also shown to be required for the expression of the circadian glow peak in *Gonyaulax polyedra* [19]. The conversion of melatonin to 5MT, due to the observed induction of a melatonin-deacetylating aryl acylamidase, is suggested to be the crucial step in the encystment response of this species [12, 15, 17, 18]. Since no high-affinity binding sites for melatonin were detected in *Gonyaulax* (Masson-Pévet M., Balzer I., unpubl. data), signalling may involve 5MT formation; this metabolite has strong effects on proton transfer from an acidic vacuole system, and the effects on encystment as well as on bioluminescence can be mimicked by protonophores [13, 15, 17]. Moreover, inhibitors of monoamine oxidase, which cause the accumulation of 5MT to cyst-inducing concentrations [12, 15], also elicited the two responses [13–15, 17]. Therefore, the question is how findings on rises in 5MT, signalled by proton transfer and effects on calcium and inositol phos-

phates, as found in *Cryptothecodinium* [38, 39], can be matched; that is, in which temporal and functional sequence such events will interact.

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