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Melatonin: presence and formation in invertebrates

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Abstract. In vertebrates, it is now clearly demonstrated that the pineal gland is implicated in conveying photoperiodic information via the daily pattern of melatonin secretion. Invertebrates, like vertebrates, use photoperiodic changes as a temporal cue to initiate physiological processes such as reproduction or diapause.

How this information is integrated in invertebrates remains an unsolved question. Our review will be an attempt to evaluate the possible role of melatonin in conveying photoperiodic information in invertebrates.

It is now well demonstrated in both vertebrates and invertebrates that melatonin as well as its precursors or synthesizing enzymes are present in various organs implicated in photoreceptive processes or in circadian pacemaking. Melatonin, serotonin or N-acetyltransferase have been found in the head, the eyes, the optic lobe and the brain of various invertebrate species. In some species it has also been shown that melatonin is produced rhythmically with high concentrations reached during the dark period. Moreover, the physiological effects of melatonin on various periodic processes such as rhythmic contractions in coelenterates, fissioning of asexual planarians or reproductive events in flies have been reported in the literature.

All these results support the hypothesis (refs 36, 37) that melatonin is not solely a pineal hormone but that it may be an evolutionary conservative molecule principally involved in the transduction of photoperiodic information in all living organisms.

Key words. Melatonin; day-night rhythm; N-acetylserotonin (NAS); N-acetyltransferase (NAT); invertebrates; photoreceptors; circadian pacemakers; photoperiod.

Introduction

Most living organisms use annual changes in seasonal variables, such as photoperiod, temperature, rainfall, and food supply, to regulate physiological functions such as reproduction, migration, molt of skin, fur and plumage, and diapause.

In vertebrates living in temperate regions it is now clearly demonstrated that the pineal gland is implicated in conveying the photoperiodic message via the daily pattern of melatonin secretion. Photoperiodic changes, acting via the nervous system, alter the temporal pattern of melatonin fluctuations; these changes in melatonin secretion may constitute a hormonal signal which drives photoperiodic responses[18,19,29].

Invertebrates, like vertebrates, use the photoperiod as a temporal cue to initiate physiological processes such as reproduction or diapause[10,15,44]. This implies that they are able to discriminate between long and short days. How is this information integrated in invertebrates? The question remains unsolved, but there are now some data suggesting that melatonin could be involved in such processes. Melatonin then would be an evolutionary conservative molecule[16] principally involved in the transduction of photoperiodic information in all living organisms[36,37,3].

This review will be an attempt to evaluate the physiological significance of melatonin and its possible role in conveying photoperiodic information in invertebrates.

Melatonin presence and synthesis in invertebrate organs

The indoleamine serotonin, which is a precursor of melatonin, has long been known to be present in invertebrate tissue[39], and is also known to be involved in numerous physiological processes[38].

In invertebrates indolamine biosynthesis follows the same pathway as in vertebrates. Tryptophan is hydroxylated to 5-hydroxytryptophan (5-HTP) which is then decarboxylated into 5-hydroxytryptamine or serotonin (5-HT)[20]. As in vertebrates, it has also been demonstrated that 5-HT metabolism follows two main routes: N-acetylation, involving N-acetyltransferase (NAT) activity giving N-acetylserotonin (NAS) the direct precursor of melatonin in the vertebrate pineal gland, and oxidative deamination involving monoamine oxidase (MAO) activity. According to Evans and Fox[12] the major enzymatic pathway, at least in insects, is N-acetylation. For two decades[8], NAT and NAS have been known to be present in the nervous tissues of the fly, *Drosophila*.

Moreover, in insects, the presence of the enzyme hydroxyindole-O-methyltransferase (HIOMT) which is implicated in the formation of melatonin from NAS, has recently been suspected. It has been reported for example that antibodies raised against the pineal enzyme HIOMT bind cockroaches cells, constituting evidence they have an enzymatic characteristic of the pineal gland of vertebrates[34].

On the other hand, it is now well established that melatonin is not unique to the pineal gland, but is present and synthesized in other organs sensitive to environmental information, especially the retina[35].

For these reasons, the possible presence and synthesis of melatonin has long been suspected in invertebrates. Preliminary studies focused on photoreceptive structures.

The first evidence of the presence of melatonin in invertebrates was reported in the compound eyes of an insect, the locust, *Locusta migratoria*[36]. Melatonin was demonstrated by radioimmunoassay (RIA) and the result has been validated by gas-chromatography-mass spectrometry (GCMS). In insects killed in October under a 9 h light – 15 h darkness schedule, melatonin concentrations ranged from 10.5 to 26.5 pg/eye with highest values found during the night and lowest values at the end of the light period (table). These concentrations vary depending on the time of the year[37]. To test if the presence of melatonin is a common feature of all invertebrate species we extended this study to other classes of invertebrates: crustacea and molluscs[37]. Melatonin has been identified by RIA in the compound eye (eye + eye stalk including the optic lobe) of the crab, *Carcinus maenas*, and in the retina of the sepia, *Sepia officinalis*. In both species melatonin is present in high concentrations (2100 to 2800 pg/retina in Sepia and 2700 to 3650 pg/eye in the crab). These concentrations are in the same range of those observed in the retina of birds[27]. However in crab killed in May, we were not able to find significant day-night changes in the ocular melatonin concentrations over a 24 h period. Since the crab lives in the intertidal zone, we have also checked for variations of melatonin concentration correlated to the tidal cycle. We were not able to find any temporal relationships between melatonin fluctuations and the tidal cycle. Nevertheless, important changes were observed depending on the time of the year (3400 pg/eye in May versus 143 pg/eye in November).

Reviewing the literature it is now increasingly evident that the presence as well as the synthesis of melatonin in photic and/or cerebral structures appears to be a common feature of all invertebrates (table).

In another insect, the face fly, *Musca autumnalis*, Wetterberg et al.[40] have demonstrated that melatonin is present in the brain and that it fluctuates rhythmically, with peak values found during the dark period of the day-night cycle. In the cockroach brain and/or optic lobe, Binkley[6] showed NAT activity of around 20 nmoles/brain/hour which is comparable to that observed in chicken pineal glands during nighttime. NAT does not fluctuate rhythmically in the cockroach, while melatonin concentrations appear to be high during the night and low during the day.

Even in a very primitive metazoan, the planarian, *Dugesia dorotocephala*, cephalic melatonin has been identified by RIA and high performance liquid chromatography (HPLC) with electrochemical detection[22]. Sexual as well as asexual planarians contain measurable amounts of melatonin in their heads (presumably brain and/or eyes; 31 to 307 pg by RIA and 18 to 288 pg by HPLC).

In most studies, cephalic levels of melatonin appear to be higher during the scotophase than during the photophase. This indicates that melatonin synthesis is probably modulated by photoperiod as it is in vertebrates. A melatonin-like activity has also been reported in whole tissues of a coelenterate, *Renilla koellikeri* (Anctil et al., cited in ref. 1).

Very recently[41] melatonin and NAT activity have been reported in the optic lobe of the giant freshwater prawn, *Macrobrachium rosenbergii*. NAT activity in this species appears to be five to eight times higher than that of pineal glands of rats treated with isoproterenol (a β-adrenergic agonist). In animals sampled at 3 h intervals during a 24 h period, NAT did not differ between daytime and nighttime. Surprisingly, melatonin measured by RIA in this species appears to be higher during daytime than during nighttime. Moreover these authors[42] also report that one month of continuous light significantly increased NAT activity in the optic lobe of the prawn, compared to that of animals raised under constant darkness or 12 h light – 12 h darkness. Conversely, melatonin did not differ significantly in the different experimental groups.

The biosynthesis of various indole derivatives including melatonin has been also reported recently in the flies, *Drosophila melanogaster* and *Drosophila simulans*[13], in isolated heads or the whole organism, by using tritiated labeled precursor. After incubation with 5-HTP, radioactivity was found in spots comigrating with 5-HT and NAS, with a tenfold increase in the amount of radioactivity incorporated in heads compared to whole animals. When 5-HT was used as tritiated precursor, radioactivity was found in spots comigrating with NAS and melatonin. When NAS was used as precursor, the radioactivity comigrated with melatonin. The identification of the melatonin-like peak was checked by thin layer chromatography (TLC), GCMS and RIA. From these results it is clear that some structures in the head of the fly are able to synthesize melatonin.

From the literature it appears that the occurrence of melatonin is not restricted to vertebrate and invertebrate organisms. Recently Pöggeler et al.[31] have demonstrated that in the unicellular alga, *Gonyaulax polyedra*, melatonin exhibits a daily rhythm of high amplitude, with a maximum as great as in the vertebrate pineals (about

Table. Identification of melatonin, its precursors or the enzymes implicated in its synthesis in invertebrates

Compound	Species	Organ	Technique	Concentrations	Day-night variations	References
Melatonin	Insects					
	Locust *Locusta migratoria*	compound eyes	RIA GCMS	10.5 to 26.5 pg/eye 2.5 to 6.5 pg/mg eye	Night peak	36, 37
	Cockroach	brain and/or optic lobe	RIA	20 to 150 pg	Night peak	6
	Flies					
	Musca autumnalis	brain	RIA	162 + 39 nmoles/brain	Night peak	40
	Drosophila melanogaster	head	Radioenzymatic assay + TLC	39.5 + 5.3 pmoles/g	Not studied	13
	Drosophila simulans	whole organism	GCMS – RIA			
	Crustacea					
	Crab *Carcinus maenas*	eyes + eye stalk	RIA	2700 to 3680 pg/eye	No variations	37
	Prawn *Macrobrachium rosenbergii*	optic lobe	RIA	47 to 73 pg/mg eye 0.5 to 5.5 pg/ng protein	Day peak	41
	Mollusca					
	Sepia *Sepia officinalis*	retina	RIA	2100 to 2800 pg/retina 2.9 to 3.5 pg/mg retina	Not studied	37
	Plathelminthes					
	Planaria *Dugesia dorotocephala*	head	HPLC RIA	31 to 307 pg	Night peak	22
	Coelenterates					
	Sea pansy *Renilla koellikeri*	whole tissue	RIA	1 ng/g	Not studied	1
NAS	Insects					
	Fly *Drosophila melanogaster*	head whole organism	Radioenzymatic assay + TLC	16.3 + 0.4 nmoles/g	Not studied	13
	Cockroach *Periplaneta americana*	cerebral ganglion	HPLC	0.2 to 1.4 ng/cerebral ggl pair	Not studied	33
	Crustacea					
	Crayfish *Pacifastacus leniusculus*	brain	Radioenzymatic assay + TLC	1.6 ng/ml homogenate	Not studied	9
NAT	Crustacea					
	Crayfish *Pacifastacus leniusculus*	brain	Radioenzymatic assay	545–555 pmoles/min/mg protein	Not studied	9
	Prawn *Macrobrachium rosenbergii*	optic lobe	Radioenzymatic assay	1.0–1.5 nmoles/hr/µg protein	No variations	41
	Insects					
	Cockroach	brain and/or optic lobe	Radioenzymatic assay	20 nmoles/brain/hr	No variations	6
HIOMT	Insects					
	Cockroach	optic lobe	Immunocyto chemistry			34

2.5 ng/mg protein). In this alga, NAS and HIOMT, as well as another 5-methoxyindole, 5-methoxytryptamine, were also detected[2].

When comparing these different data, it appears that melatonin, as well as the enzymes involved in its synthesis, are present in various invertebrate species and even in unicellular organisms such as *Gonyaulax*, in concentrations comparable to those observed in the pineal gland of vertebrates.

In many species melatonin fluctuates rhythmically with high levels at night and low levels during the day, suggesting that its synthesis is modulated by the light/dark cycle as in the vertebrate pineal gland. All these results corroborate the hypotheses of Gern[16] and of Vivien-Roels et al.[37] that melatonin could be an evolutionary conservative molecule. The results also raise the question of the significance of melatonin in organs implicated in photoreceptive processes as well as the physiological role of this compound in invertebrates.

Physiological significance of melatonin in invertebrates

It is now generally believed that in many invertebrate species the eyes are the photoreceptors, perceiving light for the circadian system, while the oscillators are generally located in the optic lobe or the brain (refs 6, 14, 32). There is evidence that the light receptors and the photoperiodic clocks are located in the head[14].

In the cockroach, for example, blanchening or excising the compound eyes blocks the entrainment of the locomotor activity to light, as does transsecting the optic nerves that project from the eyes to the optic lobe. Bisecting the brain produced arhythmia in *Periplaneta americana*, indicating a neural location for the oscillator (refs 6, 32). Further evidence has been provided by Page[24,25] that the optic lobes in cockroaches are the site of the pacemaker. Since serotonin as well as melatonin are present and rhythmically synthesized in the optic lobe of the cockroach there is some evidence that these compounds could be involved in rhythm regulation. Moreover, serotonin in the cockroach is able to phase-shift the circadian rhythm of locomotor activity[26].

In flies, where melatonin as well as NAS are present in the head, it has been reported[32] that the daily eclosion rhythm (which is entrained by exposing pupae to light cycles) is lost after excision of the brain, suggesting that either the circadian pacemaker or the entraining mechanism is within the brain.

In crustacea little is known about circadian oscillators. A circadian pacemaker which controls the sensitivity of the eye to light via a specific neurotransmitter is known to be present in the brain of a merostoma, *Limulus*. On the other hand, the organ of Bellonci, a structure located in the optic lobe of crustacea, has been shown to be composed of 'pinealocyte-like' cells[11,4]. This structure has been shown to contain serotonin, NAS and melatonin[4,41]. Serotonin which is present in the *Aplysia* eye is able to shift the phase of the *Aplysia* eye rhythm[6]. Even in the unicellular alga, *Gonyaulax polyedra*, there is good evidence that those single cells contain all the machinery to generate circadian rhythms and to respond to light, and that methoxylated indoleamines such as melatonin and 5-methoxytryptamine may act as mediators of photoperiodicity[3]. From this overview, it appears that the organs that have been identified as light receptors or containing circadian pacemaker activity are able to synthesize melatonin in a rhythmic manner that is modulated by photoperiod.

In most species studied to date (invertebrates as well as vertebrates) the synthesis of melatonin is under environmental control. Specifically, melatonin synthesis is a diurnal biochemical event which is entrained by the light-dark cycle. This suggests that there could be a similar function for melatonin in invertebrates and vertebrates. It is now wellknown that the photoreceptor cells found in the retina and in the pineal organ of lower vertebrates are the cells responsible for melatonin synthesis[17]. In the vertebrate eye, it has been reported that melatonin may be implicated in the control of pigment aggregation, disk shedding, response to daily changes of illumination[5,30], the control of intraocular pressure[7], and the inhibition of dopamine release by amacrine cells[23]. If one compares vertebrate and invertebrate cones or rods and the microtubules of invertebrate rhabdomeres, it appears that both are membranaceous processes of retinal or retinular cells[43]. On the other hand, in the optic lobe of crustacea the organ of Bellonci, which synthesizes melatonin, also contains cells that are quite similar to pineal photoreceptor cells. Although there are no experimental data directly supporting it, it appears nevertheless highly probable that in the invertebrate eye melatonin is also synthesized by the photoreceptor cells. If this is true such a hypothesis would support and extend the concept of Gern et al.[17] that melatonin synthesis is a common attribute of ciliate photoreceptor cells and that it may be a fairly consistent phylogenetic feature. Whether melatonin acts by regulating activities within the photoreceptor cells, or acts on neighbouring cells as a neuromodulator or neurohormone[28] as in the vertebrates eye, remains to be determined. Results concerning the mode of action of melatonin in invertebrates are very scarce, but there are a few data indicating that, as in vertebrates, melatonin could mediate the influence of photoperiod or of the light-dark cycle.

In the planarian, *Dugesia dorotocephala*, an effect of melatonin on asexual reproduction has been reported[21]. Asexual planarians exhibit a distinct day-night rhythm of fission clearly dependent on photoperiod, fission occurring at night. This rhythm is broken by constant light (LL), constant darkness (DD), or decapitation. Fission rate increases after exposure to LL or after decapitation. This indicates that the planarian head may release a substance which inhibits fission. Moreover these authors have shown that fission of decapitated planarians is suppressed by continuous treatment with melatonin in culture water. In decapitates treated with melatonin during daytime, fission occurs only during night; in decapitates treated during night, fission occurs only during daytime. These results are consistent with the hypothesis that melatonin is synthesized and stored in the planarian brain during the dark phase and released during the light phase. They also suggest that melatonin released from the head brain (and/or eyes) mediates the influence of environmental photoperiod on fission.

In insects there is also evidence that melatonin could influence reproduction. Finochiaro et al.[13] reported that female *Drosophila melanogaster* injected with 1 nmole melatonin showed a markedly decreased receptivity and decreased egglaying ability. In the unicellular alga, *Gonyaulax*, Balzer and Hardeland[3] have also shown that melatonin as well as 5-methoxytryptamine provoked cyst formation when given in a non-inducing photoperiod, suggesting that these compounds may play a role in the transduction of photoperiod information in this unicellular species.

A recent paper[1] reports a physiological effect of melatonin in the modulation of rhythmic contractions in a coelenterate, *Renilla koellikeri*, via cyclic guanosine monophosphate (cGMP). The authors show that, in this colonian antozoan, melatonin consistently and reversibly depressed the amplitude of rhythmic contractions, and that melatonin alters the amplitude of rhythmic contractions in the direction opposite to that of 5-HT which potentiates rhythmic contractions in this species. By using analogues of cGMP as well as phosphodiesterase inhibitors these authors show that melatonin depresses peristalsis through the action of intracellular cGMP.

Conclusions

From this brief survey of the literature, it is clear that the presence of melatonin as well as its rhythmic synthesis appears to be a common feature of all invertebrate species and probably of all living organisms. It is now increasingly evident that melatonin is an evolutionary conservative molecule[37] and that, according to Gern's hypothesis[16], a primitive function for melatonin is dark adaptation. During evolution, the daily night pulse of melatonin may have been used by animals in the control of circadian rhythm and/or in the transduction of photoperiod information. Experimental data obtained in primitive species such as *Gonyaulax* or planarians support such a hypothesis. Many questions remain open: what is the precise location of melatonin synthesizing cells? Does melatonin act in the cells where it is synthesized or is it released as in vertebrates? Are there specific receptors or binding sites for melatonin in invertebrate tissues? Is melatonin directly involved in the control of circadian rhythm?

Further investigations, especially comparative studies, are needed to answer those questions and to evaluate more precisely the physiological role of melatonin in invertebrates.

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