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Photoreceptors in the pineal of lower vertebrates: Functional aspects

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Summary. The pineal of lower vertebrates characteristically contains true and modified photoreceptors with functional und structural homologies to retinal photoreceptors. Afferent nerves convey photic information from the pineal to sensory areas of the brain stem. Light also influences synthetic activity within the organ, controlling the rhythm in melatonin production which is generated endogenously. The molecular mechanisms underlying this rhythmic event are described and the hypothesis advanced that the pineal transduces several forms of environmental stimulus involved in the regulation of rhythmic function.
Key words. Circadian rhythm; pineal; melatonin; c-AMP; fish.

Introduction

In vertebrates, as in most living organisms, many functions are rhythmic in their occurrence. Rhythms with a periodicity of 24 h (nyctohemeral) or one year (annual) represent major components of the adaptation of organisms to their environment. Such synchronization to environmental factors is mediated by the circadian system which is composed of sensors and of circadian oscillators, and which drives a number of behavioural and physiological functions.

The pineal organ of vertebrates is a component of the circadian system, primarily influenced by the light/dark (LD) cycle[2,4,5,16]. It elaborates rhythmic signals, amongst which is the hormone melatonin which is considered as an internal 'zeitgeber' of many organisms (see Underwood; Armstrong, this issue). Melatonin secretion is low during daytime and high during night-time. Despite this apparent homogeneity within the vertebrate phylum, the pineal and its chief cells display a distinct evolutionary trend[2,4,5]. Typical photoreceptors are mainly found in the pineal of ectotherms. During the course of evolution, they are gradually replaced by modified photoreceptors (e.g., reptiles, birds) and then by the pinealocytes stricto sensu (snakes, mammals). These stages of differentiation exhibit a corresponding plurality in the mechanisms involved in the photic control of the production of the melatonin and other signals. The general acceptance is that the direct control by light, as seen in the pineal of primitive vertebrates, has been completely replaced in mammals by an indirect one mediated by the retina of the lateral eyes[2,4,5,16]. Most of our knowledge on the mechanisms of control of melatonin production by the pineal refers to studies on the rat (Sugden, this issue) and on the chick. The present review outlines the most essential functional characteristics of the pineal photoreceptor cells as they emerge from recent studies on lower vertebrates, in particular lampreys, fish and frogs.

Functional organization of the pineal organ

The cyto-architecture of the pineal is rather consistent among lower vertebrates[2,13]. Schematically, three principal cell types make up the pineal parenchyma: photoreceptor cells which are synaptically linked to second-order neurons (which constitute bineuronal chains), and glial (interstitial) cells. The neurons convey their axons to brain centers via a pineal tract[4,5,13]. Structurally analogous to the retinal cones, the photoreceptors show a classical polarized and segmented organization consisting of an outer segment, the photoreceptive pole, an inner segment, a cell soma and a synaptic process, the neurotransmitter pole[4,5,13]. Interestingly, the presence of photoreceptors, with a more or less reduced photoreceptive pole, has also been reported in the pike and in lampreys[1,6] possibly signalling the dominant cell type of more developed forms. In the pike the so-called modified photoreceptor does not contact neurons but abbuts the basal lamina, close to pericapillary spaces, an arrangement reminiscent of the mammalian pinealocyte.

Photosensitivity and elaboration of nocturnal nervous signals

In addition to the structural criteria, several other lines of evidence indicate that the pineal photoreceptors transduce photic information into signals. Molecules involved in phototransduction commonly located in the vertebrate retina have also been localized in pineal photoreceptors (opsin, vitamin A, the α subunit of transducin and arrestin[2,3]). More direct evidence has been provided by electrophysiological studies[14]. In ectotherms, spike discharges can be recorded extracellularly from nerve fibers of the pineal tract or from single neurons of the pineal vesicle. Increasing the levels of steady exposure to white light results in a decrease of the impulse frequency of these neurons (e.g., in the frog and in the pike[6,14]). Illumination of the pineal with white or monochromatic light induces several types of responses:

a) Spike discharges of neurons are usually inhibited by all visible wavelengths (achromatic response) or, very rarely, inhibited with short wavelengths and stimulated with longer ones (chromatic response).

b) Photoreceptors hyperpolarize (lampreys[15]; pike and trout: personal observations). In the frog and in the pike, photoreceptors generate early receptor potentials, which result from charge displacements at the level of the photopigment, as well as slow potentials (= electropinealogram[6,7,14]). In the pike pineal, slow potentials but not spike potentials can be recorded from isolated medial regions, where the modified photoreceptors predominate and neurons are extremely scarce, as well as from the distal and proximal parts of the organ, which both contain bineuronal chains[6]. In some species, the spectral sensitivity of the response shows striking similarities to the spectral sensitivity of their lateral eyes (for details see[6,7,14]). Thus, the pineal of lower vertebrates responds directly to light via its photoreceptors. It acts as a dosimeter of solar radiation and as an indicator of daylength[6,14]. The typical photoreceptors elaborate, mainly in darkness, a nervous message transmitted to the second-order neurons via an excitatory neurotransmitter, the effects of which can be mimicked by L-aspartate and L-glutamate[4]. In this sense the pineal organ of lower vertebrates contrasts significantly with that of mammals. However, the production of the hormonal signal, melatonin, displays considerable conservatism.

Indole metabolism and melatonin synthesis by pineal photoreceptor cells

From studies in endotherms, it is known that pineal serotonin is synthetized from tryptophan in two enzymatic steps; hydroxylation followed by a decarboxylation (Sugden, this issue). Monoamine oxydase (MAO) converts serotonin to a series of deaminated products. Alternatively, acetylation of serotonin by a specific N-acetyltransferase (NAT) opens the melatonin synthesis pathway, which is completed by the action of the hydroxyindole-O-methyltransferase (HIOMT). HIOMT may also methylate serotonin and its deaminated products to give 5-methoxyindoles[16].

A number of studies have shown the involvement of the fish pineal in indole metabolism[10,11,13]. The use of combined (immuno)cytochemical, radiobiochemical and radioautographic techniques support the proposal that the photoreceptor cells of the pike and the goldfish contain and metabolize melatonin and other indoles[8].

Rhythmic melatonin production by pineal photoreceptors

In lampreys and fish the pineal content of several indole-like compounds, including serotonin, N-acetylserotonin and melatonin, detected immunocytochemically or radioimmunologically, displays fluctuations over the LD cycle with a nocturnal peak[8,11]. Cultured pineals of trout[12] and of pike[10] release low amounts of melatonin during daytime, and high amounts during night-time. Whether HIOMT is responsible for the daily variations of melatonin production, has long been a matter of discussion[11,13]. This does not seem to be the case at least in fish. In the pike, the rhythmic melatonin production reflects – in vivo and in vitro – daily changes of NAT activity, synchronized to the LD cycle, with light exerting inhibitory effects on both NAT activity and melatonin release (fig. 1)[8,10]. However, cyclical changes in NAT activity and melatonin release are more than a passive response to ambient illumination. Pineals of pike cultured in constant darkness maintain a high amplitude rhythm of NAT activity and of melatonin release for at least three days (fig. 2). A low amplitude rhythm of NAT activity may also be observed in constant light, both in

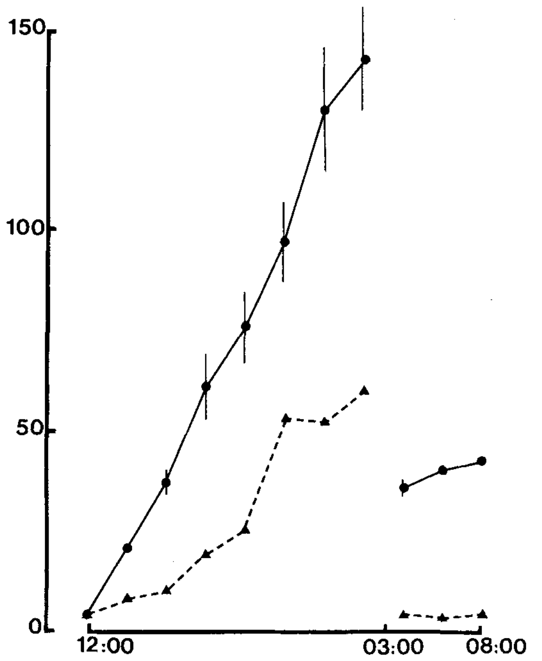


Figure 1. Melatonin production (ng/ml) by superfused pike pineals (22 °C) in the absence (– – – – –), or presence (———) of forskolin ($3 \cdot 10^{-5}$ mole/l). Darkness, given at 12.00 (midphotophase) does not induce an immediate rise in melatonin production in the absence of forskolin, whereas a linear increase is observed in its presence. In both cases, light given at midscotophase (03.00) exerts an inhibitory effect, which is more pronounced in the presence of forskolin (in collaboration with C. Thibault, unpublished).

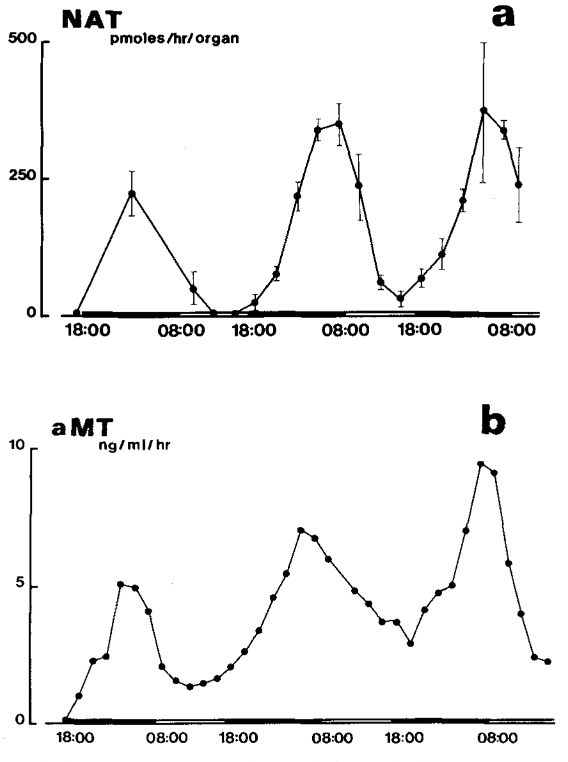


Figure 2. In the pike, free-ranging variations *a* in NAT activity (static organ culture) and *b* in melatonin release (one superfused pineal), are maintained for at least three 24-h cycles under continuous darkness. The shaded bar corresponds to the subjective scotophase (from Falcón et al.[10]).

vivo and in culture[8, 10]. Unexpected darkness at midday does not induce an immediate increase in melatonin production, indicating the presence of a refractory period during the subjective day (fig. 1)[10]. In contrast to the pineal of the pike, trout pineals release continuously high or continuously low amounts of melatonin when they are cultured respectively in constant dark or in constant light[12]. These results indicate that the pike pineal, but not the trout one, contains a circadian oscillator driving the rhythm of NAT activity, which is synchronized by the LD cycle. An intrapineal circadian oscillator has also been localized in two, out of three, lizards investigated and in the chick (see Underwood, this issue). It is remarkable that in the chick very small pineal fractions or cells, as well as fractions of pike pineals containing either bineuronal chains or modified photoreceptors, still release melatonin in constant dark for at least three days (Falcon, unpublished). It is therefore hypothesized that cells of the photoreceptor type might represent, at least in some species, miniaturized circadian systems. In addition to being the photoreceptive unit important for entrainment and the biosynthetic machinery for the production of indoles, the hands of the clock, they could also contain a circadian oscillator, the clock itself[4, 5]. As such, they offer enormous potential for studies of the cellular biology of circadian function in vertebrates.

Molecular mechanisms involved in the control of melatonin production

In this field, research is just at its beginnings. This and the following sections will refer to preliminary data that we have obtained in the pineal of the trout and of the pike. In these species, non-hydrolysable analogues of cyclic AMP (c-AMP), or agents which are known to increase intracellular levels of this second-messenger (either by stimulating adenylcyclase or by inhibiting the c-AMP phosphodiesterase) lead to an increase in NAT activity and/or melatonin release in pineals cultured in the dark (figs 1, 4). Under these conditions, the light-mediated inhibition of NAT activity and melatonin release are attenuated. Increases in c-AMP accumulation and NAT activity have been measured as a function of temperature, in the presence of the adenylate cyclase stimulator forskolin, in cultured pike and trout pineals. In both species, the curves have similar profiles (fig. 3). A maximal rate of synthesis occurs at around 15 °C. As temperature increases or decreases from this level, the rate of enzymatic activation declines rapidly. Such responses may lead to changes in amplitude of the melatonin signal, but it remains to be determined whether temperature pulses may also exert phase or period control over the pineal oscillator. It is tempting to suggest that these effects of temperature may represent the physiological basis to the entraining effects of temperature cycles in lizards and other species (Underwood, this issue). Altogether, these results strongly suggest that c-AMP is one

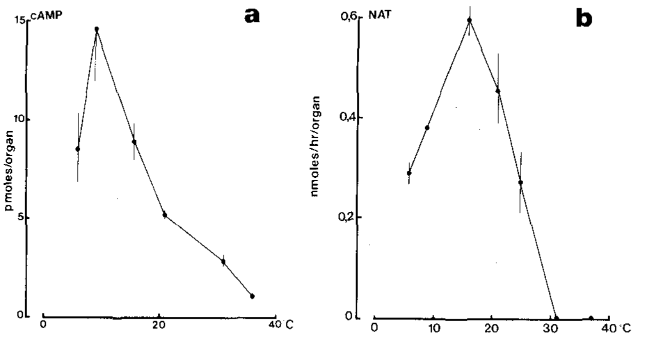


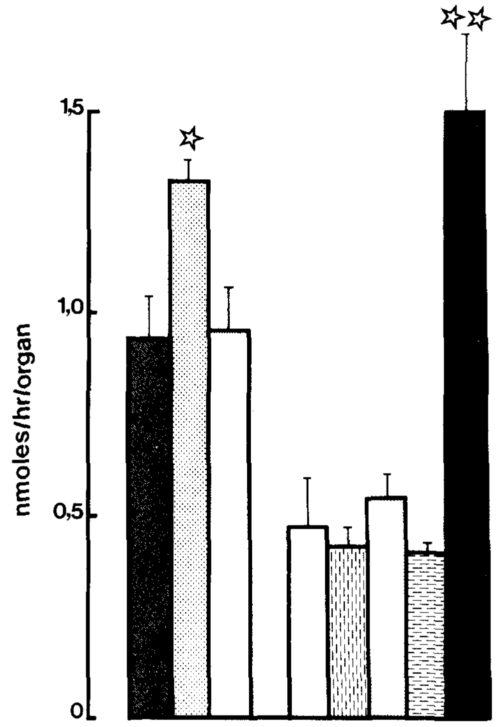
Figure 3. In trout pineals, c-AMP accumulation *a* and NAT activity *b* measured after 6 h of culture in darkness, and in the presence of forskolin (10^{-5} mole/l), are dependent upon temperature; both curves have similar profiles (in collaboration with C. Thibault, unpublished).

of the second messengers involved in the control of melatonin production by pineal photoreceptor cells, and that temperature affects directly this production. It remains unclear as to whether the inhibitory effects of light might involve a photoactive c-AMP phosphodiesterase.

Do photoreceptors transduce inputs other than the photoperiodic one?

From cytochemical[9] and pharmacological studies (in preparation), we believe that adenosine produced by the pike and trout pineals modulates c-AMP levels and NAT activity. Removing extracellular adenosine with adenosine deaminase (ADA) resulted in an increase in NAT activity in trout pineals (fig. 4). NAT activity was also elevated in pike pineals in the presence of a blocker to the plasmalemmal adenosine carrier system. Finally, adenosine analogues used in the nanomolar range lowered c-AMP levels and NAT activity of cultured pike and trout pineals. The opposite was the case at higher (μmolar) concentrations (figs 4, 5). Stimulatory effects probably involved cell surface, low affinity α_2 receptors positively linked to adenylate cyclase. Inhibitory effects may have been mediated by high affinity α_1 receptors, negatively coupled to adenylate cyclase (in preparation). Adenosine is a catabolite of adenylic nucleotides (including c-AMP) and of S-adenosylmethionine the co-factor of HIOMT. It is therefore tempting to suggest that adenosine, co-released with melatonin, might act locally in a feed-back mechanism to regulate melatonin production.

The nocturnal rise in NAT activity and melatonin production of cultured pike pineals can be inhibited, in a dose-dependent manner, by the α_1 agonists cirazoline and phenylephrine. Furthermore, membrane preparations of pike pineal organs saturably bind the α_1 antagonist (125 I)-heat. This binding could be displaced by α_1, but not by α_2 analogues (Falcon and Olcese, unpublished). Taken together, these results suggest that the pineal of some fish might be under control of catecholamines of still unknown origin.



Forskolin	10^{-5} mole/l	+	+	+	−	−	−	−	−
ADA	10 U/ml	−	+	−	−	+	−	+	−
NBTI	10^{-4} mole/l	−	−	+	−	−	+	−	+
CLAD	$5 \cdot 10^{-5}$ mole/l	−	−	−	−	−	−	+	+

Figure 4. Effects of adenosine deaminase (ADA), nitrobenzylthioinosine (NBTI: blocks the adenosine transmembrane carrier), and of chloroadenosine (CLAD: adenosine analogue), on NAT activity of trout pineals cultured for 6 h in darkness, and in the presence (left), or the absence (right), of forskolin. NAT activity was twice higher in the pineals treated with forskolin (ADA potentiated the effect of forskolin), suggesting that under these conditions endogenous adenosine inhibited melatonin production. NBTI and CLAD had no effect when used alone but stimulated NAT activity when used in combination. This suggests that CLAD, an adenosine α_2 agonist, was acting through cell surface receptors to stimulate NAT activity only when the nucleoside permeant was blocked. ($n = 5 \pm$ SEM; Student's t-test: ☆, $p < 0.02$ and ☆☆, $p < 0.005$; in collaboration with C. Thibault, unpublished).

Summary and concluding remarks

In the pineal organ of lower vertebrates, typical and, when present, modified photoreceptors, are influenced primarily by the duration, intensity and spectral composition of lighting during the 24-h cycle. Both elaborate and release melatonin during the dark phase. Typical photoreceptors appear as multimessenger cells because they also release an excitatory neurotransmitter mainly during darkness, which affects the activity of second-order neurons, and thereby influences brain centers. The interplay between neural and humoral regulation of circadian function may have parallels with other vertebrate classes, including mammals (see Underwood; Armstrong; Morgan and Williams, this issue). Our preliminary data obtained on the fish pineal also suggest that photoreceptors might be multi-effector cells which receive information other than that provided by the daily alternation of light and dark. These sources of informa-

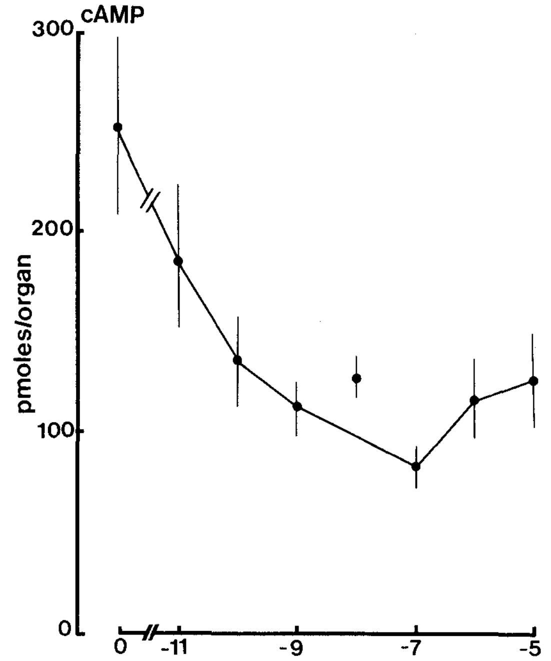


Figure 5. Dose-dependent effect of cyclohexyladenosine (CHA: adenosine analogue) on c-AMP accumulation in trout pineals cultured for 3 h in darkness, in the presence of forskolin (10^{-5} M), adenosine deaminase and nitrobenzylthioinosine. The α_1 adenosine agonist CHA inhibited c-AMP accumulation in a dose-dependent manner when extracellular adenosine was removed and when the nucleoside carrier was blocked. This suggests that CHA acted through cell surface receptors negatively coupled to adenylate cyclase. ($n = 5 \pm$ SEM; in collaboration with C. Thibault, unpublished).

tion concern external (temperature) as well as internal (chemical) factors. The exact nature, origin and modes of action of the chemical messengers remain to be determined. Finally, the presence of intrapineal circadian oscillators driving the melatonin production in the pike, and its absence in the trout, raises a number of interesting questions concerning the cellular localization of the oscillators and the mechanisms of their entrainment by external as well as internal factors. This variability within taxa is echoed throughout the phylogenetic scale and illustrates the pronounced flexibility and lability of the pineal complex. The pineal photoreceptor cells of lower vertebrates share a number of analogies with the retinal ones and undoubtedly pineal photoreceptors represent an interesting model for the study of the photoneuroendocrine properties of photoreceptor cells in general. A better knowledge of their properties might also be very helpful in understanding better the nature of the functional evolution undergone by their phylogenetical derivatives in higher vertebrates. Such complete understanding awaits a more extensive appreciation of the role of light and other periodic variables in the ecology of individual species.

Acknowledgments. The authors received financial support from the University of Poitiers, the CNRS, INSERM (grant No. 876007) and the 'Fondation Langlois', Rennes, France. We wish to express our deep thanks to C. Thibault, M. Hastings and J. L. Blazguez for their valuable help.

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