Pineal Transducers in the Course of Evolution: Molecular Organization, Rhythmic Metabolic Activity and Role*

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Summary. Data on the cell biology of pineal transducers (chief cells: typical and modified photoreceptors, pinealocytes) which belong to the paraneuron family, are reviewed in the vertebrate series. In spite of major changes throughout phylogeny, it is proposed that pineal chief cells share a common feature: they somehow transform the information derived from the light/dark cycle into daily rhythms of neural (an excitatory neurotransmitter) and/or hormonal (melatonergic) output and appear invariably involved in the temporal organization of physiological and behavioral processes.

Pineal transducers—mammalian pinealocytes and their forerunners, the submammalian photoreceptor cells—correspond to pineal chief cells. They belong to the paraneuron family (FUJITA et al., 1988).

Recent investigations in physiology and cell biology allow to propose that, in spite of an evident diversity in their fine structure, pineal transducers share common features in molecular organization and function. To illustrate this viewpoint, the photosensitivity, the production of signals, the circadian rhythmicity of metabolic activity and the plurality of translation mechanisms of information derived from light/dark (L/D) cycles into signals will be successively examined in pineal transducers, throughout vertebrate phylogeny. Several pineal functions and their recurrence in the course of evolution allow us to propose that pineal transducers are involved in phasing temporal events.

DIRECT AND/OR INDIRECT PHOTOSENSITIVITY

In ectotherms, the bineuronal chains—which consist of typical photoreceptor cells synaptically connected to second-order neurons projecting into the brain—are gradually replaced by modified (or rudimentary) photoreceptors mainly in sauropsids, and then by pinealocytes (mainly in snakes and mammals) (COLLIN, 1969; COLLIN and OKSCHE, 1981; COLLIN et al., 1986a; OKSCHE et al., 1987). These cells have been schematized in Figures 1–3. In a few species, it has been shown that photoreceptors and sparse pinealocytes may project their processes directly into the brain (KORF and EKSTROM, 1987). Pineal transducers clearly share structural homologies and utilize common molecules as their signal substances. It is apparent that several types of photoreceptors and pinealocytes exist, though the modalities in the evolution of pineal transducers remain to be clarified in detail (COLLIN, 1985).

Electron microscopic, electrophysiological, microspectrophotometric and immunocytochemical studies have indicated that typical photoreceptors respond to the L/D information. Their outer segment, endowed with a variable number of saccules (or disks), contains the membrane receptor protein opsin and the vitamin A chromophore of photopigments as well as cytosolic proteins (α-transducin and arrestin) also involved in phototransduction, as shown in retinal photoreceptors (for reference see COLLIN et al., 1986a; KORF and EKSTRÖM, 1987; VIGH-TEICHMANN

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et al., 1987). Photoreceptor responses depend on intensity, spectral composition and the action duration of light (MEISSL, 1986). Indeed, bineuronal chains operate over a wide range of light intensities, thereby sending to the brain messages related to the ambient light level. Two types of responses (achromatic and chromatic) have been recorded on second-order neurons (MEISSL, 1986; MORITA et al., 1987). However, the achromatic (lumiance) responses are the more common: with light stimuli of all wavelengths and ultraviolet spectrum, the responses are inhibitory. The spectral sensitivity distribution of the luminance neurons varies slightly among different species, with peak wavelengths between 500-540 nm, sometimes maxima at 560-580 nm in frogs or 620 nm in pikes (see MEISSL, 1986).

Pineal photoreceptors depolarize under darkness and hyperpolarize under light stimulation (MEISSL, 1986; MORITA et al., 1987). Depolarization induces the release of an excitatory neurotransmitter: in the frog at least, aspartate and glutamate mimic the action of the transmitter and depolarize second-order neurons (MEISSL in COLLIN et al., 1986b). (Fig. 1b). Hyperpolarization reduces the release of the transmitter (MEISSL, 1986; MORITA et al., 1987) (Fig. 1b). Photoreceptor cells appear as luminance detectors and function as indicators of daylength and dosimeters of solar radiation (MEISSL, 1986; see also MORITA, this volume).

Projections of afferent neurons in the brain have been investigated in only a few species, and the role of their messages is still poorly understood. Projections to the mesencephalic tegmentum may serve in the control of behavioral thermoregulation, and of phototactic reactions; those to the hypothalamus might be involved in circadian and neuroendocrine functions (ref. in COLLIN et al., 1986a).

Modified photoreceptors are directly and indirectly sensitive to L/D information. In the course of vertebrate phylogeny, the outer segment becomes atypical (either disks are absent or very much reduced in number; membrane-whorls may also be found) (COLLIN and OKSCHE, 1981). However, opsin-, vitamin A-, \( \alpha \)-transducin- and arrestin-like molecules can be found in the outer segments and other cell compartments (COLLIN et al., 1986a; KORF and EKSTRÖM, 1987 and ref.). Although intracellular recordings are still absent, direct photosensitivity has been recognized from in vitro experiments indicating that direct illumination suppresses the nighttime increase of serotonin-N-acetyltransferase activity, an enzyme (see later) involved in the synthesis of melatonin (DEGUCHI, 1981). Furthermore, the action spectrum of the photosensitivity resembles the absorption spectrum of rhodopsin, and light induces stereoisomeric changes in vitamin A (DEGUCHI, 1981; KORF and EKSTRÖM, 1987). Besides this direct photosensitivity, modified photoreceptor cells are also influenced by environmental lighting via retinal photoreceptors connected to neural pathways projecting into the pineal (see later).

Pinealocytes are indirectly sensitive to L/D information. In adult mammals, the outer segment is absent or reduced to a simple, sometimes bulbous, cilium. The functional significance of the opsin immunoreactivity found in a few pinealocytes or, in contrast, the arrestin immunocytochemical labeling of numerous pinealocytes in various species, is still enigmatic. However, L/D information indirectly influences the metabolic activity of pinealocytes as indicated by studies on melatonin production under different lighting conditions; here a complex neural pathway starting at the retina and ending in the pineal is involved (see later).

According to the types of pineal transducers, the L/D information is thus differently perceived throughout phylogeny. Before analyzing the mechanisms of this major change, as well as its consequences, data on the metabolic activity of transducers shall be presented.

**KNOWN AND HYPOTHESIZED SIGNALS**

Depending on the lighting conditions, an excitatory neurotransmitter may be released (see above), probably from synapses of the ribbon type, connecting typical photoreceptors to second-order neurons (Fig. 1b). Because this neurotransmitter is utilized by typical photoreceptors of lampreys and apparently by other ectotherms (MEISSL in COLLIN et al., 1986a; MORITA et al., 1987), it may be considered an evolutionarily ancient signal. It is unknown whether this transmitter is or is not yet present and released for other functions in modified photoreceptors and pinealocytes which replace typical photoreceptors (mainly from reptiles) and become independent of second-order neurons. In any case, neural messages conveyed to the brain via the second-order neurons, gradually disappear in amniota. This represents another major change which precedes the loss of direct photosensitivity.

During the last two decades, a long series of papers have substantiated the assertion that pineal transducers are responsible for indole metabolism (COLLIN, 1969–1985; COLLIN and OKSCHE, 1981; COLLIN et al., 1986a, b). From biochemical investigations car-
ried out mainly in endotherms, it has been shown that in the pineal, serotonin (5-HT) is synthesized from tryptophan (Try). Acetylation of 5-HT by a specific N-acetyltransferase (NAT) to give N-acetyl-serotonin (N-ac-5-HT) opens the melatonin synthesis pathway which is completed by the action of hydroxyindole-O-methyltransferase (HIOMT). Alternatively, monoamine oxidase (MAO) converts 5-HT into a series of deaminated products. HIOMT can also act on 5-hydroxytryptophan (5-HTP), an intermediary product between tryptophan and 5-HT, and on 5-HT and its deaminated products to give a series of 5-methoxyindoles. All enzymes of indole metabolism seem to be active in the cytosol except the MAO, present mainly in the outer membrane of mitochondria (COLLIN, 1979; COLLIN et al., 1986b).

In a few representative vertebrates, in vivo and in vitro radioautographic and chromatographic experiments have shown that 3H-Try, 3H-5-hydroxytryptophan and 3H-5-HT are selectively metabolized in fish photoreceptors and modified photoreceptors of the pike and of some lizards and birds. Irrespective of whether the experiments were performed either throughout the year or during the daily L/D cycle (unpublished data of JUILLARD et al., in the parakeet), no qualitative change in the labeling could be observed. In addition, cytochemical and immunocytochemical studies have shown the localization of: 1) 5-HT (or 5HT-like indole) to be in typical and modified photoreceptors and pinealocytes of several representative vertebrates; and 2) N-ac-5-HT-like indole to be in typical (pike) and modified (pike, parakeet) photoreceptors. Although a melatonin-like compound was found in pineal cells of many species, the immunoreactive cells could not be determined, except in lamprey and pike photoreceptors and in modified pike photoreceptors. The recent production of antibodies against HIOMT (the last enzyme in the pathway of melatonin synthesis) has provided direct evidence that avian modified photoreceptors and mammalian pinealocytes are involved in the synthesis of melatonin and probably the other 5-methoxyindoles (KUWANO et al., 1983; WIECHMANN et al., 1985; GUERLOTTE et al., 1988; VOISIN et al., 1988). As far as MAO activity is concerned, it was found in pineal transducers, glial (supportive) cells and, when present, in sympathetic efferents (COLLIN, 1979).

Comparative studies have suggested that most cells (if not all?) of a given category of transducers are involved in indole metabolism. Data obtained at the organ level referring to the presence of 1) NAT activity (in pike: FALCÓN et al., 1987, 1989), 2) HIOMT activity (lamprey and fish: COLLIN et al., 1986b; FALCÓN et al., 1987) and 3) radioimmunoassayable melatonin (lampreys and fish pineals: FALCÓN et al., 1987, 1989; GERN, personal communication), strengthen the opinion that melatonin and possibly the other 5-methoxyindoles are ancient signals, more precisely neurohormones which maintained in pineal transducers throughout phylogeny.

The production of secretory granules by the Golgi apparatus and their proteinaceous content (which has been shown in amniotes) also constitutes a common property of pineal transducers, from lampreys up to mammals (COLLIN, 1979, 1981). These dense-cored vesicles are mainly found in the transducer's processes, oriented towards the perivascular spaces. In amniotes at least, both indoles and proteinaceous secretion(s) coexist in the same transducer (5-HT may be also taken up by secretory granules). It has been suggested that the secretory granules might convey an active neuropeptide or neuropeptides (COLLIN, 1981).

**RHYTHMIC (CIRCADIAN) METABOLIC ACTIVITY**

Cellular compartments and organelles (examined in fish photoreceptors and mammalian pinealocytes) and molecules (investigated in fish and mainly in pineals of endotherms) display significant quantitative changes during the 24 h L/D cycle (ref. in COLLIN et al., 1987). For example, the volumes of the cytoplasm, of the nucleus (and nucleolus), of the endoplasmic reticulum and of dictyosomes, as well as the number of secretory granules and the number and length of synaptic ribbons, all display daily variations. The contents in RNA, nucleotidic second messengers, lysosomal enzymes, rhodopsin kinase, serotonin, N-acetyl-serotonin, melatonin, 5-hydroxyindole acetic acid, as well as NAT activity and β-adrenergic receptors density, also vary markedly during the 24 h cycle (COLLIN et al., 1987; FALCÓN et al., 1987, 1989; BINKLEY, 1988). All these daily oscillations display peak rates either during daytime or during nighttime. Although a majority of the above-mentioned molecules remain to be localized in the pineal tissue, and precise correlations are needed between the different types of fluctuations, the data as a whole tend to reflect variations in the general metabolic activity of the pineal transducers.

The rhythms of NAT activity and/or melatonin content measured in representatives of vertebrate classes often reveal the following common features:
Figs. 1-3. Diagrams representing the pineal of the pike (1a) chiken (2a) and rat (3a), as well as the direct and/or indirect pathways by which environmental lighting (hv) influences these organs. RHP: retinohypothalamic projection. (see text for further information). Pike typical photoreceptors connected to second order neurons (1b), chicken modified photoreceptors (2b) and rat pinealocytes (3b) are shown under light and dark conditions. Schematic daily time profiles are represented for N-acetyltransferase activity (NAT) and melatonin (MEL) contents. Daily variations of the release of the excitatory neurotransmitter (EN) and noradrenaline (NA), respectively from photoreceptors and sympathetic endings, are also shown. For each species, the mechanisms of photoregulation of melatonin synthesis are discussed in the text. Note that the intracellular localization of a circadian oscillator (OSC) is purely hypothetical. AC adenylate cyclase, cAMP cyclic adenosine 3': 5'-monophosphate, ATP adenosine triphosphate, BL basal laminae, CI cilium, CP cellular process, CS cellular soma, Gs G protein, IS inner segment, mRNA messenger ribonucleic acid, N nucleus, OS outer segment, PIP proteins involved in phototransduction, PKC protein kinase C, RS ribbon synapse, SP synaptic process, T transcription.
both increase considerably during nighttime, irrespective of the nocturnal or diurnal behaviour of animals and of their habitat. The duration of NAT activity and melatonin production, as well as their nocturnal amplitude, depends on the length of the darkphase (ref. in BINKLEY, 1988; COLLIN et al., 1987). Furthermore, in the pike and endothermic species, the rhythmicity of melatonin production depends on that of NAT but not on HIOMT activity. In the trout, as well as in endotherms, it has been proved that melatonin production depends, as does the release of the excitatory neurotransmitter, on the intensity, spectral composition and duration of light (for reference see COLLIN et al., 1987, 1988; REITER, 1985).

Furthermore, it has been shown that the rhythmicity of some of the parameters studied persists under constant conditions. For example, the daily oscillations of pineal NAT activity and melatonin content, or those of melatonin released in the blood or in culture media, persist in constant darkness in the pike, lizards (Anolis), chicks and rats (COLLIN et al., 1987; BINKLEY, 1988; FALCÓN et al., 1989). These fluctuations, are endogenous, truly circadian (a period close to 24 h under constant conditions), i.e., generated by circadian clocks (or oscillators). The clocks are either intra- or extrapineal (depending on species studied) and are entrained (synchronized) by environmental cues. Although the L/D information appears to be a powerful synchronizer, the influence of other environmental cues (e.g., temperature in ectotherms) must be taken into consideration (for reference see COLLIN et al., 1987; BINKLEY, 1988). The characteristics of the rhythms (amplitude, position and duration of the peak, period length) differ when observed under natural or constant conditions.

The circadian oscillator or oscillators involved in the photoregulation of melatonin biosynthesis in the pike, chick and rat, provide a timed program. Indeed, there exists a refractory period, approximately coinciding with the lightphase, during which darkness does not initiate a rise in melatonin. However, light imposed unexpectedly at any time during the darkphase causes a rapid drop in melatonin production (BINKLEY, 1988 and COLLIN et al., 1987 for reference; FALCÓN et al., 1987, 1989). By contrast — in the absence of intra- and extrapineal oscillator in the trout — light or darkness provided unexpectedly during the 24 h cycle causes an inhibition or an induction of melatonin secretion, respectively (GERN in COLLIN et al., 1988 and in preparation).
SPECTRUM OF MECHANISMS TO CONVERT THE L/D INFORMATION INTO A RHYTHMIC MELATONINERGIC SIGNAL

In the course of vertebrate evolution, the pineal transducers have gradually changed their mode of decoding the L/D information but have maintained the daily rhythm of melatonin secretion.

Pike and trout:
The way the L/D information controls melatonin synthesis is achieved directly in the pineals of both fish (Fig. 1a). Through the molecules involved in phototransduction, light hyperpolarizes photoreceptors and inhibits the release of the excitatory neurotransmitter as well as melatonin production (Fig. 1a). An intrapineal oscillator is apparently either involved (pike) or not (trout) in the inhibition of melatonin synthesis. In Figure 1b, it is hypothesized that many intrapineal oscillators exist (one oscillator in a photoreceptor; see below for the chick). In darkness, the photoreceptors are depolarized, the excitatory neurotransmitter is released and the synthesis of melatonin is induced either via a circadian oscillator (pike) or not (trout). The molecular mechanisms involved in the inhibition and induction of melatonin synthesis are under investigation.

Chick:
The rhythm of melatonin synthesis is driven both by an extrapineal and an intrapineal oscillator entrained by L/D information, which in these conditions is perceived directly and indirectly by the pineal. As in fish, the intrapineal circadian timing system is synchronized by light crossing the braincase (Fig. 2a). Here light energy is decoded by the photosensitive pole of the modified photoreceptors (Fig. 2b). It has been put forward that the extrapineal oscillator might be localized in the suprachiasmatic nuclei and entrained by L/D information via the retina and the retino-hypothalamic pathway. As in the chick, noradrenaline released from pineal sympathetic endings is the final signal of the extrapineal circadian clock. In contrast to the chick, however, noradrenaline is here released at night (Fig. 2b), and acts on the 2α- and β1-adrenoceptors of pinealocytes: the 2α-receptors considerably amplify the effects of the β1-adrenergic stimulation at the cyclic AMP levels, resulting in a large increase in the production of this second messenger. Melatonin synthesis is regulated by cyclic AMP which causes a large increase in NAT activity. Cyclic AMP has two probable types of effects on the NAT activity: 1) on transcription, by inducing the synthesis of a protein (either NAT or an activating enzyme recycling existing NAT molecules); and 2) on stabilization of NAT and preventing its inactivation (Fig. 3b). The regulation of melatonin synthesis appears relatively similar in rats and humans (for reference see BINKLEY, 1988; COLLIN et al., 1988). However, to inhibit the nocturnal melatonin synthesis, a considerably higher light intensity is necessary in humans, compared with other mammalian species (ARENDT, 1985; REITER, 1985). This fact suggests that "man's pineal function has evolved in such a way as to resist the social variations of light and dark that we impose upon ourselves" (ARENDT, 1985).
From the few currently investigated species, our knowledge of the mechanisms involved in the translation of L/D information into a melatonnergic output appears very incomplete. The complexity of these mechanisms is also increased by the influence of internal factors (not discussed herein). A comprehensive view of melatonin photoregulatory mechanisms depends also on our knowledge gained from other species, taking into account the various ways they have adapted to their environment. In spite of these various information gaps, however, it can be proposed that phototransduction (in submammalian vertebrates) is gradually relayed by chemo- (adreno-) transduction (in amniotes). Consequently, the circadian oscillations of melatonin (and the time-keeping functions of this molecule: see below) have been saved in the course of evolution (Figs. 1b, 2b, 3b). A general property of pineal transducers emerges: they somehow translate the L/D information into rhythmic neural and/or melatonnergic outputs. As we shall now see, pineal transducers are invariably involved in the temporal organization of physiological and behavioral processes.

PINEAL FUNCTIONS RECURRING THROUGHOUT PHYLOGENY

Immediately after its synthesis, melatonin is released mainly into the bloodstream. However, besides the neurohormonal actions, pineal melatonin may presumably have autocrine and paracrine roles.

In most latitudes, the ratio of light to dark in the environment fluctuates with the seasons of the year. Thus, the nocturnal melatonin pulse of the proper shape, amplitude and duration has an essential role in time-keeping (for reference see RALPH, 1983; BINKLEY, 1988; COLLIN et al., 1988). Therefore, pineal transducers appear — via their melatoninergic output — to be involved in aligning physiological and behavioral processes with a changing environment. Because of the extreme regularity of melatonin secretion, the pineal is associated with daily and seasonal rhythms. Although still incomplete, current physiological data obtained mainly from pinealectomized vertebrates and from melatonin administration, tend to indicate a continuity in the pineal control of physiological and behavioral rhythms. Several examples illustrate this (for reference see COLLIN et al., 1988).

Ectotherms display daily rhythms of skin colour changes: the dorsal skin takes on a night pallor. In a few ectotherms, the involvement of the pineal (or melatonin) on the rhythmic changes in the distribution of melanosomes within melanophores (responsible for the color changes) has been shown.

The pineal is involved in the daily rhythms of locomotor activity. In some species of vertebrates, this organ appears as a circadian oscillator which somehow regulates other brain centers controlling locomotor activity. In other species, the pineal may be one of several independent oscillators, perhaps with melatonin acting as a hormone that synchronizes the phases of two or more independent oscillators with that of the pineal. In some birds, melatonin can entrain free running rhythms or cause phase-shifting. In lizards, it also modifies the circadian activity. Pharmacological doses of melatonin can change the direction of the re-entrainment of locomotor activity in rats after phaseshifts, and entrain free-running activity-rest cycles.

The pineal complex (pineal plus supernumerary organs in some ectotherms) is involved in the circadian rhythmicity of body temperature, as observed in amphibians, reptiles, birds and possibly neonatal mammals. In some mammals pinealectomy reduces their ability to maintain torpor (the pineal is involved in the initiation, continuation and arousal from daily torpor events). In addition, the pineal is involved in hibernation as has been evidenced in ground squirrels.

Although data are still incomplete in submammals, the vertebrate pineal appears to participate in mediating photoperiodic effects on seasonal reproductive processes. Its main role is in cueing reproductive events, so they occur during the correct season. For example, in long-term experiments, pinealectomized hamsters and sheep become aphotoperiodic: the reproduction cycle is not synchronized with the annual environmental cycle, and the birth of young cannot be achieved under the most propitious conditions. When suitably administered, melatonin is able to induce the reproductive responses, appropriate to a given daylength, in pinealectomized and intact long-day breeders (hamster) and short-day breeders (sheep). Melatonin is currently used to optimize seasonal functions in seasonal breeders.

The role of the human pineal is still obscure. Although alterations of the melatonin rhythm are associated with several pathological states (psychiatric diseases, retinal dysfunction, pineal tumors, endocrinopathies, hormone-dependent tumours, insomnia, etc.), current research is largely in the phenomenological stage (ARENDT, 1985). According to ARENDT (1985), “if melatonin is able to enhance synchronization of circadian rhythms or speed up resynchroniza-
tion, following a disruptive event, it has considerable potential use in clinical situations where disturbed rhythmic function is present, if such disturbance is important to well-being" (ARENDT, 1985). Jet-lag, shift work, certain types of insomnia, cecity, and affective diseases represent situations where abnormal phase-relationships of the circadian system are present. After melatonin administration, hypnotic and sedative effects as well as an adaptation to rapid time-zone change could be recognized (ARENDT, 1985; COLLIN et al., 1988). In two thirds of the travellers, melatonin alleviated jet-lag after eastward and westward flights (ARENDT, cited in COLLIN et al., 1988). Phototherapy is a successful treatment for seasonal affective disorder, especially winter depression (ref. in ARENDT, 1985).

CONCLUSIONS

Pineal chief cells convert information derived from L/D cycles into daily rhythms of neural and/or hormonal outputs. In the course of evolution, the major changes concern the mode of decoding this information (phototransduction → adrenotransduction) and the gradual disappearance (in amniotes) of the nervous messages conveyed, via the second-order neurons, to the brain. An excitatory neurotransmitter (neural output), an indolergic hormonal output (e.g. melatonin) and a still enigmatic proteinaceous secretion have developed very early in the course of evolution and may coexist in photoreceptors. In modified photoreceptors and pinealocytes, both the melatoninergic output and a proteinaceous secretion have been maintained and coexist in the same cell. Further data are needed before concluding that all pineal transducers are multimessenger cells. In order to ensure the circadian metabolic activity and particularly the nocturnal production of melatonin, vertebrates have developed a variety of strategies.

As a whole, data on pineal cell biology and physiology throughout phylogeny allow us to propose that pineal transducers contribute to the shaping of daily rhythms (e.g., skin color change, locomotor activity, body temperature) and to the cueing of annual events (e.g., reproduction, thermal acclimatization etc.). Finally, these important components of the circadian system play a key role in the optimization of the relationships between the organisms and their environment.

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