MELATONIN—THE HORMONE OF DARKNESS

O. Vakkuri
Department of Physiology, University of Oulu, Finland.

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Contents

Summary

Melatonin, the pineal hormone of darkness, was originally found and chemically characterized to N-acetyl-5-methoxytryptamine in bovine pineal extracts in the late 1950s. Since then melatonin has been studied more and more intensively and not only in humans and several animal species but lately also in plants. After its first-described biological effect, i.e. skin-lightening effect in lower vertebrates, melatonin was shortly known as a rhythm marker due to its circadian biosynthesis and secretion pattern in the pineal gland: melatonin is synthesized and secreted during the night, i.e. the dark period of the day. This circadian rhythm is endogenously regulated by the biological clock in the suprachiasmatic nuclei of the hypothalamus. Environmental light has a clear inhibiting effect on melatonin biosynthesis, continuously entraining the melatonin rhythm so that endogenous and exogenous rhythms are maintained in the same phase. The entraining light information is transmitting via the eyes and the retinohypothalamic tract to the suprachiasmatic nuclei and then via the paraventricular nuclei to superior cervical ganglia from which along the sympathetic tract finally to the pineal gland. According to recent studies the stimulatory effect of light on the suprachiasmatic nuclei is GABAergically reversed in these nuclei so that melatonin synthesis and secretion is every day activated during evening-night transition. Melatonin is secreted into circulation in which the circadian rhythm, high nocturnal and low daytime concentrations, can be continuously monitored. It is metabolized mainly in the liver by

6-hydroxylation and excreted as sulfate and glucuronide conjugates of 6-hydroxymelatonin. The physiological effects of melatonin are based on plasma membrane M1 receptors which have been characterized widely in different tissues, especially in the suprachiasmatic and some other hypothalamic nuclei as well as the *pars tuberalis* of the pituitary gland. The sleep-inducing effect is perhaps the best-known one of its effects. This effect is in close connection with melatonin-induced lowering of body temperature at night. In general melatonin has been linked to nocturnal damping of cell growth and body metabolism, e.g. gastrointestinal activity. Its antioxidant activity with respect to cancer and immune functions has been lately intensively studied.

1. Introduction

The discovery of melatonin was originally based on skin color experiments in amphibians. Bovine pineal extracts were found to contain a substance which lightened amphibian skin by aggregating melanophores. This substance was isolated and chemically characterized to an indole compound, N-acetyl-5-methoxytryptamine (Figure 1). This breakthrough in the late 1950s initiated an intensive study series which soon disclosed the biosynthesis of melatonin and its circadian character in the pineal gland. Melatonin was found to be typically synthesized at night, the dark period of the day. Hence it is now called the pineal hormone of darkness or the night hormone, sometimes also the clock hormone. Currently we know that the circadian rhythm of melatonin in the pineal gland, measurable in blood, urine and saliva is endogenous driven by the biological clock, the suprachiasmatic nuclei (SCN) of the hypothalamus, but it is continuously entrained by environmental light. Although subsequent studies have managed to declare the metabolism of melatonin and also melatonin receptors our knowledge concerning the physiological effects of melatonin especially in human body is still rather limited.

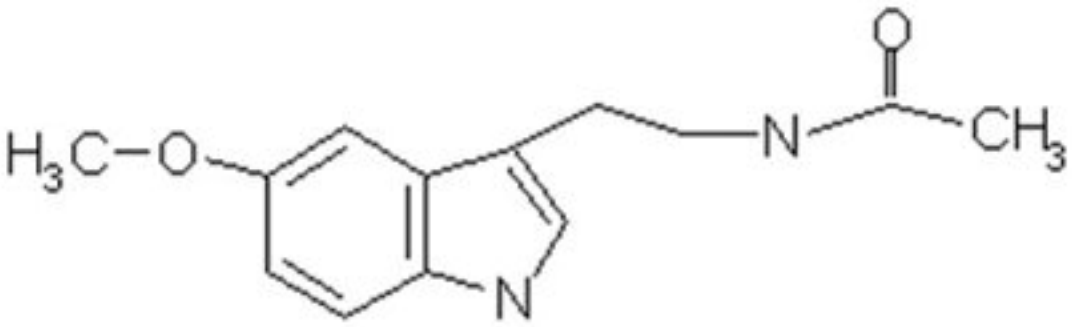

Figure 1. Melatonin is chemically N-acetyl-5-methoxytryptamine, a derivative of tryptophan amino acid.

2. Melatonin as Pineal Hormone of Darkness

Melatonin is synthesized in the pineal gland from tryptophan in four reaction steps: 1) via 5-hydroxylation from tryptophan to 5-hydroxytryptophan; 2) via decarboxylation to 5-hydroxytryptamine (serotonin); 3) via N-acetylation to N-acetylserotonin; and 4) via O-methylation to melatonin. These steps are catalyzed four special enzymes of which

N-acetyltransferase (NAT, step 3) is the most important regulating the circadian expression of melatonin biosynthesis in the pineal gland. It has been shown that light inhibits NAT activity breaking pineal melatonin production and secretion. This light-dependence has been observed in humans and in all animal species studied, also in nocturnal animals, leading to a clear circadian rhythm of pineal melatonin.

In both human and animals studies it has been shown that the melatonin rhythm is circadian also in continuous darkness, i.e. in conditions without any external day-night variation. These observations refer to an endogenous (intrinsic) character of the melatonin rhythm. Interestingly, the endogenous rhythm is not exactly 24 hrs as the exogenous day-night rhythm but a little longer (≤25 hrs). In continuous darkness the melatonin (and also other) rhythmicity is delayed ≤1 h/day leading to desynchronization of endogenous and exogenous rhythms. Normally external light-dark variation of 24 hrs, i.e. morning light, is every day pacing (shortening) the endogenous rhythm to the exogenous rhythm of exactly 24 hrs. According to recent studies the synchronization of endogenous rhythms with the external rhythm is important to both mental and physical health (see below).

How does environmental light information reach the pineal gland? In mammals light is transmitted via the eyes and retinohypothalamic route first to the SCN, the biological clock of the organism, then to the paraventricular nuclei (PVN) of the hypothalamus and via descending intermediolateral column fibers of the spinal cord and back via ascending presynaptic sympathetic fibers to the superior cervical ganglia from which finally via postganglionic sympathetic pathways to the pineal gland (Figure 2). Recently it has been shown that the first (=retinohypothalamic) part of this tract is separate from the optic nerve fibers although they both leave the retina at the blind spot running together to the hypothalamus. Quite recently melanopsin, a new photopigment for this rhythmic tract, has been found in special light-sensitive ganglion cells of the inner retina.

Light information is transmitted from the melanopsin-containing ganglion cells of the retina to the SCN glutaminergically (excitatory transmission). Here light paces (entrains) the circadian activity of these nuclei but at the same time it mobilizes gamma aminobutyric acid (GABA) transmission so that light excitation is blocked in forthcoming pathways (A-D in Figure 2). This GABA block is eliminated every evening during light-dark transition. Hence the rest of this rhythmic pathway is first vasopressinergically from the PVN via descending intermediolateral column fibers of the spinal cord and finally noradrenergically activated via ascending autonomic sympathetic neural pathways leading to the nocturnal stimulation of pineal melatonin biosynthesis and secretion.

When the activity of the pineal gland is governed by circadian light-dark variation the gland can be regarded as a neuroendocrine transducer: when reacting to neurally informed light-dark transition in the evening it starts to produce and secrete a hormone, melatonin, into circulation. This nocturnal hormone secretion can be seen as a melatonin pulse, a message of darkness (or night) which is the important circadian information to the whole organism.

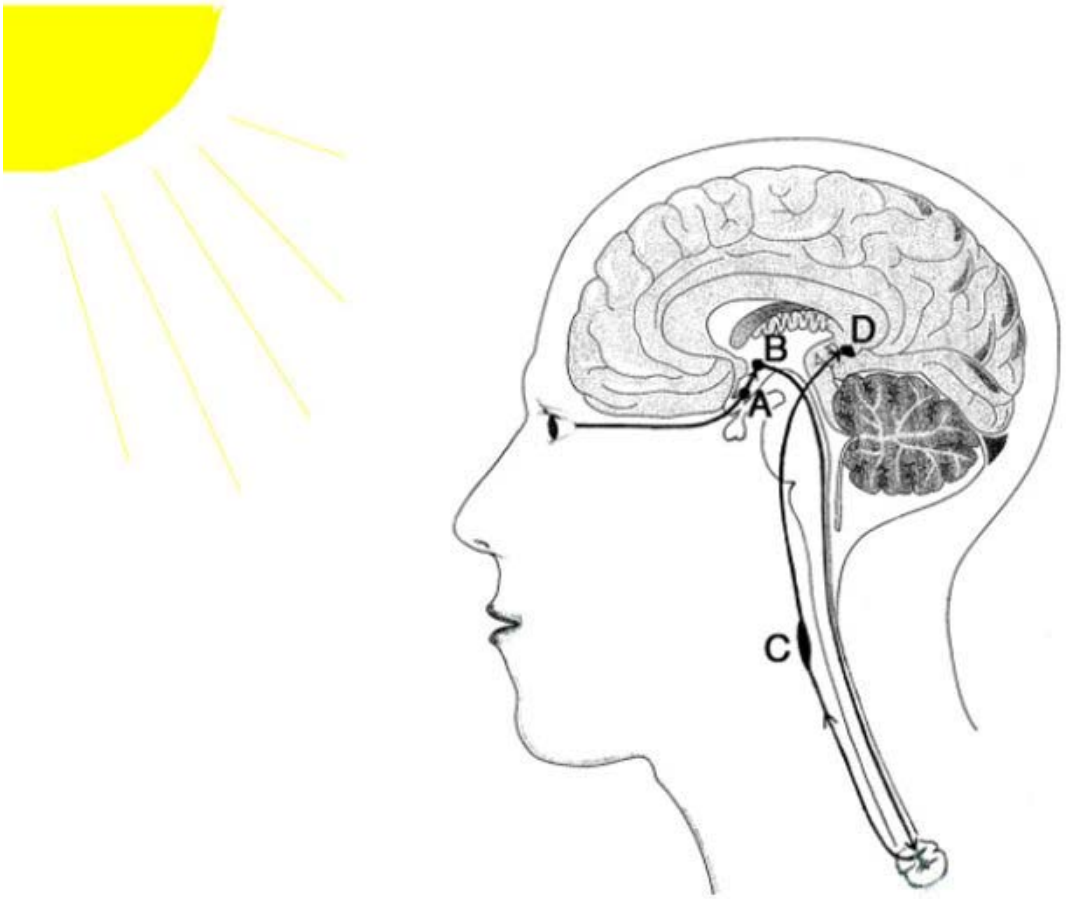

Figure 2. Regulation of pineal melatonin synthesis by environmental light. The first phase of activation by light rays in the eyes uses melanopsin, a specific ganglion cell pigment. This stimulation is conveyed along with optic nerves and transmitted glutaminergically to the suprachiasmatic nuclei (A). After that the stimulation is GABAergically abolished into the paraventricular nuclei (B) leading to cessation of pineal melatonin synthesis (the entraining effect of light). During light-dark transition in the evening the GABAergic block is eliminated allowing first vasopressinergic transmission of dark information via descending intermediolateral column fibers of the spinal cord and then after acetylcholinergic transmission in the spinal cord via ascending sympathetic preganglionic pathways to the superior cervical ganglia (C). Finally the dark information is noradrenergically transmitted into the pineal gland (D).

3. Melatonin in Other Tissues

After the original pineal finding melatonin has been screened throughout other mammalian, avian and also many lower vertebrate tissues and biological fluids. Using very sensitive immunoassay methods, melatonin is measurable in many tissues, especially in night-sampled tissues, but so far melatonin biosynthetic activity has been described in addition to the pineal gland only in mammalian retina and gut (enterochromaffin cells). Also in these tissues melatonin is fluctuating cyclically with high nocturnal and low daytime levels. Although the absolute melatonin amounts are exceeding the pineal ones, it is suggested that melatonin is not secreted from these

tissues into circulation but instead, melatonin has a local, exactly unknown significance in extrapineal tissues. Consequently, the pineal gland seems to be the crucial organ in that it sends circadian information (nocturnal melatonin pulse) to all other tissues of the body.

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Biographical Sketch

Olli Vakkuri was born in August 1952 in Kalajoki, Finland. After passing the matriculation examination at Kalajoki Yhteiskoulu in 1972, he studied zoology, botany, chemistry and biochemistry, genetics, geography, and finally physiology as a main discipline at the University of Oulu. The Master of Science was achieved in 1979 and The Licentiate degree in 1984. At that time he immersed himself in pineal and melatonin research under professor Juhani Leppäluoto, the head of the Department of Physiology. The first challenging scientific goal was to develop a sensitive and specific radioimmunological assay method for melatonin analyses, because up to 1980s pineal and melatonin studies were hampered by the lack of sensitive melatonin assays. The project produced the first radioiodinated melatonin (^{125}I-melatonin which was shown to be 2-iodomelatonin, i.e. iodination was taken place at C-2 position of the indole ring) which was the first direct iodination product of an indole compound (tryptophan amino acid and its derivatives). This radioligand was found to be beneficial both in radioimmunoassay and later also receptor use. It gave more analytical power to measure the low daytime concentrations of circulating melatonin but much more - the new radioligand greatly promoted melatonin receptor physiology: it made it possible to show high-affinity melatonin receptors first on amphibian skin melanophores and later also widely on animal and human cell membranes. Vakkuri completed his Ph.D. thesis ("Melatonin in human and animal tissues: an analytical and physiological study") in 1986. After that he continued melatonin research, focusing especially on the seasonal secretion of melatonin in humans, because Oulu, far in the north (65°N), offers favourable conditions (long summer and short winter days) to study seasonal phenomena. Based on these and other findings Vakkuri was awarded a Docent in Physiology in 1991. Since then he has also carried out various other projects, e.g. on ouabain.