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Melatonin: An endogenous chronobiotic key regulator of biological clock

Melatonin and biological clock

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Abstract

The internal mechanism through which a behavioral activity of organism repeats in a predictable pattern and is coordinated with the presence and absence of light is called biological rhythm generated by the internal pacemaker, or 'circadian clock'. Circadian rhythms are in synchronous with the light-dark cycle of the environment. This type of well conserved rhythmic behavior like sleep-wake cycle, enzyme level and their associated metabolic pathways are synchronized with external environment by neuronal and circulating humoral cues. The suprachiasmatic nucleus (SCN) or 'central clock' in the anterior hypothalamus is the time keeper of biological rhythms, which perceives photic signals from environment through retino-hypothalamic tract to coordinate different oscillators present in 'peripheral clocks' in almost all tissues throughout the body. It is composed of loops and is regulated by an interconnected network of transcription-translation feedback loops (TTFL). In this system, transcription factors induce the transcription of clock-controlled genes (CCG), viz. *Clock, Bmal1, Per1, Per2, Per3, Cry1, Cry2, Cry3, Dec1, Dec2* and *Rev-erbα,* and their products function as repressors and negatively regulate their own expression. The neuroendocrine system involved in the regulation of circadian timing as well as the production of the pineal hormone melatonin (*N*-acetyl-5-methoxytryptamine). It is produced cyclically in the pineal glands in response to the ambient light-dark cycle, with a maximum value during the dark phase. This indolamine is considered as a 'chronobiotic' molecule, or 'hormone of darkness' or 'biological night', which is exclusively involved in signaling the 'time of day' and 'time of year' in all central and peripheral clocks. Thus, this hormone acts as an endogenous chronological pacemaker or Zeitgeber to adjust the timing of biological rhythms as calendar function. Thus, the present review summarizes the rhythmicity of various physiological functions and their interactions with different 'clock genes' regulating the synthesis of melatonin.

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1. Introduction

Melatonin (*N*-acetyl 5-methoxytryptamine) was initially discovered in bovine pineal preparations. The study began in the early 1900s when McCord and Allen discovered that treating tadpoles of *Rana pipens* with crude acetone extracts of bovine pineal glands produced skin blanching, allowing sight of enlarged viscera through the dorsal body wall [1]. In 1958, Lerner and his colleagues identified and purified the pineal indole component responsible for the skin blanching in tadpoles and they used the term 'Melatonin' for this pineal substance in reference to McCord and Allen's first discoveries [1, 2]. It is a phylogenetically conserved ancient molecule found in all species, ranging from microbes to wide variety of animals and plants [3]. Melatonin is widely distributed molecule, has a broad range of physiological and therapeutic effects in all living systems, including synchronization of rhythmic body functions with the environment; regulation of the sleep-wake cycle, mental state, brain functions, and reproduction; control of body temperature, oxygen consumption, and locomotor activity; protection against oncogenic and immunological affects; scavenger of free radicals [4]. Melatonin is synthesized predominantly from the pineal gland in mammals and other vertebrates in a rhythmic manner in synchronization with the environmental light-dark cycle. It is also produced in some extra-pineal sources which include tissues and organs such as retina, gastrointestinal tract, Harderian gland, extraorbital lachrymal gland, gall bladder, bone marrow cells, lymphocytes and platelets etc. Melatonin is generally synthesized during night regardless of the diurnal or nocturnal activity of the organisms and this feature of melatonin is conserved among all vertebrates investigated so far. Considering this feature melatonin is regarded as a "chronobiotic" substance which is generally involved in providing with information about the length of the night or time of the day or the time of the year to all tissues and cells in our body [4, 5]. This review is an attempt to focus on the role of melatonin in the regulation of biological rhythms as well as to bring together all pertinent information concerning the melatonergic

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system and its rhythmicity in relation to the circadian clock regulating mechanism.

2. Biological Rhythms

Biological rhythm is the internal process of a living organism's behavioral activity that repeats in a predictable pattern and is synchronized with the presence and absence of light. It is created by the internal pacemaker or 'clock'. Animals' sleep-wake cycle, females' menstrual cycle, leaves shifting orientation in plants, stomata opening and shutting in plants, and the arrival of fruits and flowers in each season are all instances of biological rhythm. In 1935, two German zoologists, Hans Kalmus and Erwin Burning, revealed the existence of these endogenously controlled cycles in the fruit fly *Drosophila melanogaster* [6]. These rhythms are classified according to their frequency, duration, and amplitude. Additionally, these are defined by their recurring shifting pattern over time. Every living thing has biological rhythms, which are governed by its built-in internal biological clock. Endogenous neuronal and hormonal mechanisms regulate biological rhythms. In living systems, several forms of biological rhythms exist. Ultradian rhythms are shorter than a day or a 24 hr time frame. For example, respiratory cycles, brain electrical activity, sleep, and several endocrine functions are ultradian rhythms in origin [7]. The majority of hormones are released in pulsatile into the blood from the endocrine glands. For instance, aldosterone and cortisol both have a peak release every four hours in humans, as well as, luteinizing hormone (LH), is secreted every 30 minutes in rats, every 60 minutes in monkeys, and every 180 minutes in mice [8]. Rhythms which occur with a time period of about a day are termed as circadian (*circa*, about; *dian*, day). Under constant conditions circadian rhythms can run freely with a time period slightly shorter or longer than 24 hrs. One of the best examples of circadian rhythm is the sleep-wake cycle in animals. In humans, the sleep-wake cycle in free running conditions is almost close to 25 hrs whereas in case of mouse it is close to 23 hrs [7].

Infradian rhythms are biological rhythms that last longer than a day and often last more than 28 hrs. Circannual rhythms are an example of such rhythms. Circannual rhythms are created endogenously and have a duration of approximately one year or twelve months. Annual behavioral changes occur in animals such as insect pupation, migration, reproduction, and hibernation. Endogenous clocks termed 'circannual clocks' regulate these changes in animals. The circannual clock can be reset when an animal migrates [9].

3. Circadian Rhythms and the Clock System

Circadian rhythms are synchronized with environmental light-dark cycles. The circadian clock is an endogenously controlled time keeping mechanism that produces these cycles. The circadian clock in mammals is hierarchical, with a central clock in the suprachiasmatic nucleus (SCN) of the hypothalamus and peripheral clocks in the liver, lungs, kidney, heart, skeletal muscles, and adipose tissue [10]. In an organism, SCN is the master clock or coordinator [11]. However, it is impacted by external or internal elements such as food consumption, day/night cycles, temperature, etc. Nearly every tissue and cell in the body has a molecular circadian clock. These clocks may operate freely in any setting [12]. The circadian clock regulates sleep-wake cycles, hormone release, metabolism, and other processes [13]. It regulates the functioning of several cells and organs in time. The circadian clock is controlled genetically and when mutations occur in it, changes occur in the rhythmic behavior of the organisms [14].

The central clock present within SCN mainly regulates the diurnal rhythms of melatonin production and the neuroendocrine functions of an organism. The peripheral clocks also plays very essential role in different tissues and organs, as liver and pancreatic clocks can control the liver homeostasis, ovarian clock have the ability to control ovulation in female reproductive cycle, clock present in skin have the wound healing ability and the ventromedial hypothalamus clock can control the energy expenditure [15]. Circadian rhythms are also responsible for the daily food intake of organisms because it occurs regularly with a constant time of the day. Availability of food also acts as a synchronizer for controlling the rhythms in the gastro-intestinal (GI) tract. This rhythmic activities in the various segments of gut like muscular contractions are also regulated by the synthesis of enteric hormones (ghrelin, CCK etc.) and different clock genes. For example, three cycles of rhythmic slow waves occur in stomach, twelve in duodenum, seven to ten in the jejunum and ileum, and twelve in the colon, per minute as calculated in mammals [16]. In the cardiovascular system, the physiological functions show distinctive diurnal rhythms and melatonin acts as a synchronizer of this circadian rhythm [17]. Circadian clocks located in the hypothalamus, pituitary, ovary and testis as well as in uterus regulating the timing and rhythmicity of hormone release and tissue sensitivity in the hypothalamic-pituitary-gonadal (HPG) axis [18]. Disruption of the circadian clock and the clock genes induce the expression of pro-inflammatory cytokines as clock controlled mitochondrial functions are disturbed leads to the formation of inflammasome and administration of melatonin abrogates altered clock induced innate immune system and mitochondrial homeostasis [19]. Thus, synchronization between central and peripheral clocks occurs by the coordination of neurons, hormones and body temperature. The disruption of these clocks can lead to the

development of cancers as well as various other disorders in metabolism, cardiovascular and nervous system [12].

3.1. The Molecular Mechanism of Circadian Clock System

The circadian clock is controlled through a network of interlocking transcription-translation feedback loop (TTFL) mechanism. Generally, the TTFL system consists of two arms, a positive arm, where the transcription factors promote the transcription of clock-controlled genes (CCGs) and in the negative arm the products of positive arm act as a repressor and negatively regulate their own expression. There are at least ten genes found to be responsible for circadian rhythms which include *Clock, Bmal1, Per1, Per2, Per3, Cry1, Cry2, Cry3, Dec1, Dec2* and *Rev-erbα*. *Dec1* and *Dec2,* these two genes also possess conserved E-Box elements in their promoter region and are induced by CLOCK/BMAL1. DEC1 and DEC2 also repress the transcriptional activation of *Per* and *Cry* genes [20]. According to various researchers, *Bmal1* is the most studied component of the molecular circadian clock among the core clock genes and it is found that dysfunction of BMAL1 affects both cellular and behavioural circadian rhythms in a most dramatic manner jeopardizing the circadian clock [21].

In mammalian system, two nuclear transcription factors, “circadian locomotor output cycle kaput” (CLOCK) and the “brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein1” (BMAL1) initiate the positive loop of the clock system. CLOCK and BMAL1 together form a heterodimeric transcriptional activator [22], which translocates into the nucleus where it binds to specific E-box (′CACGTG′) elements of CCGs promoters and promotes their transcription. The mammalian circadian machinery includes; Period (Per1, Per2 and Per3), Cryptochrome (Cry1 and Cry2), RAR-related orphan receptor alpha (RORα) and nuclear receptors reverse erythroblastosis virus-alpha (REV-ERBα) nuclear transcription factors [23, 24]. In the cytoplasm the PER proteins are phosphorylated by a group of casein kinase-1 proteins CK1δ and CK1ϵ, which make it stable and allow it to dimerize with CRY [25]. The CRY protein is considered as the rate limiting repressor of transcription [26], which translocates into the nucleus, where it binds to BMAL1 and repress its functions, thereby completes the circadian loop [27]. For CRY to bind with BMAL1, it is acetylated first at lysine-537. This acetylation is facilitated by CLOCK, which has an intrinsic acetyl transferase activity [28] (Fig. 1).

3.2. Regulation of Clock Genes Expression

In mammals, a group of ubiquitin ligase has been identified that promotes the proteolysis of different clock proteins, thus helps in the maintenance of circadian rhythms [29]. Cry1, Cry2 proteins are stabilized by F-box proteins such as FBXL3 and FBXL21, which mediate protein degradation by adding ubiquitin chains to their target proteins. The fast degradation of different CRYs is mediated by FBXL3 enzymes, whereas ubiquitination by FBXL21 leads to slow degradation of CRY proteins [18]. FBXL3 binds to the FAD-binding domain (FADD) of mammalian CRY protein and induce its proteolysis [30]. A loss of function mutation in FBXL3 results in stabilization of CRY protein [31]. In mammals two ubiquitin ligases i.e. β-TRCP/FBW1A and β-TRCP/FBW1B have been identified that facilitate the proteolysis of Period (PER) [32]. β-TRCP1/2 bind to phosphorylated PER and induce polyubiquitination which ultimately leads to its degradation [33]. REV-ERBα levels are also regulated by ubiquitination. In cytoplasm, glycogen synthase 3β (GSK-3β) stabilizes the level of REV-ERBα. It is established that ubiquitin ligase HUWE1/ARF-BP1 and PAM (MYCBP2) are associated with the proteolysis of REV-ERBα, therefore it helps in the maintenance of Bmal1 expression [34]. UBEA acts as the E3 ubiquitin ligase of BMAL1 [35]. De-ubiquitination is also considered to be an important process in the regulation of circadian clock machinery [36]. Interestingly a common de-ubiquitinase enzyme USP2 has been reported for the clock proteins like cryptochrome (CRY) [37], period (PER) [38] and BMAL1 [39, 40].

4. Pineal Hormone Melatonin: Chemical Synchronizer of Biological Rhythms

Melatonin is produced primarily in the pinealocytes of the pineal gland and is crucial for setting the biological rhythms of the body as a self-sustaining "clock" [41]. The pineal organ is found near the skin in lower vertebrates such as fish and amphibians and works as a light sensor. The eyes detect the light signal, which is subsequently sent to the pineal through the optic nerve of the retino-hypothalamic tract (RHT). In higher vertebrates, the pineal organ in brain operates as a conventional endocrine gland, but in lower vertebrates, such as frogs and, more particularly, fishes, it serves as both a secretory and sensory organ [42]. In teleost and other non-mammalian vertebrates, the pineal organ is made of an end vesicle that is contained inside a window below the skull that allows light to enter and excite photoreceptor cells [43]. Non-mammalian vertebrates have a pineal organ that is made of cone-like photoreceptor cells that are structurally similar to retinal cones and also has secretory capabilities similar to the mammalian pineal gland [44]. Plasma and pineal melatonin levels fluctuate in lockstep, with the lowest levels occurring during the day or light phase and the greatest occurring during the night or dark phase. The photoreceptor cells manufacture retinal melatonin, which acts primarily in an autocrine or paracrine way and is normally associated with the retinal circadian clock [45]. In addition to the

regulation of biological rhythms, melatonin also protects cells by scavenging the free radicals as this indoleamine can readily enter into all of our body's cells, tissues, and organs through the phospholipid bilayer of the plasma membrane due its lipophilic nature within the living systems during evolution [46].

4.1. Biosynthetic and Catabolic Pathway of Melatonin

Melatonin is synthesized from aromatic amino acid L-Tryptophan (L-Trp) which is taken up by the pinealocytes of the pineal gland from the circulation. The biosynthesis of melatonin is a four steps phenomenon. It begins with the hydroxylation of tryptophan by '*tryptophan hydroxylase*' (TPH) and is converted into 5-Hydroxytryptophan (5HTP). It is then decarboxylated by '*aromatic amino acid decarboxylase*' (AAAD) into 5-Hydroxytryptamine (5HT) or serotonin. Serotonin is then acetylated by '*arylalkylamine-N-acetyltransferase*' (AANAT) into *N*-acetylserotonin (NAS). The last step in the biosynthesis pathway of melatonin converts *N*-acetylserotonin into melatonin or *N*-acetyl-5-methoxytryptamine by the enzyme '*hydroxyindole-O-methyltransferase*' (HIOMT) [47]. AANAT is a key enzyme as considered as the rate limiting enzyme in this biosynthesis pathway [48] (Fig.1).

Circulatory melatonin is primarily metabolized in the liver by the enzyme cytochrome P450 monooxygenases (mainly CYP1A2) and is converted into 6-hydroxymelatonin which may conjugate with sulphate to form 6-sulfatoxymelatonin by sulphotransferase ST1A3 and finally eliminated through the urine, or it may undergo conjugation with glucoronic acid to form 6-hydroxymelatonin glucuronide [49]. Urinary measurement of 6-sufatoxymelatonin can be used to determine the pineal melatonin production. In the central nervous system (CNS), melatonin is catabolized to form *N*-acetyle-*N*-formyl-5-methoxy-kynurenine (AFMK) via oxidative reactions by the enzymes indoleamine-2, 3-dioxygenase (IDO) and myeloperoxidase (MPO). AFMK acts as the primary active metabolite of melatonin. AFMK is further deformylated to form *N*-acetyl-5-methoxy-kynurenine (AMK) by kynurenine formamidase [50] (Fig.1).

4.2. Rhythmicity of Melatonin

Melatonin is synthesized primarily in the pineal in a cyclical manner in accordance with the ambient light-dark cycle, with a peak value during the dark phase and a minimum value during the day. The rise in melatonin synthesis throughout the night is attributed to the increase in AANAT activity. By contrast, HIOMT activity is nearly constant throughout the light-dark (LD) cycle. In general, serotonin levels are highest during the day and lowest at night [51]. Melatonin production lasts longer in winter, when nighttime hours are typically longer, than in summer, when nighttime hours are shorter [52]. The primary environmental element regulating melatonin synthesis in the pineal and retina is light. The RHT transmits light information primarily to the hypothalamic suprachiasmatic nucleus (SCN), from which nerve fibres transfer the signal to the paraventricular nucleus (PVN), then to the superior cervical ganglia (SCG), and finally to the pineal gland [4].

There are primarily three distinct forms of nocturnal melatonin rhythms found in teleosts: Type-A, Type-B, and Type-C. The A-type rhythm is characterized by a definite peak in the late-dark phase, as seen in Atlantic cod (*Gadus morhua*) [53] and haddock (*Melanogammus anglefinus*) [54]. As found in Nile tilapia (*Oreochromis niloticus*), B-type rhythm is characterized by a distinct peak in the mid-dark period [55] and C-type rhythm is defined by a definite peak in the early-dark phase or shortly after the commencement of the dark period, as observed in Atlantic salmon (*Salmo salar*), Rainbow trout (*Onchorhynchus mykiss*) [56], and the majority of other teleosts [42]. Although research on carp indicated that the nocturnal pattern of serum melatonin profiles might change across A- and B-types within the same species depending on the species' reproductive phase [57, 58]. The explanation for the differential nocturnal melatonin patterns reported in various animals is unknown. It may be related to seasonal variations in a number of external elements, the species' behavior and habitat, light availability, temperature, and salinity. Additionally, it may be determined by their self-sustaining endogenous clock system. The duration of the melatonin synthesis from pineal gland is highly dependent on the length of night, and its amplitude is strongly reliant on the ambient temperature. Daily light of a sufficient intensity and duration, or perpetual darkness, can induce melatonin cycles to phase shift. The amount of light necessary to inhibit melatonin production differs among species and may also change according to the species' history of past light exposure [59, 60].

4.3. Molecular Regulation of Rhythmic Melatonin Synthesis

The rhythmicity of melatonin is dependent upon the rhythmicity of *aanat* gene and its expression. In mammals and birds, only one *aanat* gene is present. Teleost fish is special among vertebrates because they possess two types of *aanat*, termed *aanat1* and *aanat2*. *Aanat1* is expressed specifically in the brain and retina, whereas *aanat2* is expressed in the pineal [5]. In teleosts, more specifically there are even three types of *aanat* genes viz., *aanat2* and two types of *aanat1*, termed *aanat1a* and *aanat1b*. The *aanat2* gene is expressed exclusively in fish pineal organ whereas both *aanat1a* and *aanat1b* genes are expressed in

the retina, nervous system and other peripheral tissues [44, 61]. In primates and ungulates, the transcription of *aanat* gene and the translation of its mRNA are found at a constant rate constitutively and generally show no variations with the environmental light-dark conditions but the post-translational modification of the AANAT protein is responsible for the changes of its activity in a diurnal cycle [44].

After receiving the light information from SCN, the paraventricular nucleus (PVN) signals superior cervical ganglia (SCG) to release of norepinephrine at night which subsequently stimulates the α1- and β1- adrenergic receptor of pinealocytes, which results into an increase in intracellular calcium concentration and the activation of enzyme adenylate cyclase (AC) as well (Fig. 1). This activation leads to a rapid increase of intracellular second messenger cAMP concentrations [62]. Increased level of cAMP subsequently results into the activation of protein kinase A (PKA) which then exerts its effect on AANAT. During night AANAT is phosphorylated to form phosphorylated AANAT (pAANAT) by PKA and pAANAT binds with 14-3-3 regulatory proteins which prevents it from undergoing proteasomal degradation due to which AANAT remains catalytically active and thus results into subsequent synthesis of melatonin [63]. Exposure to light during the day lowers cAMP levels, which then leads to de-phosphorylation of AANAT and breakdown of the AANAT/14-3-3 complex, due to which catalytic activity of AANAT subsequently drops following proteasomal degradation of the enzyme [64, 65] (Fig. 1). The retinal AANAT is generally protected against breakdown during the day [66]. In other group of mammals, the rhythm of *aanat* gene expression in 24 hrs cycle varies and plays a dominant role which in turn is responsible for AANAT activity and the diurnal rhythm of melatonin synthesis [67]. In rats, the mRNA levels of *aanat* gene in the pineal gland increases up to more than 100 fold during the night just within the few hours after the onset of darkness during 24 hrs light-dark cycle [68]. And in case of avian pineal gland the mRNA levels of *aanat* changes parallel with the activity of AANAT protein and with melatonin levels [69, 70].

4.4. Melatonin Receptor Distributions in the 'Clock' System

Melatonin exerts its effects via two membrane-bound G-protein coupled receptor isoforms, MT1 and MT2, as well as a nuclear receptor from the retinoic acid orphan receptor family, ROR/RZR [71]. The MT1 and MT2 receptors are found in a variety of tissues and organs including the retina, pars tuberalis (PT), suprachiasmatic nucleus (SCN), cerebellum, hippocampus, kidney, intestine, lungs, ovary, testis, mammary glands, adrenal gland, lymphocytes, gall bladder, skin, adipocytes, and blood vessels [72]. These two receptor subtypes exhibit considerable variation in terms of affinity, tissue distribution, and participation in second messenger pathways. Additionally, another type of melatonin receptor, MT3, has been identified that functions similarly to quionone reductase and may be involved in mediating antioxidant effects of melatonin [73]. Mel1a, Mel1b, and Mel1c were the previous names for these three receptor subtypes. Two Melatonin receptor subtypes are found in mammals, MT1 and MT2, whereas three subtypes are found in non-mammalian species [74]. In mammals, the SCN is commonly referred to as the central rhythm generation system or "clock." Circadian clock genes are located in the SCN, and their expression regulates melatonin production in a rhythmic fashion. Along with the hypothalamic SCN, the anterior pituitary PT contains the highest concentration of melatonin receptors in the majority of mammalian species studied so far. The SCN and the PT are widely regarded as conserved sites of high receptor expression [51]. Melatonin receptors and their mRNA expressions in the brain and the retina also shows variations along with the environmental light dark conditions which helps in processing the light information and are regulated by circadian clock and light [75].

4.5. Melatonin Mediated Regulation in Clock Genes Expression

Melatonin is considered as an "endogenous synchronizer" and is associated with the regulation of different biological rhythms of living organisms. Melatonin acts on the feedback loop mechanism of circadian clock and controls the phase shifting of neuronal firing in SCN [76, 77]. It was observed that, in rat, injection of melatonin in the SCN at the end of the subjective day altered the expression of *Per1, Per3* and *Bmal1* during the second subjective night. From this novel experiment, scientists concluded that melatonin acts on the clock genes expression post-translationally rather than post-transcriptionally [78]. From a similar kind of experiment, it was concluded that melatonin regulates the clock gene expression at the transcriptional level [79]. It was found that the expression of *RORα* is regulated by melatonin independent manner whereas, the expression of *REV-ERBα* was found to be melatonin dependent and its mRNA levels are phase shifted after melatonin administration during the second subjective day [80]. On the basis of above mentioned observation, it appears that there is a strong co-relation between melatonin and the expression levels of various clock and clock associated genes. But the overall mechanism regulated by melatonin is yet not known and further research needs to be done for the clear and better understanding of the functions and mechanism on this aspect.

5. Photoperiods and Clock Genes

Photoperiod plays a vital role in the regulation of several clock genes associated with rhythmic secretion of melatonin from the pineal and several other peripheral organs. The production of melatonin and its rhythm is primarily regulated by systems which include circadian oscillators, light detectors and melatonin synthesizing enzymes. The organization of the melatonin rhythm generating systems varies greatly among vertebrates including mammals. In mammals, the system involves retina as a photodetector, SCN as a master clock which controls expression of genes regulating various biological rhythms, and the pineal gland as a primary site of all the enzymes for melatonin biosynthesis [81]. While in case of lower vertebrates, the system involves photoreceptor cells of the pineal which contains all the three components for the generation of melatonin rhythms i.e. photodetector, clock and enzymes for melatonin biosynthesis [44, 43]. Zebrafish (*Danio rerio*) is considered as one of the best studied teleost models in which the molecular clock mechanism is methodically studied. There are three *Clock*, three *Bmal*, four *Per* and seven *Cry* genes have been identified so far of which only *Bmal1a, Bmal2, Clock1a, Per1a, Per2, Per3, Cry1a* and *Cry3* are expressed in the pineal organ [82]. Study on the same have demonstrated the association between different melatonin biosynthetic enzyme genes and clock associated genes in the ovary with that of whole brain in normal and altered photoperiodic conditions where it was found that in normal and altered photoperiodic conditions, the ovary of has its own machinery of melatonin biosynthesis and is associated with central oscillating system in the brain [83]. Several studies have demonstrated the presence of clock genes in the pineal and brain and their regulation in several vertebrates [84-86]. In Atlantic salmons (*Salmo salar*), the rhythmic expression of pineal and liver *Per1-like* increased at night and brain *Per-1 like* increased at dawn, just before the onset of light, whereas the pineal *Cry2* expression peaked at midnight and the brain *Cry2* peaked at dawn. However, the peak levels of *Clock* expression occurred during the beginning of the light phase, whereas in the liver the peak value of *Clock* expression occurred during the late night [87]. Similar results were also obtained in Goldfish (*Carassius auratus*) retina where the *Per1* expression increased at midnight [88]. It was observed that during the first subjective night after the administration of melatonin to rats kept in constant darkness caused phase advancement of *Rev-erbα* and *Bmal1* mRNA expression in the SCN [80]. Administration of melatonin at the end of the subjective day to rats kept in constant darkness reduces amplitude of the expression of *Per1* and increases the expression of *Cry1* in PT and administration of melatonin in the middle of the subjective day also caused an increase in *Cry1* expression [89]. In a separate study on pubertal Soay sheep (female), it was observed that the expression of *Per1, Cry1* and *Rev-erbα* were rhythmic and varied with altered photoperiodic conditions in PT, whereas the expression of *Clock, Bmal1* and *Cry2* did not vary with photoperiodic treatment [90]. Rats maintained under long photoperiod (LD 16:8) or under short photoperiod (LD 8:16) affected phase and amplitude of the clock genes mRNA. During the daytime mRNA levels decreased for *Bmal1* and *Clock* whereas *Per1* and *Cry1* increased but their expression reversed at night after the onset of darkness. Also the amplitude of *Per1, Cry1* and *Bmal1* mRNA was found to be higher during short photoperiod as compared to the long photoperiod [91]. In the retina of rat, several clock genes showed daily rhythmicity in a photoperiod dependent manner which includes *Per1, Per2, Per3, Clock, Dec1, Dec2* and under constant darkness only the *aanat* as CCG is found to be expressed constitutively [92].

6. Involvement of 'Biological Clock' in Physiological Functions

Reinforcement as well as stabilization of rhythmic physiological functions are mostly regulated by the endogenous synchronizer, often termed as the "hormone of darkness", melatonin, acts as a time-setter of endogenous biological clock, thus inaugurate time cue or "Zeitgeber" in circadian oscillators present in every cell and organ of the body as a chronological pacemaker [93]. The role of 'biological clock' in the regulation of various physiological functions are well studied in different non-mammalian species especially in fish. However, the involvement of melatonin in controlling seasonal rhythmicity such as, reproduction is well studied in fish. To govern this synchronization between the annual gonadal cycle and the factors like habitants, the pineal organ, the lateral eyes, and the SCN all play in an interactive role [46]. Direct or indirect involvement of melatonin in controlling ovarian physiology has been evident from several carefully controlled experimental studies as melatonin can modulate hypothalamo-pituitary axis to regulate sex steroidal hormone level or alleviate endogenous stress by functioning as a scavenger of free-radicals over a reproductive cycle [94, 95]. Temporal pattern of melatonin in the regulation of ovarian reproductive cycle in association with the stress axis has been evident earlier for the first time in fish [96]. Recent research indicates that the seasonal peak of serum melatonin in carp *Catla calta* is negatively associated with the plasma 17β-E_2 and 17α, 20β-diOH-progesterone peak values [97].

7. Conclusion

Melatonin since its discovery has been implicated in a wide variety of physiological processes of vertebrates. The past few decades have witnessed an enormous progress in understanding the mechanism of action of this endogenously synthesized pleiotropic molecule in the regulation

of various body functions. Melatonin being a chronobiotic molecule, is associated with the regulation of biological rhythms in a wide range of vertebrates which ultimately helps living organisms to adjust or modulate their physiological actions in synchronization with the environmental photoperiodic conditions. Data obtained from different carefully controlled studies from the recent years have recognized its multifaceted effects and its potential as a therapeutic molecule which can be implicated in the treatment of a diverse number of diseases and abnormalities. The synthesis and secretion of melatonin and its regulation in the pineal gland of vertebrates is already well established but the source and distribution of this chronobiotic molecule in extra pineal tissues and organs are not yet clearly understood. Also the proper mechanism and the role of melatonin in the regulation of central and peripheral clocks also remain to be fully defined and thus further studies are required to fully demonstrate the mechanism of action of melatonin in the regulation of various biological rhythms in living organisms which will help us to understand the implications of this pleiotropic molecule in the various fields of studies.

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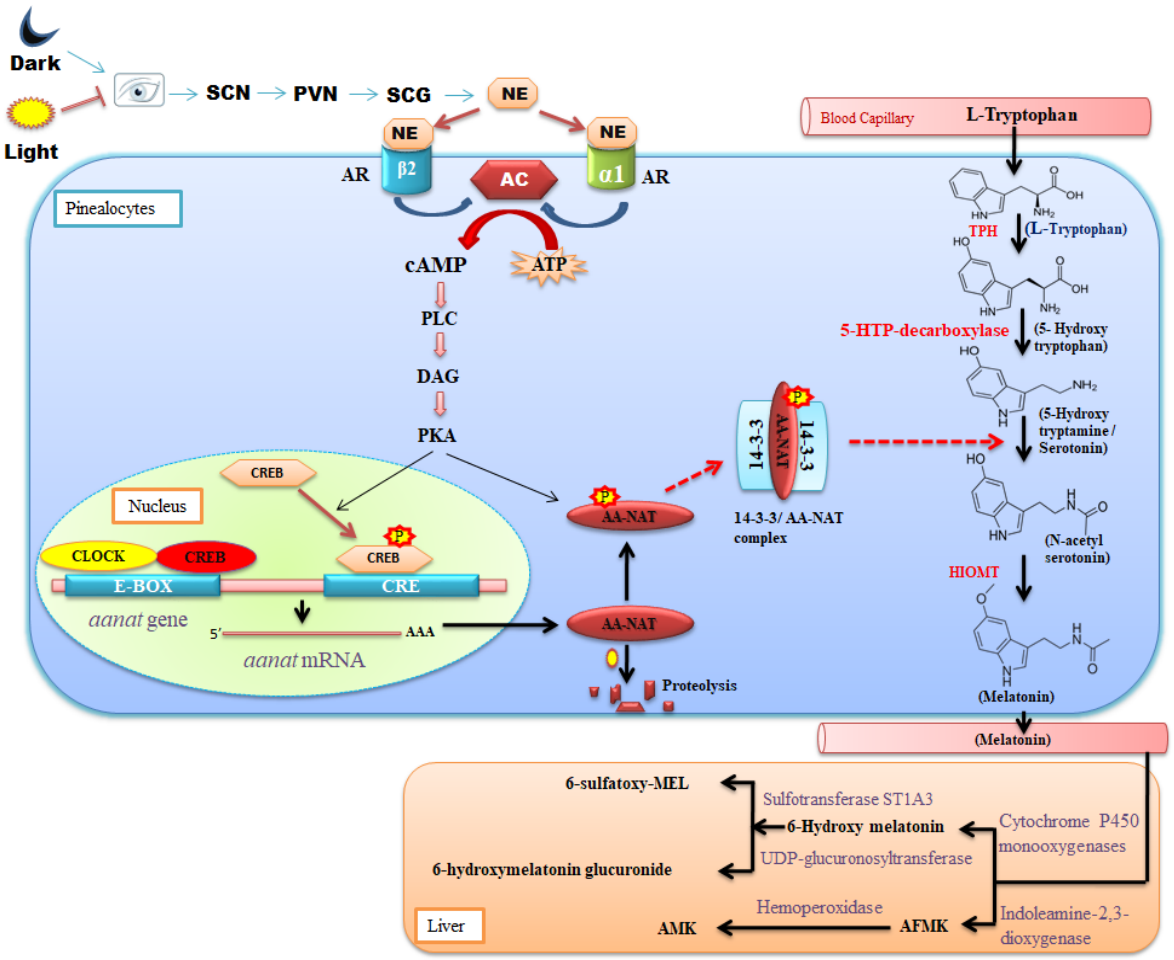

Figure Legend

Fig. 1. Synthesis and Regulation of melatonin in the pineal gland of vertebrates.

Nocturnal release of NE from the SCG occurs after receiving signals from PVN of the hypothalamus which leads to the stimulation of α1 and β1 AR of pinealocytes and results into the activation of AC and PKA. Activated PKA leads to phosphorylation of AANAT to form phosphorylated AANAT (pAANAT) at night. pAANAT then binds with 14-3-3 regulatory proteins and forms a complex and prevent the proteasomal degradation of catalytically active AANAT which further synthesize melatonin at night. Whereas, the light information travels through the retino-hypothalamic tract (RHT) to the pineal and exposure of light prevents the noradrenergic stimulation and thereby inhibits the synthesis of melatonin during the daytime.

Abbreviations: AANAT: Arylalkylamine-N-acetyltransferase; AC: Adenylate cyclase; AFMK: *N*-acetyle-*N*-formyl-5-methoxy-kynurenine; AMK: *N*-acetyl-5-methoxy-kynurenine; cAMP: 3′,5′-cyclic adenosine monophosphate; AR: Adrenergic receptor; CREB: cAMP-response element binding protein; DAG: Diacylglycerol; HIOMT: Hydroxyindole-O-methyltransferase; NE: Norepinephrine; PKA: Protein kinase A; PLC: Phospholipase C; PVN: Paraventricular nucleus; SCG: Superior cervical ganglia; SCN: Suprachaismatic nucleus; TPH: Tryptophan hydroxylase