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Review Article

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Melatonin Receptors in Amphibians and Reptiles: A Comparative *in silico* Study

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Abstract

The melatonin receptors play an important role in the regulation of circadian rhythms, behavioural and physiological processes in amphibians and reptiles. In the present study, the comparative bioinformatics analysis of the different subtypes of melatonin receptors (MTs) in amphibians and reptiles is performed in order to assess the evolution of these receptors, as well as their conservation in terms of their structure and function. For this purpose, the amino acid sequences of the different subtypes of MT receptors are retrieved from the publicly available database, National Centre for Biotechnology Information (NCBI), and analysed using Multiple Sequence Alignment (MSA) and phylogenetic tree analysis as well as structure homology modelling. The analysis demonstrates that there is a high degree of conservation of transmembrane domains, which implies that the functionality of these domains is preserved, whereas the variability of loops indicates adaptation to environmental conditions.

Keywords: Amphibian, Bioinformatics, Melatonin, Reptile, Receptor

Introduction

The neurohormone melatonin (N-acetyl-5-methoxytrptamine) is mainly secreted from the pineal gland, (it's located in epithalamus, between the two thalami, attached to third ventricle). The production of it is modulated through Suprachiasmatic Nucleus (SCN), known to be the master circadian clock.

Darkness favour melatonin production while light hinders it. At nighttime, the release of norepinephrine causes receptor stimulation in the pineal gland to increase cAMP concentrations, which stimulates the activity of aryl alkylamine N-acetyltransferase (AA-NAT) and this enzyme is essential for melatonin regulation [17]. It's an acetylated product of serotonin, synthesis of it is initiated by conversion of tryptophan to serotonin which is then converted into melatonin by the action of enzyme called arrow alkylamine in acetyl transferase "AA-NAT" [46]. While melatonin is primarily synthesized in the pineal gland, it is also expressed in different parts of the central and peripheral nervous [1]. Melatonin plays a vital role for regulating circadian rhythms, sleep, and photoperiodism. The response of organisms to variations in day duration, known as photoperiodism, aids in seasonal adaptation, including changes to metabolism, breeding status, and coloration [17]. Through encoding information of day length, & detecting these signals organism regulates migration and seasonal behaviour (*Pfeffer et al.,* 2022).

It influences other physiological roles as well, such as the regulation of the immune system, moods, timing of puberty onset, and hypnosis [23]. Disruption in melatonin is associated with development of varies sleeping as well as circadian disorder [40]. Melatonin acts via two main G protein-coupled receptors (GPCRs): MT1

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and MT2, which are encoded by genes named MTNR1A and MTNR1B, respectively [19]. Receptors are found on the surface of the cell and help in the initiation of intracellular signalling pathways leading to various cellular response [37]. MT1 are highly expressed in SCN & regulate sleep/wake rhythm while MT2 are less abundant and play more limited role [9,14]. Also, it influences maturation of oocyte (through enhancing quality of it), improving fertilization rates, and promotes embryonic development [35]. It's present in ovarian follicle as well, and highlights systemic endocrine role & regulatory functions (*Brzezinski, et al.,* 2021). The third receptor MT3 is found in birds and reptiles [5]. This MT3 receptor present in lower vertebrates for instance Xenopus, evolved into GPR50 in therian mammals, losing melatonin binding ability while gaining regulatory functions [8]. Initially in early vertebrates there are four genes of MT receptors found, but Gene loss and gene duplication events resulted in diversification of receptors among various species, while fishes have extra copies due to gene duplication [26]. Melatonin in amphibians is responsible for regulating circadian rhythm, embryogenesis, and pigmentation and also participates in adaptive pigmentation through melanin formation (*Vazquez, et al.,* 2019) [28] Studies shows that in amphibians melatonin producing enzyme HIOMT is active in brain region, especially in retina, & pineal gland helps regulate light driven rhythm. Its role in reproduction varies across species (*Vlaming & Olcese*,2020). In reptiles, reproduction is seasonal and it is influenced by environmental cues. Melatonin generally inhibits reproductive activity, helps to regulate seasonal transition by signalling non- breeding period [33]. It could be considered one of the best-preserved hormones that play vital roles in the regulation of circadian rhythms and homeostatic processes in amphibians and reptiles.

Role of Melatonin Receptors in Amphibians and Reptiles

Amphibian Reproduction is Controlled by Melatonin-Driven Photoperiodic Signal

The secretion of melatonin is affected by the environmental conditions as well as fluctuation in the body temperature (*Mendez et al.,* 2025). In contrast to birds, they do have specialised photo-sensitive which detects the photoperiodism, and the light signals help in releasing thyroid stimulating hormone (TSH) which influence their breeding activity (*Nakane & Yoshimura*, 2019). whereas in mammal's photoperiodic light signals are detected by retina which activate suprachiasmatic nucleus (SCN), carries information to brain region where TSH (thyroid stimulating hormone) is secreted to aid in reproductive activity (Nakane & Yoshimura, 2019). Therefore, melatonin production fluctuates with photoperiod and serves as an intrinsic clock in respond to seasonal changes in the environment [6]. The environment affects the processes involved in reproduction. For example, lighting conditions regulate the development and regression of gonads in vertebrates. In addition, amphibian reproduction depends on seasonal rainfall, percentage of Relative Humidity (%RH) and body temperature [4]. Frogs have light-sensitive melatonin signaling that can be triggered by artificial lighting conditions at night. The pineal gland is involved in the conversion of photoperiod information into physiological processes such as metabolism, growth, reproduction, and stress responses [16]. Melatonin is produced in the night as a response to darkness and regulates nocturnal behaviour. At a cellular level, melatonin acts on the hypothalamic-pituitary-gonadal axis through the regulation of GnRH, responsible for the regulation of LH and FSH hormones [38].

Roles of Melatonin in Controlling Thermoregulation, Reproduction and Seasonal Activity in Reptiles

Reptiles possess broader diversity of receptor which helps them in their biological activities (thermoregulation, seasonal activity cycle, reproduction). Through its receptor, melatonin influences neuroendocrine system, this signalling initiate circadian rhythm, metabolic activities, reproductive activity, seasonal behaviour, body temperature regulation [18]. The increase in melatonin levels is often linked to daily rhythmic activity, aids in reptiles adapting to seasonal environmental circumstances. The diversity of receptors affects thermoregulation [25]. reptiles rely on external factors, e.g., temperature and light intensity, to control their physiology [41]. Light intensity inhibits melatonin production, while its absence activates it and serves as a photoperiodic factor [18]. The rhythmic pattern synchronizes biological events with seasonal changes. Reproduction time depends on environmental cues such as temperature and photoperiod [43]. Temperature plays a significant role in embryonic development due to behavioural thermoregulation [24]. Seasonal changes affect the physiology of reptiles, during colder periods reptiles exhibit reduced activity and allocate in heat conservation, while in warmer periods there is increase activity seen in them [20]. Rise in melatonin levels lead behavioural changes, feeding habits, and low metabolic activities e.g., hibernation or aestivation [41]. It regulates the hypothalamus-pituitary-gonad axis, facilitating reproduction under optimal conditions [43]. Thermal conditions affect metabolic reactions, enzymatic activity, and behaviours such as basking and burrowing [30]. Transient receptor potential (TRP) channels play a role in thermal sensing in reptiles, responding to thermal variations inside and outside the body [21].

Evolutionary Divergence of Melatonin Receptors in Amphibians and Reptiles

Genomic studies Indicate Variation in the Duplications and Losses of Receptor Genes Among Amphibians and Reptiles

Melatonergic receptors have evolved as a result of duplication and loss events. This class of GPCR-type receptors include MT1, MT2, Mel1C/GPR50, and MTNR1A-like. These receptors differ from each other in terms of sequences, functions, and distributions [11]. MTNR1A-like receptor found in teleosts and reptiles, suggesting evidence that vertebrates initially had four receptor subtypes, also shows that early vertebrates possess more complex melatoninergic system [31]. Over the time some lineage species lost certain receptor genes, Mel1d is one of them [11]. Comparative genomics analysis of MT receptor genes' sequences (164 sequences in total: 57 mtnr1a, 59 mtnr1b, and 48 mtnr1c) showed a conserved core and variations in receptor complements among species (*Li et al.,* 2021). Amphibians have biphasic life cycle thus having multiple paralogs for functional partitioning, where Mel1c receptor (MTNR1C) specialised for pigment aggregation, while MT1 receptor (MTNR1A) which controls circadian rhythm [31,26]. When organism transition fully terrestrial or harsher environment, process leads some genes that no longer participate ecologically, leads regressive evolution (*Emerling et al.,* 2021). comparative genomics shows a difference in the diversity of receptors in amphibians and reptiles. Amphibians have receptors that make them suitable for living in both water and on land, while receptors of reptiles perform a specific function (*Li et al.,* 2021). Moreover, the genomes of reptiles are more organized for adaptation to a terrestrial environment.

Amphibians Retain Ancestral Receptors, While Reptiles Show Lineage-Specific Adaptations to Terrestrial Life

Phylogenetic & genomic study suggests that the core melatonin system is ancient. Amphibians are intermediator between aquatic & terrestrial vertebrates as a result many of their genes are conserved to early vertebrates [29]. They live both in aquatic & terrestrial habitat so they retained more ancestral paralogs subtype compared to streamlined or specialized systems found [31]. They also maintain Mel1c (MTNR1C), receptor that other mammals lack; it is responsible for pigment aggregation and metamorphosis [2]. Since amphibians are exposed to diversified foreign entities, recent research on TLRs (Toll-like receptors) expose that it does act as sensor detector for conserved molecules and plays major role in their immune system, shows evolutionary demand has pressurized shaping melatonin system & helped strengthen their immune system as well [46]. Study shows that while transition from amphibians to terrestrial the endocrine structure (hypothalamus) is highly conserved in amphibian (Xenopus laevis) and reptiles, but when some reptilians meet harsher environmental demands, they show variation in gene expression [13]. There exists a flexible melatonergic system in amphibians but not in reptiles, where adaptation is more oriented towards thermoregulation and water retention. Reptiles are dependent on constant temperatures on land, making them independent of aquatic habitats [11,43]. One study suggested that in amphibians, their epidermal barrier genes exhibit both conserved and diversified genes which includes Epidermal Differentiation Complex (EDC) genes suggesting transitional stage, while in reptiles their epidermal barrier genes are more specialised and advanced comparatively (preventing water loss), thus they have scaly skin (*Sachslehner& Eckhart*, 2022).

Bioinformatics Insights

Phylogenetic Analysis and Sequence Alignment

The phylogenetics analyses indicates that the 7 transmembrane helical structure helps in the receptor folding, activation mechanisms, evolutionary classification [10]. The structure contains residues like TM3, TM5, TM6 & lastly TM7 which are highly conserved across vertebrates from amphibians to mammals [7]. Also, it contains 3 extracellular and intracellular loops [36]. When talking about ECL particularly ECL2, its flexible, as a result significant functional divergence is observed, while the Intracellular loops (ICLs) show conservation to compatible signalling with G- proteins [36]. The extracellular loops (ECLs) acts as a gatekeeper for the melatonin ligand; studies suggests that the divergence in these loops lead to species-specific variations with adaptive roles (*Okamoto et al.,* 2024). In amphibians and reptiles, this divergence is due to past genomic events like genome duplication [26]. Through structural & phylogenetical analysis, highly conserved transmembrane domains are observed [3] Additionally, this implies that ancestral genome duplication is one of the causes of diversification of melatonin receptor in various vertebrate species [26].

Structural Modelling

Despite the fact that the MT1 & MT2 have a conserved G- Protein Coupled Receptor (*Wang et al.,* 2022). homology modelling studies shows that minor alterations in amino acids can result in variation that affect the binding pocket, polarity, and binding of melatonin, and in receptor sensitivity [36]. These structural differences suggest ecological adaptations amphibians exhibits receptor flexibility that may be because of adaption to maintain signalling efficiency during low- energy conditions [31]. While reptiles show more rigidity, for stability under high thermal stress [43]. Furthermore, structural modelling highlights that melatonin receptor sensitivity is not influenced solely by final binding rather the flexibility of specific receptor regions, particularly the ECL2 "lid" which do cover the binding pocket, and this may vary across species influencing the receptor sensitivity; more open conformation will highly sensitive towards low melatonin level and (help amphibians for survival in dim & moist conditions) while rigidity in lid lead lower sensitivity and higher concentration of melatonin for activation (*Wang et al.,* 2022) [36].

Functional Implications

Amphibians: Melatonin Signaling Tightly Integrated with Reproductive Cycles and Seasonal Breeding

Melatonin plays a pivotal role in reproductive cycles and seasonal breeding in amphibians, its secretion is dependent upon light/dark cycles, thereby encoding information about photoperiodism and seasonal changes [40]. It's associated with regulation of circadian rhythm and other biological activities in amphibians. Breeding in them is linked with externals cues for an instance environment, temperature, light, rainfall (*Wright,* 2022). In seasonal breeding it translates day length information into endocrine response, and its dependant on duration of night time. Reproductive effects of melatonin are done through interaction with Hypothalamic Pituitary

Gonadal (HPG) axis which releases Gonadotropin-Releasing Hormone (GnRH) which plays major role in reproduction [35]. Also, it contributes in oocyte maturation (which is highly sensitive to oxidative stress caused by ROS/RNSs) due to exhibiting antioxidative properties it neutralizes ROS, through that it creates antioxidant defence mechanism [34]. Studies shows that changes in melatonin level due to light can cause effect in the reproductive activity (ensuring favourable timing for reproduction for survival) and endocrine responses [40]. It influences the production of neurosteroid 7 α- hydroxy pregnenolone which affects behavioural, physiological, and endocrine responses (and helps in synchronization of reproductive activity with optimal environmental conditions) which detects environmental changes and change hormonal activity accordingly [37].

Reptiles: Greater Emphasis on Thermoregulation and Adaptation to Arid or Variable Environments

The reproductive physiology in reptiles is strongly connected to thermoregulation and changes in environment; they are ectothermic organisms (rely on external environmental cues to regulate their body temperature), which helps them in shaping their timing of reproduction and environmental adaptations, resulting in thermoregulation [25,41]. Reptiles exhibit specialized structure through which they are able to detect the environmental fluctuations (thermoreception) and enables their thermoregulation behaviour such as basking in light, seeking shade, or burrowing [21], and helps them in maintaining their circadian rhythm. Unlike endothermic vertebrates (where photoperiod plays a major role), reptiles are more dependent upon the temperature for maintaining their internal body temperature; thus, temperature plays a critical role in the determination of the timing of their reproductive activity [33] by affecting their hypothalamic-pituitary-gonadal (HPG) axis Neural control in reptiles includes integration of photoperiodic and thermal cues within the hypothalamus, which leads to secretion of important hormones such as GnRH [43]. Melatonin helps in synchronizing internal biological activities with external environmental conditions and ensuring the favourable reproductive timing to increase the chances of offspring survival [40].

Conclusion

The comparative bioinformatics approach to the study of melatonin receptors in amphibians and reptiles proves to be extremely productive. It highlights the high degree of evolutionary conservation of specific amino acid sequences in melatonin receptors found in both these organisms. However, the presence of variations in extracellular and intracellular regions of melatonin receptors in amphibians and reptiles shows the specific adaptation peculiarities in each of these groups. The processes of gene duplication and divergence explain the evolution of various types of melatonin receptors from a phylogenetic perspective.

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