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Mecanismos sistêmicos e locais sobre a modulação da produção de melatonina no contexto do eixo imune-pineal

Systemic and local modulatory mechanisms of melatonin production in the context of the immune-pineal axis

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Resumo

O Eixo Imune-Pineal (EIP) é a comunicação entre a pineal e o sistema imunológico durante as respostas imunes. Citocinas inflamatórias e padrões moleculares associados a patógenos/perigos bloqueiam a síntese de melatonina da pineal, permitindo a migração de células imunes para o local da lesão enquanto induz sua produção por células imunocompetentes. Uma melhor compreensão dos mecanismos responsáveis de ajustar a síntese de melatonina pineal e extra-pineal melhoraria nossa capacidade de modular esse sistema em condições fisiopatológicas. Na presente Tese, determinamos o perfil cíclico do sistema melatonérgico em células imunes, como as células do sistema imune e pineal respondem aos sinais imunológicos e como a ativação desequilibrada do EIP pode induzir uma doença inflamatória crônica. Primeiro, demonstramos que fagócitos e linfócitos T da medula óssea (MO) e baço apresentam ritmos diários de enzimas melatonérgicas e produzem melatonina após estimulação com LPS e IL10. A IL10 é uma citocina anti-inflamatória que exerce seus efeitos ativando a via STAT3, um fator de transcrição capaz de interagir e regular a via NFκB. A IL10 leva à ativação de (P)STAT3/NFKB na pineal, células da MO e esplenócitos, aumentando a síntese de melatonina. A IL10 também reduziu a síntese de melatonina nas células peritoneais. Consequentemente, variações nos níveis de IL10 durante as respostas imunes podem ser um fator regulador da síntese de melatonina pineal e extra-pineal. Finalmente, usando um modelo animal de inflamação crônica (artrite reumatóide, AR), comparamos a ativação do EIP em animais resistentes (RES) e que desenvolveram AR. A conversa cruzada adrenal-pineal foi alterada em animais com AR, levando a uma diminuição da relação 6-sulfatoximelatonina/corticosterona. Essa razão foi inversamente correlacionada com o escore inflamatório (composto pelas citocinas IL-1β, MCP-1, IL-2 e IL-4) e o tamanho da lesão inflamatória, fornecendo fortes evidências de que a interação positiva adrenal/pineal é um mecanismo precoce evitando a cronificação inflamatória. Em conclusão, a presente Tese aumenta nossa compreensão dos mecanismos que ajustam a síntese de melatonina pineal e extra-pineal ao local e tempo corretos durante as respostas imunes.

Palavras-chave: Eixo Imune-Pineal, Melatonina, Glicocorticoides, Citocinas, Inflamação Crônica, STAT3, NFκB, Células do Sistema Imune.

Abstract

The immune-pineal axis (IPA) is the communication between the pineal and the immune system during immune responses. Inflammatory cytokines and pathogen/danger-associated molecular patterns block pineal's melatonin synthesis, allowing the migration of immune cells to the injury site while inducing its production by immunocompetent cells. A better understanding of the mechanisms responsible for adjusting pineal and extra-pineal melatonin synthesis would improve our ability to modulate this system in pathophysiological conditions. In the present Thesis, we determined the cyclic profile of the melatonergic system in immune cells, how pineal and immune system cells respond to immunological signals and how the unbalanced activation of IPA might induce a chronic inflammatory disease. First, we demonstrated that phagocytes and T-lymphocytes of the bone marrow (BM) and spleen show daily rhythms of melatonergic enzymes and produce melatonin following LPS and IL10 stimulation. IL10 is an anti-inflammatory cytokine that exerts its effects by activating the STAT3 pathway, a transcription factor capable of interacting with and regulating the NFκB pathway. IL10 leads to (P)STAT3/NFKB activation in the pineal, BM cells, and splenocytes, increasing melatonin synthesis. IL10 also reduced melatonin synthesis in peritoneal cells. Consequently, variations in IL10 levels during immune responses may be a regulatory factor of the pineal and extra-pineal melatonin synthesis. Finally, using an animal model of chronic inflammation (rheumatoid arthritis, RA), we compared IPA activation in resistant (RES) and animals that developed RA. The adrenal-pineal crosstalk was altered in RA animals leading to a decreased 6-sulfatoxymelatonin/corticosterone ratio. This ratio was inversely correlated with an inflammatory score (composed of the cytokines IL-1β, MCP-1, IL-2, and IL-4) and the inflammatory lesion's size, providing strong evidence that adrenal/pineal positive interaction is an early mechanism avoiding inflammatory chronification. In conclusion, the present Thesis increases our understanding of mechanisms adjusting pineal and extra-pineal melatonin synthesis to the right place and time during immune responses.

Keywords: Immune-Pineal Axis, Melatonin, Glucocorticoids, Cytokines, Chronic Inflammation, STAT3, NFκB, Immune System Cells.

Initial Considerations

In vertebrates, melatonin is produced rhythmically by pinealocytes in the pineal gland (Simonneaux & Ribelayga, 2003; Carrillo-Vico et al., 2013; Da Silveira Cruz-Machado et al., 2017; Markus et al., 2018). Melatonin production is carried out from the hydroxylation and decarboxylation of the amino acid tryptophan in serotonin, which is acetylated by arylalkylamine-N-acetyltransferase (AANAT) to form N-acetylserotonin (NAS). In the final step of the biosynthetic pathway, the acetylserotonin-O-methyltransferase enzyme (ASMT) converts NAS into melatonin. The synthesis is triggered by the sympathetic noradrenaline (NA) released exclusively at night and the consequent activation of β1-adrenergic receptors (β1-AR) located in the membrane of pinealocytes. Once synthesized, melatonin is released into the bloodstream reaching central and peripheral tissues. Although ASMT is the enzyme with the lower catalytic activity of the pathway, AANAT is considered the key enzyme of melatonin synthesis because its rhythmical expression profile reflects the coordination imposed by the biological clock located in the suprachiasmatic nuclei.

The nocturnal β1-AR activation increases AANAT activity by the cAMP-PKA-CREB pathway, inducing the transcription (e.g., rodents) and protecting the enzyme from proteasome degradation (e.g., humans). Other transcription factors might also adjust the transcription of the Aanat gene (Cecon et al., 2010; Fernandes et al., 2016; Markus et al., 2018; Barbosa Lima et al., 2019). In this sense, the expression and nuclear translocation of p50/p50 homodimer of the nuclear factor κB (NFκB) block noradrenaline-induced Aanat transcription (Ferreira et al., 2005; Cecon et al., 2010). Interestingly, NFκB p50/p50

homodimer is constitutively translocated to the nucleus of pinealocytes during the light phase and is abruptly reduced at the beginning of the dark phase in the pineal gland (Cecon et al., 2010). While in an inactivated state, NFκB localizes in the cytosol complexed with the inhibitory protein IκBα but, once activated by a variety of signals that lead to IκBα phosphorylation and subsequent dissociation, NFκB translocate into the nucleus and binds to specific DNA sequences (Ghosh et al., 1998; Hayden & Ghosh, 2008). NFκB family has five members: RelA (or p65), RelB, cRel, p50, and p52. Of these members, p65, RelB, and cRel possess a transactivation domain (TAD) and induce gene transcription, while the TAD- p50 subunits typically work as gene repressors (Zhong et al., 2002; Guan et al., 2005; Yu et al., 2009; Elsharkawy et al., 2010). NFκB TAD+ dimers increase melatonin synthesis in the pineal (mainly p50/p65 and p65/p65 NFκB dimers) and phagocytes (p50/p65 and cRel/p65 NFκB dimers) by increasing Aanat transcription (Muxel et al., 2012, 2016; Markus et al., 2013, 2018; Barbosa Lima et al., 2019). In contrast, NFκB TAD- dimers are associated with melatonin inhibition (mainly p50/p50 NFκB dimers) by blocking Aanat transcription (Ferreira et al., 2005; Markus et al., 2013; Muxel et al., 2016; Barbosa Lima et al., 2019).

The pleiotropic effects of melatonin are exerted through four principal mechanisms: binding to membrane receptors, binding to nuclear receptors; interacting with intracellular proteins; and receptor-independent radical scavenging (Hardeland, 2008; Mahmood, 2019). Receptor-mediated effects are triggered by three subtypes of known melatonin receptors (MT). Subtypes MT1 (pM binding affinity) and MT2 (nM binding affinity) are G-protein-coupled receptors (GPCRs), while MT3 (μM binding affinity) is a quinone reductase that

has not yet been characterized in mammals. Melatonin has amphiphilic properties that allow it to permeate the cellular membrane by passive diffusion. Intracellularly, melatonin might also function through the nuclear retinoic acid receptor-related orphan receptors (ROR) family. Although whether or not melatonin is an actual ligand of these receptors is still controversial, it is clear that melatonin or its metabolites can indirectly modulate ROR expression and function (Slominski et al., 2016; Ma et al., 2021). Regarding non-receptor-mediated effects, melatonin binds to intracellular calcium-binding proteins such as calmodulin and calreticulin (nM binding affinity) (Hardeland, 2008; Argueta et al., 2022). It also acts as a free radical scavenger interacting with hydroxyl radicals and neutralizing hydrogen peroxide, singlet oxygen, peroxynitrite anion, nitric oxide, and hypochlorous acid (Reiter et al., 2000). Nevertheless, melatonin protects the cell from oxidative damage by activating membrane and intracellular MT that stimulates the transcription of antioxidative enzymes such as superoxide dismutase, glutathione peroxidase, and glutathione reductase, increases the efficacy of the mitochondrial electron transport chain and reduces electron leakage (Reiter et al., 2000; Hardeland, 2008).

Melatonin receptors have been found in the brain, retina, cardiovascular system, liver, colon, skin, kidney, and immune system cells (Pandi-Perumal et al., 2008; Emet et al., 2016). In addition to the pineal, measurable levels of melatonin and its synthetic enzymes (AANAT, PAANAT, and ASMT) are also found in extra-pineal tissues, including the brain, retina, gastrointestinal tract, airway epithelium, spleen, bone marrow (BM) and different cells of the immune system (Acuña-Castroviejo et al., 2014; Córdoba-Moreno et al., 2020). Coordinated but independent pathways regulate pineal and extra-pineal

melatonin production in healthy conditions. However, when the immune system is activated, immunomodulatory signals such as pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) trigger a more intrinsic communication among melatonin sources. In this sense, in the early phase of the immune activation, transcriptional factors as NFκB blocks pineal melatonin synthesis and redirects it towards immune cells at the site of injury (Markus et al., 2013). Increased sympathetic tone together with high levels of glucocorticoids (GC) also contribute to the blockade of pineal melatonin synthesis in this phase. On the other hand, once the inflammatory focus is resolved, the sympathetic tone decreases and high GC levels modulate the return of pineal melatonin (Lopes et al., 2001; Couto-Moraes et al., 2009; Markus et al., 2018). The bidirectional communication between the immune system and the pineal gland is known as the immune-pineal axis (IPA) (Markus et al., 2018) (Figure1).

The immune system is a complex network of different types of cells, tissues, and organs that help maintain/restore the body's homeostasis. In this Doctoral Thesis, the melatonin pathway of different cells and organs of the immune system was studied to evaluate the mechanisms that adjust melatonin synthesis in different tissues and scenarios. We also seek to demonstrate how an unbalanced IPA activation might lead to the chronification of an inflammatory process. The results found in this work increased our understanding that melatonin is differentially synthesized before, during, and after IPA activation depending on the cellular environment to fulfill and coordinate specific functions related to the locations (Figure1).

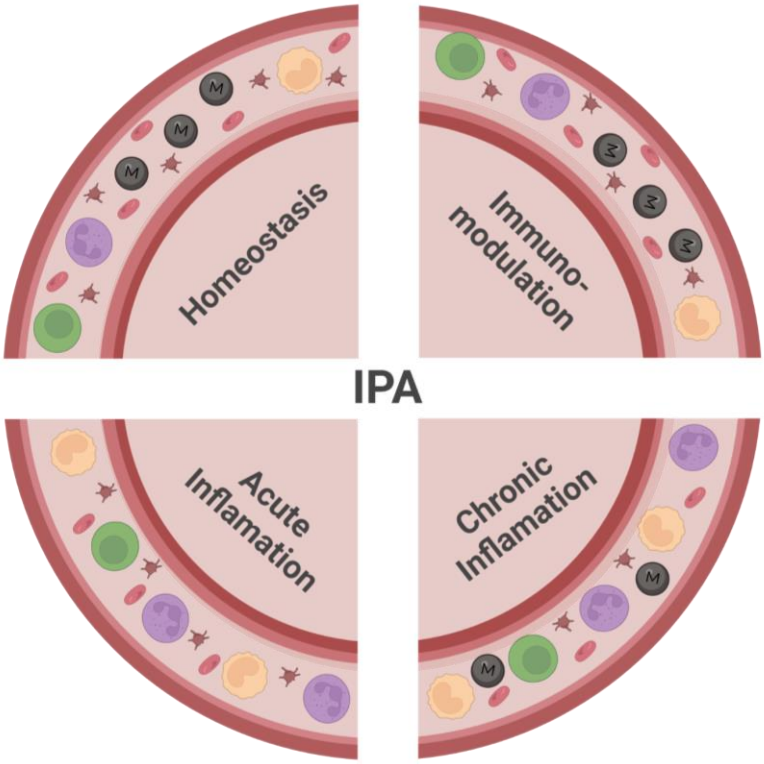

Figure 1. Immune-Pineal Axis (IPA). Communication between the pineal and the immune system occurs in the different phases of the immune surveillance/response: homeostasis, immunomodulation, acute inflammation, and chronic inflammation. During these phases, circulating levels of pineal melatonin regulate the nocturnal migration of leukocytes

Conclusions

The activation of the immune response is a necessary process that allows the body to defend itself against malignant agents, where the correct assembly of the response is going to be crucial to determine the success of the recovery of homeostasis. An exacerbated response can lead to an immunological lack of control, triggering chronic inflammation (e.g., rheumatoid arthritis) or immunosuppression. On the other hand, a diminished response would not cover all the requirements, leaving the organism unprotected and free to be controlled by the pathogen. The Immune-Pineal Axis is the communication between the pineal and the immune system that is established when the immune response is activated and shows that directing melatonin synthesis from the pineal to the injury site is a critical step that will allow a balanced immune response.

Under basal conditions, β1-AR stimulation, GC release from the adrenal gland, and the daily variation of NFκB control pineal melatonin synthesis. This melatonin regulates various circadian functions, including maintaining endothelial cells in a non-reactive phenotype that inhibits the expression of adhesion molecules and controls cellular migration. When the immune system is activated, pineal melatonin synthesis is blocked by the increase in GC levels with the α1 + β1-AR simultaneous adrenergic stimulation or by PAMPs and DAMPs, allowing leukocyte migration. At the injury site, PAMPs, DAMPs, and proinflammatory cytokines (such as TNF) induce melatonin synthesis by activating the NFκB pathway TAD+ in active immune cells. This melatonin, added to that already produced locally, reaches intracellular concentrations where it mainly performs anti-inflammatory functions and helps to prevent

oxidative damage. Once the inflammatory focus is resolved (during the recovery phase), or when the sympathetic tone is reduced (only β1-ARs are activated - during the recovery phase or in a chronic inflammation process), pineal melatonin levels return thanks to GC-induced GR activation and NFκB inhibition. During chronic inflammation, high levels of TNF continue to induce melatonin synthesis at the site of injury, and the relationship established between pineal melatonin and GC levels is decisive for controlling inflammation. Finally, in the recovery phase, TNF-induced melatonin synthesis might be blocked by IL10 by activating the TAD-/ NFκB and STATs pathways, and the body returns to a homeostatic phase.

Another important aspect of this Thesis is the more profound understanding of the intracellular mechanisms that regulate melatonin synthesis in the pineal and extra-pineal sources. In addition to the GR and NFκB interactions described above, the role of the STAT family (mainly STAT1 and STAT3) appears to be crucial in differentially regulating melatonin synthesis. According to cell type and the phase of the immune response, STAT3 activation can interact and activate the inductive or inhibitory pathways of NFκB, adjusting DNA binding and thus regulating AANAT and ASMT transcriptions and melatonin synthesis.

Thus, future works should focus on finding strategies to redirect melatonin synthesis to the right place and time. In this sense, it is first necessary to understand the integrative mechanisms in each organismic context and develop strategies and parameters for measuring immunological factors in the blood and pineal/urinary melatonin levels.

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