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Original Article

Assessment of melatonin-alpha adrenergic receptor complexes by molecular docking analysis

Avaliação dos complexos melatonina-receptores alfa-adrenérgicos por análises de docagem molecular

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Abstract

The pineal melatonin (N-acetyl-5-methoxytryptamine) is a molecule associated in a way or another with probably all physiological systems, aiming to fulfil its functional integrative roles in central nervous system activity, sleep and wakefulness cycles, energy metabolism and thermoregulation, immune, reproductive, endocrine, cardiovascular, respiratory and excretory systems. Within this context, the present study aimed to assess *in silico* the formation of complexes between ligand melatonin and other potential receptor proteins by molecular docking analyses. The main steps established in this experimental procedure were: a) search and selection of the 3D structure of the melatonin from DrugBank; b) search and selection of 3D structures of other target receptor proteins using STRING, protein BLAST and database PDB; and c) formation of the complexes between melatonin and receptors selected using AutoDock4.0 server by molecular docking analyses. High reliability score and significant similarity were only identified between type 1B melatonin and alpha-2A adrenergic receptor. Thus, molecular docking assays were carried out using ligand melatonin and crystallographic structures of the alpha-2A adrenergic receptor coupled to an antagonist (ID PDB 6kux) and a partial agonist (ID PDB 6kuy) available in the database PDB. Binding energy values of -6.79 and -6.98 kcal/mol and structural stability by non-covalent intermolecular interactions were predicted during the formation of complexes between melatonin and alpha-2A adrenergic receptor 6kux and 6kuy, respectively. In this way, the findings described in current study may indicate strong interactions between melatonin and adrenoceptors, suggesting its possible partial agonist effect on the activation of the alfa-2A adrenergic receptor.

Keywords: ligand-receptor complex, binding energy, intermolecular interactions, *in silico* prediction.

Resumo

A melatonina pineal (N-acetil-5-metoxitriptamina) é uma molécula associada de um modo ou outro com provavelmente todos os sistemas fisiológicos, visando cumprir seus papéis funcionais integradores na atividade do sistema nervoso central, ciclos de sono e vigília, metabolismo energético e termorregulação, sistemas imunológico, reprodutivo, endócrino, cardiovascular, respiratório e excretor. Assim, o presente estudo objetivou avaliar *in silico* a formação de complexos entre o ligante melatonina e outras proteínas potenciais receptoras por meio de análises de docagem molecular. As principais etapas estabelecidas neste procedimento experimental foram: a) busca e seleção da estrutura 3D da melatonina a partir do banco de dados DrugBank; b) busca e seleção de estruturas 3D de outras proteínas receptoras-alvo utilizando STRING, proteína BLAST e o banco de dados PDB; e c) avaliação da formação dos complexos entre melatonina e receptores selecionados a partir do servidor AutoDock4.0 para análises de docagem molecular. Alto escore de confiabilidade e similaridade significativa foram identificados apenas entre a melatonina do tipo 1B e o receptor alfa-2A adrenérgico. Valores de energia de ligação de -6,79 e -6,98 kcal/mol e estabilidade estrutural pela presença de interações intermoleculares não covalentes foram preditos durante a formação de complexos entre o ligante melatonina e os receptores adrenérgico alfa-2A 6kux e 6kuy, respectivamente. Dessa forma, os achados descritos no presente estudo podem indicar fortes interações entre melatonina e adrenoceptores, sugerindo seu possível efeito agonista parcial na ativação do receptor alfa-2A adrenérgico.

Palavras-chave: complexo ligante-receptor, energia de ligação, interações intermoleculares, predição *in silico*.

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1. Introduction

The pineal melatonin (N-acetyl-5-methoxytryptamine) is a neurohormone that maintains circadian rhythms by synchronization to environmental cues and is involved in diverse physiological processes, such as the regulation of blood pressure and core body temperature, oncogenesis, and immune function (Genario et al, 2021). Melatonin is readily released into the bloodstream and cerebrospinal fluid, presenting immediate (non-receptor and receptor-mediated) and prospective (chronobiotic, seasonal, and transgenerational) effects that are dependent on its distinct ways of action in innumerous living organisms of different types of habitats (Amaral and Cipolla-Neto, 2018). Due to its amphiphilicity, melatonin is able to cross the cell, organelles, and nuclear membranes and directly interact with intracellular molecules in the non–receptor-mediated actions. Furthermore, melatonin also presents receptor-mediated actions that result from the interaction of this hormone with both membrane and nuclear receptors (Cipolla-Neto and Amaral, 2018). There is an abundance of reviews evaluating the effects of exogenous and endogenous melatonin on health and its biological actions have been shown to be associated with a wide variety of health outcomes in clinically and methodologically heterogeneous populations (Posadzki et al., 2018).

In silico prediction of the potential chemical interactions between a ligand and a protein at the atomic level has been carried out to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes using Bioinformatics' tools (McConkey et al., 2007, Meng et al., 2011, Muhammad et al., 2021). The growing increase of scientific knowledge about structural informatics, genomics and proteomic have contributed for drug discovery and development using computer simulation procedure to predict the possible conformation of a binary receptor-ligand complex (Chaudhary and Mishra, 2016). With the docking strategies, the druggability of the compounds and their specificity against a particular target can be calculated for further lead optimization processes (Pagadala et al., 2017). According to Cava and Castiglioni (2020), the integration of molecular docking and *in vitro* studies may accelerate the discovery and development of new drugs for treatment of several pathologies and metabolic disorders.

Within this context, the purpose of current study was to investigate *in silico* the formation of complexes between ligand melatonin and alternative target receptor proteins. The findings described in the current study may improve new insights into the identification of potential receptor proteins in response to neurohormone melatonin.

2. Materials and Methods

2.1. Search and selection of the human melatonin ligand from database DrugBank

For docking molecular analysis the structure and chemical formula of the small molecule of the human melatonin (accession number DB01065) were initially selected and downloaded in format PDB from DrugBank, a global provider of structured drug information and patient insight tools that accelerate drug research and improve healthcare delivery (Wishart et al., 2018).

2.2. Search and selection of potential receptor proteins correlated to ligand melatonin

Still according to DrugBank, two target receptor proteins activated by melatonin are yet well validated and characterized: melatonin receptor type 1A (UniProt ID P48039) and melatonin receptor type 1B (UniProt ID P49286). Amino acid sequences of the type 1A and 1B melatonin receptors were selected in format FASTA from UniProt, a comprehensive, high-quality and freely accessible resource of protein sequence and functional information (UniProt Consortium, 2021), and submitted to STRING analysis to infer protein-protein interaction networks functional enrichment analysis (Szklarczyk et al., 2019). STRING results demonstrated a direct correlation of both melatonin receptors with distinct target proteins, such as: different guanine nucleotide-binding protein subunits; pro-neuropeptide Y and pro-opiomelanocortin, with score values ranging from 0.922 to 0.940. Furthermore, STRING results for melatonin receptor type 1B exclusively demonstrated its significant interaction with alpha-2A adrenergic receptor (ADRA2A, UniProt ID P08913) in a score value of 0.920. Amino acid sequences of the type 1A and 1B melatonin receptors were comparatively aligned against the different receptor proteins indicated by STRING results using protein BLAST tools. Under these experimental conditions, the alpha-2A adrenergic receptor was the only protein sequence that yielded a significant comparative alignment to melatonin receptor type 1B with 28.64% identity and $1e^{-14}$ E-value. No significant alignments were detected for other protein receptors investigated.

Based on these findings, two crystal structures corresponding to the human alpha-2A adrenergic receptor were searched and selected from PDB database: a) PDB ID 6kux coupled to an antagonist RSC and b) PDB ID 6kuy in complex with a partial agonist. The alpha-2A adrenergic receptor has well characterized three active binding sites situated at following amino acids: aspartic acid Asp at position 128 associated with ligand binding, and two serines Ser at positions 215 and 219 implicated in catechol agonist binding and receptor activation binding (Wang et al., 1991). The aspartic acid is a non-essential amino acid that usually occurs as the negatively charged R group and its carboxyl groups are ionized at physiological pH, whereas the serine is an amino acid with a polar but neutral R group able to perform hydrogen bonds with water (Nelson and Cox, 2018). The 3D structures of both the human alpha-2A adrenergic receptors were downloaded in format PDB for further analysis.

2.3. Chemical interactions between ligand melatonin and alpha-2A adrenergic receptors by molecular docking analysis

The chemical interactions between ligand melatonin and crystallographic structures of the alpha-2A adrenergic receptors were assessed by molecular docking using AutoDock4.0 and AutoDockTools1.5.7 that provide a suite of

automated docking tools to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure (Morris et al., 2009). The preparation of the 3D structures of the receptors involved the addition of polar hydrogen and Gasteiger partial charges, and removal of non-polar hydrogen. The Lamarckian genetic algorithm (LGA) was selected for the ligand conformational search. The docking area was defined using AutoGrid, which a 50×50×50 3-D affinity grid was centered on the binding sites with a 0.375 Å grid point spacing. Under these experimental conditions, additional docking parameters were also defined as population size: 150, mutation rate: 0.02, crossover rate: 0.8, local search rate: 0.06, and maximum number of energy evaluations: 2,500,000. The other parameters were remained unchanged under these experimental conditions. The molecular docking interactions of the ligand melatonin in the active binding sites of the adrenergic receptor proteins were visualized from UCSF Chimera1.15 (Huang et al., 2014).

Only the best conformations predicted for the melatonin and alpha-2A adrenergic receptor complexes were automatically plotted for visualization of the protein-ligand interactions using program LIGPLOT (Wallace et al., 1995). The protein-ligand interactions are those mediated by hydrogen bondings and hydrophobic contacts. Hydrogen bondings are indicated by dashed lines among atoms in these interactions and their distances are automatically presented in angstroms (Å). Hydrophobic contacts are represented by arcs with spokes radiating towards the ligand atoms they contact, and the contacted atoms are shown with spokes radiating back.

3. Results

The binding energy values of -6.79 e -6.98 kcal/mol were estimated for the complexes between melatonin and adrenergic receptors 6kux and 6kuy, respectively. Inhibition constants of 7.67 and 10.46 µM were calculated for melatonin-adrenergic receptors 6kux and 6kuy, respectively. The ligand efficiency of -0.4/-0.41 and torsional energy of 1.19 kcal/mol were estimated for both the adrenergic receptors tested. Intermolecular energy of -8.17 and -7.99 kcal/mol, electrostatic energy of -0.15 and -0.03 kcal/mol, and vdW+Hbond+desolv energy of -8.02 and -7.95 kcal/mol were revealed for adrenergic receptors 6kux and 6kuy, respectively. Total internal energy of -0.35 and un-bound energy of -0.44 kcal/mol were similar for both the receptors tested. As shown in Figure 1A and B, the ligand melatonin directly targeted at the active binding sites of the adrenergic receptor proteins analyzed, as defined in the AutoGrid box for both complexes.

Hydrogen bondings and hydrophobic interactions were revealed in the complexes between melatonin and adrenergic receptor proteins (Figure 2A and B). Two hydrogen bondings were demonstrated in the ligand-receptor 6kux complex: a) a hydrogen atom linked to the heteroatom nitrogen of the indole ring of the melatonin interacted with the oxygen situated in double bonding in the carboxyl group of the amino acid Asp113 of the target receptor at a distance of 2.77 Å, and b) a hydrogen atom of the hydroxyl radical of the side chain of the amino acid Asp113 of the target receptor interacted with the nitrogen atom not belonging to indole ring of the ligand melatonin at a distance of 3.0 Å (Figure 2A). Furthermore, 12 hydrophobic interactions were identified between atoms of the ligand melatonin and of the side chains of the following amino acids of the adrenergic receptor 6kux: phenylalanine Phe391, cysteine Cys201, serine Ser200, threonine Thr118, tryptophan Trp387, tyrosine Tyr394, serine Ser204, phenylalanine Phe412, phenylalanine Phe390, tyrosine Tyr416, valine Val114 and cysteine Cys117 (Figure 2A). Furthermore, two hydrogen bondings were identified in the formation of the melatonin-adrenergic protein 6kuy complex: a) a hydrogen atom linked to heteroatom nitrogen of the indole ring of the melatonin interacted with the oxygen situated in double bonding in the carboxyl group of the amino acid Asp113 of the target receptor at a distance of 2.64 Å, and b) an oxygen atom of the amide group of the melatonin interacted with the hydrogen linked to the oxygen of the side chain of the amino acid Ser204 of the target receptor at a distance of 3.12 Å (Figure 2B). Furthermore, 10 hydrophobic interactions were identified between atoms of the melatonin and the side chains of the following amino acids of the adrenergic receptor 6kuy: phenylalanine Phe116, phenylalanine Phe391, tryptophan Trp387, phenylalanine Phe412, phenylalanine Phe390, cysteine Cys201, valine Val197, tyrosine Tyr394, serine Ser200 and valine Val114 (Figure 2B).

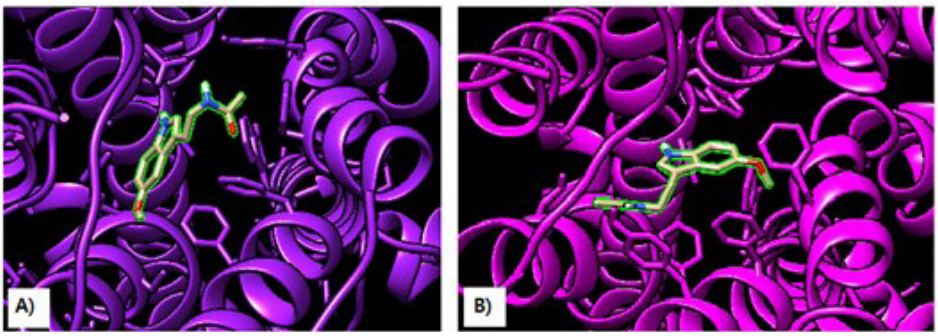

Figure 1. Molecular docking interactions of the ligand melatonin (in green) in the active binding sites of the adrenoceptors visualized from UCSF Chimera1.15. Tridimensional structures of the adrenergic receptors 6kux in purple (A) and 6kuy in pink (B).

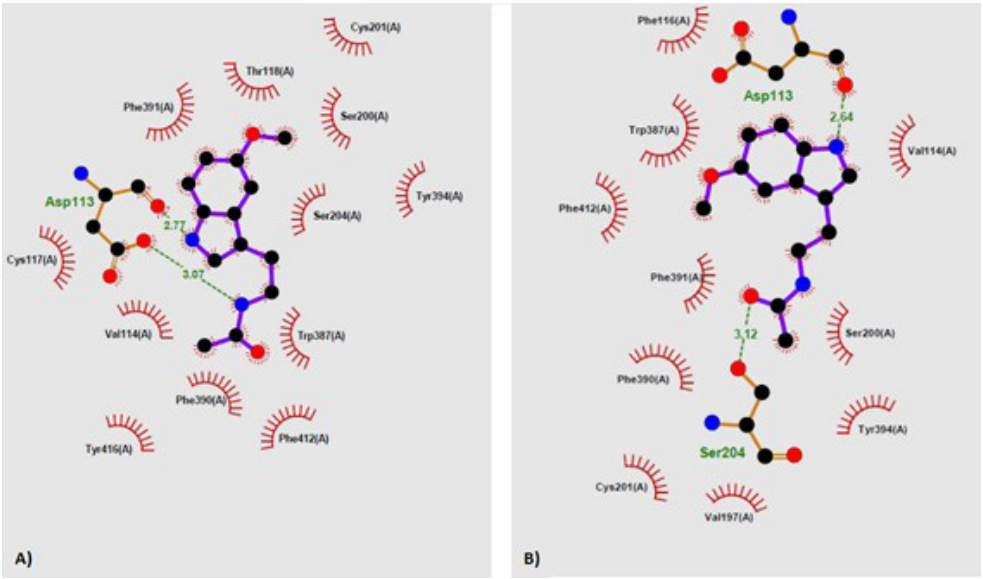

Figure 2. Two-dimensional schematic diagrams of the intermolecular interactions in the complexes between ligand melatonin and alpha-2A adrenergic receptor 6kux (A) and 6kuy (B). The ligand melatonin is positioned in the center of the diagrams and its nitrogen, oxygen and carbon atoms are indicated in blue, red and black spheres, respectively, connected to each other by solid blue lines. The amino acids Asp113 and Ser204 of the active binding sites of the receptor proteins are indicated by nitrogen, oxygen and carbon atoms represented as blue, red and black spheres, respectively, connected to each other by solid yellow lines. Hydrogen bonding interactions in the ligand-receptor complexes are represented in green dashed lines with their respective distances in angstroms Å. Hydrophobic contacts are represented in red arcs with spokes radiating towards the ligand atoms they contact, and the contacted atoms are shown with spokes radiating back.

4. Discussion

Alpha-2A adrenoceptors were predicted as potential target receptors to the ligand melatonin under these experimental conditions, revealing lowest binding energy values and structural stability by hydrophobic and hydrogen bonding interactions (Figures 1 and 2). Binding energy characterizes as the sum of the favorable and unfavorable values for the formation of a protein-ligand complex (Guimarães, 2012). According to Russel (1994), a characteristic of the spontaneous reactions is the tendency of systems to seek a state of lower energy. Within this context, more negative binding energy values are preferentially favorable for successful interactions between a ligand and a receptor protein. Furthermore, hydrophobic and hydrogen bonding interactions play a crucial role during the formation of ligand-protein complexes. Hydrogen bonding is a special interaction type involving a hydrogen atom located between a pair of other atoms having a high affinity for electrons (Rozenberg, 2002). While the hydrophobic interactions comprehend the tendency of non-polar groups or molecules to aggregate in water solution, since the water molecules of the medium tend to form a solvation layer around the hydrophobic molecules, increasing the degree of organization of the water molecules and maximizing the hydrogen bonding (Delatorre, 2015). To reverse this process and increase the entropy, hydrophobic interactions occur in order to minimize the solvent organization. Therefore, hydrophobic interactions are specially detected in the most active binding sites of the receptor proteins and exert a crucial role during formation of ligand-protein complexes.

Adrenergic receptors are transmembrane proteins located in both neuronal and non-neuronal tissues belonged to G protein-coupled receptor superfamily that mediate the actions of the endogenous catecholamines secreted in the adrenal medulla (Piascik and Perez, 2001). Alpha-adrenoceptors are especially involved in most excitatory functions (vasoconstriction, uterine musculature contraction, urethra contraction, pupil dilation, amongst others) and one important inhibitory function (intestinal relaxation) (Civantos Calzada and Aleixandre de Artiñano, 2001). Interestingly, experimental findings have revealed a possible interaction between ligand melatonin and adrenergic receptors with strong physiological implications. Crooke et al. (2013) confirmed *in vitro* the indirect action of melatoninergic compounds on adrenergic receptors and their remarkable effect upon the ocular hypotensive action in rabbits mainly of alpha-2 adrenergic receptor agonists but also of beta-adrenergic antagonists. More recently, Alkozi et al. (2020) demonstrated that α-adrenergic and melatonin receptors form functional complexes in which the C-terminal tail of the adrenergic receptor play a relevant role, identifying that the number of complexes significantly decreased in models of glaucoma and, more importantly, in human samples of glaucoma patients. Remarkably, co-instillation of melatonin resulted in long-term decreases in the intraocular pressure in a well-established animal model of glaucoma, hypothesizing that melatonin could normalize pressure in glaucoma (Alkozi et al., 2020).

These findings are crucial to understand the potential physiological functions and implications of the melatonin supplementation to address pathological dysfunctions by targeting distinct receptors and their complexes.

The best affinity energy values and satisfactory structural stability by intermolecular interactions were especially demonstrated in the formation of complexes between melatonin and alpha-2A adrenergic receptor 6kuy, whose 3D PDB structure is originally coupled to a partial agonist substrate. These findings may suggest a possible partial agonist effect of the melatonin on these adrenoceptors. Based on the preliminary data predicted in current study, additional procedures must be established to ensure the experimental validation of the melatonin-alpha 2A adrenergic receptor complexes, as well as to test the biological viability of this interaction ligand-receptor of interest and its positive effects on the treatment of distinct pathologies and metabolic disorders.

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