ANEXO 5 DP Cardinali publicaciones conjuntas

Propuesta de la Facultad de Medicina de la Universidad de Granada para el Grado de “Doctor Honoris Causa” de la Universidad de Granada del Profesor Daniel P. Cardinali de la Facultad de Ciencias Médicas de la Pontificia Universidad Católica de Buenos Aires (Argentina).

Journal of Pineal Research 3:101–109 (1986)

Diurnal Variations of Benzodiazepine Binding in Rat Cerebral Cortex: Disruption by Pinealectomy

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In a previous work, pinealectomy was found to depress benzodiazepine (BZP) receptor binding in cerebral cortex membranes of rats killed at noon. In order to assess the effect of pineal removal on diurnal variations of BZP binding site concentration and affinity, groups of intact, pinealectomized, or sham-pinealectomized rats (subjected to surgery 2 wk earlier) were killed at six different time intervals during the 24-h cycle. BZP binding was assessed by Scatchard analysis of ^{3}H-flunitrazepam high-affinity binding to cerebral cortex membranes. In intact and sham-pinealectomized rats, a maximum in BZP receptor concentration was found at midnight. Pinealectomy blunted the nocturnal peak of receptor concentration and caused a significant depression of binding site number at noon. No changes in the affinity of the binding sites for the radioligand were detected as a function of time of day or following surgery. In a dose-response experiment for melatonin ability to restore the depressed BZP receptor concentration of cerebral cortex membranes of pinealectomized rats killed at noon, a minimal effective dose of 25 μg/kg body weight was obtained. These results further support a link between pineal activity and brain BZP receptors in rats.

Key words: brain benzodiazepine binding, pineal gland, diurnal rhythms, melatonin, pinealectomy

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Changes in Gamma-Aminobutyric Acid High Affinity Binding to Cerebral Cortex Membranes after Pinealectomy or Melatonin Administration to Rats[1]

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Key Words. GABA binding · Pineal gland · Cerebral cortex · Superior cervical ganglion · Melatonin

Abstract. In order to assess the effect of pinealectomy (Px) on the diurnal rhythmicity of gamma-aminobutyric acid (GABA) high affinity binding to cerebral cortex membranes, groups of intact, Px or sham Px rats (subjected to surgery 15 days earlier) were killed at six different time intervals during the 24-hour cycle. GABA binding was estimated by Scatchard analysis of ^{3}H-GABA binding to cerebral cortex membranes prepared from individual brains; only one type of binding site with dissociation constant (K_D) about 20–50 n*M* and site number (B_{max}) about 200–500 fmol/mg protein was apparent in the assay conditions employed. In intact and sham Px rats B_{max} attained minimal values at night and increased during daylight. Px increased generally B_{max} and disrupted its normal diurnal rhythmicity, a peak in B_{max} being observed at midnight. A significant decrease of GABA high affinity binding affinity was detected at morning hours in intact rats and at late scotophase and morning hours in Px and sham Px rats. B_{max} of GABA high affinity binding in Px rats attained maximal values by 5–10 days after surgery and decreased somewhat 5 days later. Sham Px rats exhibited a transient increase in B_{max} up to 10 days after surgery, returning to normal values by the 15th day. Superior cervical ganglionectomy increased binding affinity up to 15 days after surgery without affecting B_{max}. The minimal melatonin effective dose to counteract Px-induced increase of GABA high affinity binding was 25 μg/kg body weight when given 3 h before sacrifice. Melatonin activity on GABA binding did not depend upon a direct effect on the binding sites, as shown in vitro. These results suggest a link between pineal function, melatonin secretion and GABA receptor activity in rats.

It is generally accepted that gamma-aminobutyric acid (GABA) is a major inhibitory transmitter in both invertebrate and vertebrate central nervous system (CNS) [25, 32]. GABA transmitter function involves a rapid (i.e. milliseconds) increase of postsynaptic membrane conductance to Cl^- ions following interaction of GABA with its recognition sites. The GABA receptor complex in vertebrate neurons contains a bicuculline-sensitive receptor for GABA ($GABA_A$ receptor) in association with sites for benzodiazepine and barbiturates-picrotoxin that regulate responses to GABA [25]. Benzodiazepines potentiate responses to GABA and its analogue muscimol while both benzodiazepines and barbiturates reduce the potency of the convulsant agent picrotoxin as an antagonist of GABA [19, 34]. Therefore, modulation of the GABA receptor-Cl^- ionophore complex may underly the sedative, hypnotic and anticonvulsant effects of benzodiazepines and barbiturates in vertebrates [25].

In a previous report we observed that pineal removal, a surgical procedure causing a convulsive-prone [6, 24, 28–30, 32, 33] and epileptoid state in several mammals, decreased brain benzodiazepine binding in rats, and that melatonin, a compound that causes mild sedation and torpor [35], restored benzodiazepine receptor levels [22]. Since melatonin had been reported to increase GABA concentration in brain [2, 3], we considered it worthwhile to examine the changes in brain GABA high affinity binding after manipulation of pineal activity or melatonin injection to rats.

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Functional Links Between Benzodiazepine and GABA Receptors and Pineal Activity

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INTRODUCTION

The critical role of the pineal gland in the photoperiodic circannual control of endocrine activity of certain mammals has been well established. This function is mediated by the secretion of melatonin, and the discrete, nocturnal elevation of this hormone encodes daylength information to the neuroendocrine apparatus (7). Melatonin acts on receptor sites in the brain by affecting several presumptive second messengers, including cyclic nucleotides, prostaglandins and Ca^{2+} in neuronal and glial cells (10). In mammals melatonin secretion is controlled by primary neural signals given by peripheral and central pinealopetal fibers, and secondary modulatory influences provided by several hormones (9).

Among the putative neurotransmitters found in pineal nerve endings, norepinephrine (NE) is paramount in enhancing melatonin synthesis. Injection of NE or of its β-adrenoceptor agonist isoproterenol increases melatonin production both in innervated and denervated pineal glands, and the in vitro addition of either drug to rat pineal organ cultures stimulates melatonin release, thus supporting the conclusion that NE plays a causal rather than a permissive role in diurnal melatonin rhythm in this species (4).

It must be noted however that the striking in vitro or in vivo effect of β-adrenergic stimulation on rat melatonin synthesis is either difficult to obtain or very tiny in hamsters (35), cows (12), or sheep (14,37). In this respect significant central pinealopetal connections have been described in several mammals, and non-adrenergic transmitters like different neuropeptides γ-aminobutyric acid (GABA) or glutamate may play a role in the central pathways controlling the circadian rhythm of melatonin release (12). This article summarizes the available information suggesting a

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Melatonin-Induced Oncostasis, Mechanisms and Clinical Relevance

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Abstract

Melatonin is a natural substance ubiquitously distributed and present in almost all living species, from unicellular organisms to humans. Melatonin is synthesized not only in the pineal gland but also in most tissues in the body where it may have a cytoprotective function via paracrine or autocrine effects. Melatonin is effective in suppressing neoplastic growth in a variety of tumors. The mechanisms involved include antiproliferative effects via modulation of cell cycle, ability to induce apoptosis in cancer cells, anti-angiogenic and antimetastatic effects, anti-estrogenic activity, the capacity to decrease telomerase activity, immune modulation, and direct and indirect antioxidant effects. Besides these oncostatic properties, melatonin deserves to be considered in the treatment of cancer for two other reasons. First, because its hypnotic-chronobiotic properties, melatonin use that can allow the clinician to effectively address sleep disturbances, a major co-morbidity in cancer. Second, because melatonin's anxiolytic and antidepressant effects, it has a possible application in two other major co-morbidities seen in cancer patients, i.e. depression and anxiety. This report summarizes the possible mechanisms involved in melatonin oncostasis and reviews what is known about the clinical application of melatonin as an adjuvant therapy in cancer patients.

Keywords: Melatonin; Cancer; Apoptosis; Antioxidant; Angiogenesis; Estrogen signaling pathway; Metastasis

Abbreviations: 13-HODE: 13-hydroxyoctadecadienoic Acid; AKT: Protein Kinase B; AIF: Apoptosis-Inducing Factor; Bax: B cell lymphoma Bcl-2 associated X protein; Bid: BH3 Interacting-Domain Death Agonist; Ca2+/CaM: Calcium/Calmodulin; cAMP: Cyclic Adenosine Monophosphate; Cdk: Cyclin/Cyclin-Dependent Kinase; COX: Cyclooxygenase; E2: Estradiol; EGF: Epidermal Growth Factor; EGFR: Epidermal Growth Factor Receptor; EMT: Epithelial–Mesenchymal Transition; ER: Estrogen Receptor; ERE: Estrogen Response Element; ERK: Extracellular-Signal-Regulated Kinase; ET-1: Endothelin-1; GSK3β: Glycogen Synthase Kinase 3; Hif1a: Hypoxia-Inducible Factor 1; IGF: Insulin-Like Growth Factor; IL: Interleukin; iNOS: Inducible Nitric Oxide Synthase; L1: Element 1 Retrotransposon; MAPK: Mitogen-Activated Protein Kinase; MET: Mesenchymal-To-Epithelial Transition; MT: Melatonin Receptor; mTOR: Mammalian Target Of Rapamycin; MyD88: Myeloid Differentiation Primary Response Gene 88; NF-κB: Nuclear Factor Kappa-Light-Chain-Enhancer Of Activated B Cells; NK: Natural Killer; NO: Nitric Oxide; PI3K: Phosphatidylinositol-4,5-Bisphosphate 3-Kinase; PK: Protein Kinase; RNS: Reactive Nitrogen Species; ROS: Reactive Oxygen Species; SCN: Suprachiasmatic Nuclei; Smad: Mothers Against Decapentaplegic Homolog; Sirt: Sirtuin; TERT: Telomerase Catalytic Protein Component; TGF: Transforming Growth Factor; Th: T Helper; TNF: Tumor Necrosis Factor; uPA: Urokinase-Type Plasminogen Activator; VEGF: Vascular Endothelial Growth Factor

Introduction

Melatonin is a ubiquitous methoxyindole present in most living species, including unicellular microorganisms, plants, most invertebrates and vertebrates and humans. The first function of melatonin in phylogeny may have been cytoprotective [1]. As such, melatonin could be among the natural molecules that are effective in treating neoplastic malignancies. Despite a number of studies that have established the potentiality of melatonin as an adjuvant in the treatment of cancer melatonin's importance on cancer therapy remains largely unappreciated. Several aspects of this subject have been reviewed elsewhere [2-8]. The aim of this report is to update the present knowledge on the possible mechanisms involved in melatonin oncostasis (Figure 1) and to assess what is known about the therapeutic application of melatonin in cancer patients.

Melatonin Oncostasis

Antiproliferative effects

Numerous studies have shown that melatonin has remarkable oncostatic properties and can reduce the promotion and/or progression of tumors. Its antiproliferative properties have been demonstrated in an extensive variety of tumors including breast, endometrial, prostate, colon, and ovarian cancers, choriocarcinoma, melanoma, neuroblastoma, osteosarcoma, and leukemia, with particular efficacy in lymphoproliferative tumors [9-15] (Figure 2).

Melatonin exerts direct anticancer actions by inhibiting the proliferation and growth of tumor cells. The potential signaling pathway responsible for inhibiting cell proliferation requires further investigation, but several explanations are possible, as follows.

Modulating the cell cycle: Melatonin increases the duration of the cell cycle in cancer cells by either expanding the G1 phase (thus

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