Melatonin Role in Experimental Arthritis

Daniel P. Cardinali*[1], Ana P. García[1], Pilar Cano[2] and Ana I. Esquifino[2]

[1]*Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina, and* [2]*Departamento de Bioquímica y Biología Molecular III, Facultad de Medicina, Universidad Complutense, Madrid, Spain*

Abstract: Our perception of the function of the pineal gland and its hormone melatonin has attained a new dimension during the last decade. Through melatonin, the pineal becomes a principal organ present in vertebrates involved in the control of rhythmic adaptations to daily and seasonal cycles. Melatonin is synthesized and secreted during the dark period of the light/dark cycle. The rhythmic nocturnal melatonin secretion is directly generated by the circadian clock and is entrained to a 24-hour period by the light-dark cycle. The periodic secretion of melatonin may be used as a circadian mediator to any system than can "read" the message. Melatonin acts as an arm of the circadian clock, giving a time-related signal to a number of body functions; one of them is the circadian organization of the immune response. This review discusses melatonin role in rheumatoid arthritis. Animal studies employing Freund's complete mycobacterial adjuvant (FCA) as a model of rheumatoid arthritis are described. Immune and neuroendocrine circadian rhythms were examined in FCA-injected rats, both in the preclinical phase of arthritis (2-3 days after FCA injection) as well as in the acute phase of the disease (18 days after FCA injection). In arthritic rats, the 24-h organization of immune and neuroendocrine responses becomes altered. Significant effects of immune response on diurnal rhythmicity of adenohypophysial and hypophysiotropic hormones occurred in arthritic rats. Melatonin treatment prevented alteration of 24-h rhythms of serum ACTH, prolactin and luteinizing hormone in rats injected with FCA. In addition, melatonin treatment prevented alteration of the 24-h variation in hypothalamic monoamine transmitter turnover during the preclinical phase of Freund's adjuvant arthritis in rats. A comparison between the inflammatory and immune responses elicited by physiological and pharmacological doses of melatonin in FCA arthritis is reported. Pinealectomized rats exhibited a significantly less pronounced inflammatory response, which was restored to normal by a low melatonin dose (0.3 μg/ml of drinking water), whereas a high melatonin dose (30 μg/ml) that resulted in a 50-60-fold increase in plasma melatonin, augmented the inflammatory and immune response. These results should be considered in the light of recent reports that rheumatoid arthritis patients have increased nocturnal plasma levels of melatonin and that their synovial macrophages respond to melatonin with an increased cytokine production.

Keywords: Freund's adjuvant arthritis, Melatonin, Circadian rhythms, Cytokines, Neuroimmune mechanisms, Spleen, Pineal gland, Pituitary hormones.

ADJUVANT ARTHRITIS IN RATS IS AN EXPERIMENTAL MODEL OF RHEUMATOID ARTHRITIS

Rheumatoid arthritis is a systemic inflammatory disorder that mainly affects the diarthrodial joint. It is the most common form of inflammatory arthritis and affects about 1% of the population, in a female/male ratio of 2.5/1. The disease can occur at any age, but it is most common among those aged 40–70 years. The geographic distribution of rheumatoid arthritis is worldwide, with a notably low prevalence in rural areas [1, 2].

Although it initially presents as a symmetrical polyarticular synovitis with prominent hand involvement, rheumatoid arthritis has multiple potential systemic manifestations. The clinical course of the disorder is extremely variable, ranging from mild, self-limiting arthritis to rapidly progressive multisystem inflammation with profound morbidity and mortality. Fever and weight loss can be part of the acute symptoms, while splenomegaly, vasculitis, neutropenia and amyloidosis are some of the disease's complications, which may occur in patients with long-standing disease [1, 3, 4].

Rheumatoid arthritis is a T-cell-driven autoimmune process associated with the production of autoantibodies. Rheumatoid arthritis is initiated by $CD4^+$ T cells, which amplify the immune response by stimulating other mononuclear cells, synovial fibroblasts, chondrocytes, and osteoclasts. The release of cytokines, especially tumor necrosis factor (TNF)-*a*, interleukin-1 (IL-1) and interleukin-6 (IL-6), causes synovial inflammation. In rheumatoid arthritis the inflammatory process, usually tightly regulated by mediators that initiate and maintain inflammation and mediators that shut the process down, becomes imbalanced leaving inflammation unchecked and resulting in the destruction of cartilage and bone. Joint damage derives from the degradation of connective tissue by matrix metalloproteinases and the stimulation of osteoclastogenesis by activated $CD4^+$ T cells.

*Address correspondence to this author at the Departamento de Fisiología, Facultad de Medicina, UBA, Paraguay 2155, 7o. Piso, 1121 Buenos Aires, Argentina; Tel/Fax: 54-11-59509611; E-mail: cardinal@mail.retina.ar

1568-0088/04 $45.00+.00

Efforts to develop safer and more effective treatments for rheumatoid arthritis rely heavily on the availability of suitable animal models [5]. Among these models, the rat's adjuvant arthritis is widely employed [6]. Hallmarks of this model are reliable onset and progression of easily measurable, polyarticular inflammation, marked bone resorption and periosteal bone proliferation. Induction of adjuvant disease can be done with either Freund's complete adjuvant (FCA) supplemented with mycobacterium or by injecting synthetic adjuvants [5, 6]. The pathogenesis for development of adjuvant disease following injection of mycobacterial preparations is not fully understood, although a cross-reactivity of mycobacterial wall antigens with cartilage proteoglycans occurs.

After FCA injection to rats, the inflammatory disease of the joints shows four stages in its time-course: preclinical (first week), acute (weeks 2-4), post-acute (weeks 5-8) and recovery (weeks 9-11) [7]. The preclinical stage of FCA arthritis (first week) is characterized by discrete radiological lesions of the forepaws and slight increase in the threshold for struggle triggered by foot pressure, presumably due to an impending, initially painless, stiffness. The acute stage or arthritis (weeks 2-4) is defined by signs of hyperalgesia, lack of mobility and a pause in body weight gain; during the acute period, hindpaw and forepaw joint diameters increase [7]. In the later, acute, stages of disease (day 12+), adjuvant arthritis rats are often relatively immobile due to severity of paw swelling. At day 18^{th}, an increase in scratching behavior and signs of hyperalgesia are clearly established as compared with the adjuvant's vehicle-injected group. FCA arthritis is induced most easily in inbred Lewis rats; it is also produced, to a milder extent, in Wistar and Sprague Dawley rats [8-12].

The use of the adjuvant's arthritis model offers an opportunity to study pathological changes in a variety of tissues other than the joints. Among these, central nervous system (CNS) changes are most relevant [13-15]. The major objective of our work during the last years has been to examine several circadian correlates of both the preclinical and the acute phases of arthritis in rats. The present work reviews evidence indicating that the production of melatonin, a major circulating signal for the circadian system, is affected in adjuvant arthritis and that endogenous as well as exogenously administered melatonin can modulate local and systemic symptoms in experimental arthritis.

EXPERIMENTAL ARTHRITIS DISRUPTS NORMAL CHRONOBIOLOGICAL ORGANIZATION

Symptomatology after FCA administration, like anorexia and depressed activity, is a part of a defense response to antigenic challenge and is mediated by the neural effects of proinflammatory cytokines like IL-1, IL-6, interferons (IFN) and TNF-α, which are directly involved in disease's pathophysiology [16]. Clinically, these central changes are known as "sickness behavior", i.e. the "nonspecific" symptoms (anorexia, depressed activity, loss of interest in usual activities, disappearance of body care activities) that accompany the response to infection. These "nonspecific" symptoms of infection include fever and profound psychological and behavioral changes, among them disruption of circadian structure [14]. Indeed, sick individuals show indication of decreased amplitude of circadian rhythmicity, like superficial sleep at night and hypersomnia, loss of interest and depressed activity during the day.

One possible mechanism through which changes in the circadian rhythms can occur is by modifying directly the activity of cells in the hypothalamic suprachiasmatic nuclei (SCN), the major central oscillator for 24-h rhythms. Cytokine receptors, e.g. IFN-γ receptors, have been detected in neuronal elements of ventrolateral SCN [17]. Expression of SCN IFN-γ receptors followed a 24-h rhythm, coinciding with the expression of janus kinase 1 and 2 as well as the signal transducer and activator of transcription factor 1, the main intracellular signaling pathway for IFN-γ. In an ontogenic study, SCN IFN-γ receptors were found to reach their adult pattern between postnatal day 11 and 20, at a time when capacity for photic entrainment of the pacemaker became established [18]. In a recent publication whether the intracerebroventricular administration of IFN-γ could modify 24-h wheel running activity was examined in the golden hamster. Animals received IFN-γ or saline at "Zeitgeber" time (ZT) 6 or ZT 18, with ZT12 defined as the time of light off. Intracerebroventricular administration of IFN-γ at ZT 6 produced a significant phase advance in acrophase of rhythm, an effect not seen with injection at ZT 18. IFN-γ depressed mesor value of rhythm significantly; the effect was seen both with ZT 6 and ZT 18 injections. These results support the view that the circadian sequels arising during the immune reaction can rely partly on central effects of IFN-γ [19]. A disruptive effect of systemic administration of IFN-α on the circadian rhythm of locomotor activity, body temperature and clock-gene mRNA expression in SCN has also been documented in mice [20].

In the last years we have examined a number of immune and neuroendocrine circadian rhythms in FCA-injected rats, looking for changes in the preclinical phase of arthritis (2-3 days after FCA injection) as well as in the acute phase of the disease (18 days after FCA injection). Generally, changes in circadian rhythms in lymph node immune function tended to be more profound at the preclinical phase of the disease. For example, B-cell- and T-cell-mediated mitogenic activity of lipopolysaccharide(LPS) and concanavalin (Con A), respectively, were modified in amplitude or acrophase during the preclinical phase [21] while exhibiting few or none changes during the acute phase of experimental arthritis [22]. Similarly, 24-h variations of B and T cells, as well as of $CD4^+$ (T helper) and $CD8^+$ (T cytolytic) cells became significantly changed during the preclinical phase [21, 23], with absence of changes during the acute phase [22]. In the case of lymph node cell proliferation and local autonomic nerve activity, the increase in amplitude and mesor of rhythms found in the preclinical phase of arthritis was higher than that observed as the disease progressed [24]. Therefore, the results suggest that some sort of homeostatic compensation of initial changes in circadian rhythmicity of immune changes occurs with the development of arthritis.

It must be noted that virtually all immunological variables investigated to date in animals and humans have been shown to display biological periodicity, the circadian being the best known [25-27]. Both the humoral arm and the

delayed (cellular) arm of the immune system function in a rhythmic manner. Circadian rhythmicity is revealed in circulating cells, lymphocyte metabolism and transformability, circulating hormones and other substances that may exert various actions on different targets of the immune system, cytokines, receptors, and adhesion molecules, cell cycle events in health and cancer, reactions to antigen challenge, and disease etiology and symptoms [25-27].

The bilateral anatomical location of submaxillary lymph nodes and their easily manipulable autonomic innervation allowed us to carry out the analysis of the humoral and neural mechanisms regulating the lymphoid organs. Both in immunized and in non-immunized rats a significant diurnal variation of submaxillary lymph node ornithine decarboxylase activity (ODC), an index of cell proliferation in a number of organs, including immunocompetent organs [28] and endocrine glands [29], was reported, displaying maximal activity at early afternoon [30]. Such a maximum coincided with peak mitotic responses to LPS and Con A in incubated lymph node cells [31]. A purely neural pathway including as a motor leg the autonomic nervous system innervating the lymph nodes was identified [32]. In addition, a hormonal pathway involving the circadian secretion of melatonin also plays a role to induce rhythmicity [33, 34].

As far as the changes in neuroendocrine rhythmicity during rat's arthritis is concerned, early data had indicated in FCA-injected rats that the 24-h organization of the biologic responses was altered. For example, morning-evening differences in circulating ACTH and corticosterone disappeared by days 7-21, and between days 6 and 8 after FCA injection a loss of the adrenocortical ODC circadian rhythm of activity was found [35]. In our own studies conducted during the preclinical phase of arthritis, a significant effect of immune-mediated inflammatory response on diurnal rhythmicity of circulating ACTH, growth hormone (GH), prolactin and thyrotropin (TSH) release was found, and was partially sensitive to immunosuppression by cyclosporine [36]. Further experiments indicated that hypothalamic levels of corticotrophin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), GH-releasing hormone (GHRH) and somatostatin were altered in the preclinical phase of arthritis [37]. In the median eminence, adjuvant's vehicle-injected rats exhibited significant 24-h variations for the four hypophysiotropic hormones examined, with maxima at noon. These 24-h rhythms were inhibited or suppressed 3 days after FCA injection. The administration of the immunosuppressant drug cyclosporine impaired the depressing effect of FCA injection on CRH, TRH and somatostatin content in median eminence, but not that on GHRH. The activity of cyclosporine was less evident in other hypothalamic regions examined [37]. Generally, a decrease amplitude or mesor of transmitter rhythms were detectable, mainly in anterior and medial hypothalamic regions [38].

We also examined the changes in circadian rhythms of CNS and hypophyseal hormones and neurotransmitters during the acute phase of Freund's adjuvant arthritis (i.e. 18 days after FCA administration). Differing from the relative compensation of circadian immune changes seen at this time of arthritis, changes in 24-h rhythms of neuroendocrine parameters persisted during the clinical phase of the disease [39]. Daily rhythms in plasma luteinizing hormone (LH), testosterone and TSH became suppressed or disrupted in arthritic rats. Concerning GH, the depressed mean values found in the preclinical phase of arthritis also persisted during the acute phase, as it was the case for the changes in catecholamine transmitter activity [40]. Twenty-four h variation in dopamine (DA) content were blunted in the anterior hypophyseal lobe but remained unaltered in the neurointermediate lobe [40].

MELATONIN ACTS AS AN ARM OF THE BIOLOGIC CLOCK

In mammals, circulating melatonin is synthesized primarily by the pineal gland and is released in a circadian fashion, being synthesized and secreted during the dark period of the light/dark cycle [41-43]. The rhythmic nocturnal melatonin secretion is directly generated by the circadian clock located in mammals in the SCN and is entrained to a 24-hour period by the light-dark cycle. The periodic secretion of melatonin may be used as a circadian mediator to any system than can "read" the message. In addition, direct effects of the hormone on the SCN could explain some of melatonin effects on the circadian system. Duration of melatonin nocturnal secretion is directly proportional to the length of the night and it has experimentally been demonstrated to be the critical parameter for photoperiod integration [44-46].

Melatonin is secreted by the pineal gland by simple diffusion. The lipophilicity of melatonin contributes to its easy passive diffusion across cell membranes as well as through cell layers. Radioactive melatonin administered intravenously rapidly disappears from the blood with a half-life of about 30 min. About 60-70% of melatonin in plasma is bound to albumin [47]. Most of melatonin in the general circulation is converted to 6-hydroxymelatonin in the liver, which clears 92-97% of circulating melatonin on a single pass [48]. Although several tissues can synthesize melatonin, circulating melatonin derives exclusively from the pineal gland, as it is indicated by the disappearance of melatonin from the circulation after pinealectomy [49], as shown in Fig. (**1**).

Melatonin is not only involved in the photoperiodic response but is also a direct circadian component in that it constitutes a signal driven by the clock and exporting information on time of day to (theoretically) every cell in the body. Since melatonin rhythm is an important efferent hormonal signal driven by the clock, its rhythmic secretion can therefore, be used as an internal synchronizer (or "internal Zeitgeber") [50]. Therefore, the melatonin signal can be considered an efferent hormonal signal downstream from the clock, which reflects the functioning of the pacemaker. Melatonin can be used as a mediator to impose circadian rhythmicity upon target structures, directly driving these rhythms [51].

Indeed melatonin regulates and modulates a myriad of physiological functions, including sleep, as well as circadian, visual, cerebrovascular, reproductive,

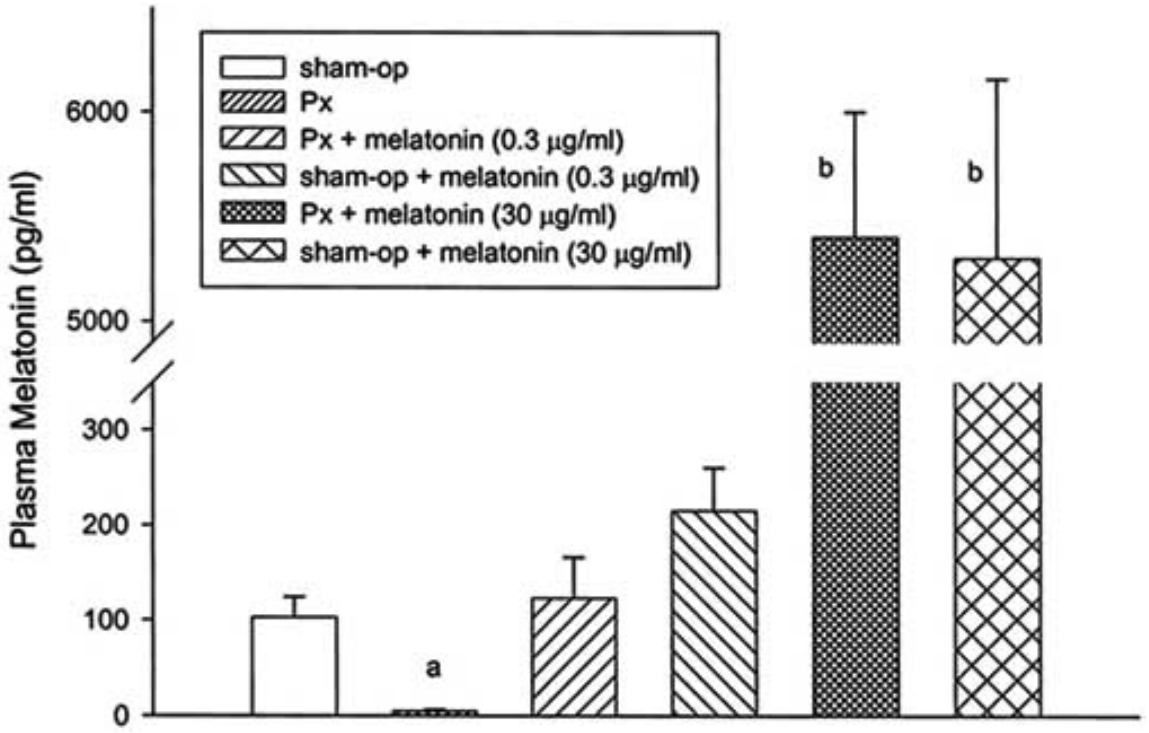

Fig. (1). Plasma values of melatonin at 0200 h in rats receiving melatonin dissolved in the drinking water at a concentration of 0.3 μg/ml or 30 μg/ml. Rats were pinealectomized (Px) or sham-operated 7 days earlier. Shown are the means ± SEM. Plasma melatonin concentration was measured by RIA. Letters indicate significant differences in a one-way ANOVA followed by a Student-Newman-Keuls test, as follows: (a) $p < 0.01$ vs. the remaining groups; (b) $p < 0.01$ vs. rats receiving none or 0.3 μg/ml melatonin.

neuroendocrine and neuroimmunological functions [42, 52-55]. Such a broad spectrum of functions and the timely fashion in which some of them are affected suggest that melatonin may signal through a variety of pathways precisely regulated.

The circadian effects of melatonin appear to be universal and, largely, similar among the diurnal and nocturnal species. This can be explained by the similarities in the temporal organization of their circadian system. Indeed, in both diurnal and nocturnal animals, SCN neurons are normally active during the day and slow down at night [56]. The activation of SCN neurons has an inhibitory effect on the pineal gland, defining a nocturnal pattern of melatonin secretion. If SCN neurons are activated at night, e.g. by environmental light perceived by the retina, melatonin production declines. Melatonin, in turn, can acutely attenuate the activity of SCN. This melatonin action is likely to support a normal decline in the activity of the SCN at night, further promoting melatonin secretion and contributing to an overall increase in the amplitude of circadian body rhythms [56]. A temporal and functional interplay between melatonin and SCN, and their response to environmental light, promote a temporal alignment of multiple circadian body rhythms with each other (internal synchronization) and with the periodic changes in the environment (external synchronization) [57].

In addition to an acute inhibition of SCN activity, melatonin administration can also produce a shift in the circadian phase of SCN activity, either advancing or delaying its onset. The direction of the phase-shift depends on the time of melatonin treatment, i.e. administration of melatonin in the late afternoon can advance the circadian clock, while early-morning treatment can cause a phase delay [56]. Studies conducted *in vitro* suggest that a chronobiological effect of melatonin, i.e. the induction of circadian phase shift, is likely to be explained by its direct effect on SCN neurons via MT2 receptors. In humans suffering from circadian sleep disorders, daily melatonin treatment can help to reinforce the circadian synchronization with the environment and entrain the physiological rhythms to a 24-hour cycle [42].

The first experiments on brain melatonin receptor sites were carried out in the late seventies by employing ^{3}H melatonin as a ligand indicating the existence of melatonin acceptor sites in bovine hypothalamic, cerebral cortex and cerebellar membranes [58]. This was followed by the discovery of the first functional melatonin receptor in a neuronal mammalian tissue, the rabbit retina [59]. Melatonin, at picomolar concentrations, inhibits the calcium-dependent release of DA from retina, through activation of a now well characterized melatonin presynaptic receptor.

The introduction of the 2-^{125}I-iodomelatonin analog, a major landmark in the melatonin receptor field [60], allowed detection of melatonin binding in several brain areas, the choroid plexus and in some brain arteries as well as in peripheral organs. Indeed, to have an organ devoid of melatonin binding site may constitute an exception rather than the rule [61-68].

The two cloned mammalian melatonin receptors so far [Mel1a [69]; Mel1b [70]], now referred as the MT1 and the MT2 melatonin receptors [71], belong to a subfamily of G-protein coupled receptors. They are defined as unique receptor types on the basis of their molecular structure and chromosomal localization[72]. A third mammalian melatonin receptor, the MT3 (previously referred as ML2) is yet to be cloned [73].

Among presumptive second messengers for melatonin action, the cAMP generating system has received paramount attention [74, 75]. The main signal transduction pathway of high affinity MT1 receptors in both neuronal and non-neuronal tissues is the inhibition of cAMP formation through a pertussis toxin sensitive inhibitory Gi protein. Coupling of the high affinity melatonin receptors to other signaling pathways has also been reported. Melatonin augmented cGMP levels, decreased Ca^{2+} influx and inhibited arachidonate acid conversion and prostaglandin synthesis (for ref. see [76]). Direct effects of melatonin on calmodulin and other intracellular proteins, nuclear receptor activity for melatonin, and the free radical scavenging properties of melatonin should be also considered.

Indeed, melatonin is a significant free radical scavenger and antioxidant at both physiological and pharmacological concentrations [77-79]. Besides its ability to directly neutralize a number of free radicals and reactive oxygen and nitrogen species, melatonin stimulates several antioxidative enzymes that increase its efficiency as an antioxidant. Melatonin suppresses the activity of singlet oxygen, superoxide anion radical, hydroxyl radical, peroxyl radical, and finally the peroxynitrite anion. Melatonin is able to protect macromolecules in all compartments of the cell from oxidative damage and especially in cell membranes due to its high lipid solubility, making them more resistant to oxidative attack [77-79].

MELATONIN IS A CIRCADIAN IMMUNOREGULATORY SIGNAL

Pineal ablation, or any other experimental procedure that inhibits melatonin synthesis and secretion induces a state of immunodepression, which is partly counteracted by melatonin in several species [55, 80-85]. Melatonin treatment increases T cell proliferation [86, 87], enhances antigen presentation by macrophages to T cells by increasing the expression of MHC class II molecules [86], activates splenic, lymph node and bone marrow cells [88, 89], stimulates antibody-dependent cellular cytotoxicity [90] and augments natural and acquired immunity [91-93]. Melatonin also stimulates natural killer (NK) cell activity [94, 95], activates monocytes [96], increases the number of Th-2 lymphocytes [97], augments $CD4^+$ lymphocytes and decreased $CD8^+$ lymphocytes in submaxillary lymph nodes [38], restores impaired Th cell activity in immuno-depressed mice [98] and augments antibody responses *in vivo* [81, 86, 91, 99, 100]. Concerning B cells, there is information on melatonin inhibition of apoptosis during early B-cell development in mouse bone marrow [101].

Besides the release of proinflammatory Th-1 cytokines, such as IFN-γ and IL-2, administration of melatonin to antigen-primed mice increased the production of IL-10, indicating that melatonin also activates anti-inflammatory Th-2-like immune responses [102]. It is not yet clear whether melatonin acts only on Th-1 cells or also on Th-2 cells. This is an important subject as the Th-1/Th-2 balance is significant for the immune response [55]. Relevant to this, melatonin treatment suppressed the subsequent *in vitro* stimulation by the mitogenic agents LPS (that stimulates B cells) and Con A (that stimulates T cells) in submaxillary lymph nodes [38]. In addition, an inhibitory influence of melatonin on parameters of the immune function has also been demonstrated, i.e. in human lymphocytes NK cell activity, DNA, IFN-γ and TNF-α synthesis, as well as the proliferation of T lymphocytes and lymphoblastoid cell lines were depressed by melatonin [103-105]. In our own studies, melatonin treatment exerted an inhibitory influence on submaxillary lymph node cytolytic, $CD8^+$ cells [21].

Melatonin possesses anti-inflammatory activity [106]. It reduces tissue destruction during inflammatory reactions by a number of means, among them scavenging of free radicals [107]. Additionally, melatonin prevents the translocation of nuclear factor-kappa B to the nucleus and its binding to DNA, thereby reducing the up-regulation of a variety of proinflammatory cytokines (for ref. see [108]). There is evidence that melatonin inhibits the production of adhesion molecules that promote the sticking of leukocytes to endothelial cells, attenuating transendothelial cell migration and edema [109].

The subcellular mechanisms involved in the immunomodulatory activity of melatonin remain to be elucidated. High affinity binding sites for melatonin have been described in membrane homogenates of thymus, bursa of Fabricius, and spleen of a number of birds and mammals [91]. High-affinity binding sites also exist in bone marrow $CD4^+$ cells. T cell activation significantly increased melatonin binding [87]. Melatonin binding sites and melatonin receptor mRNA were detected in human $CD4^+$ and $CD8^+$ cells and B cells [110].

Such a predominant effect on $CD4^+$ cells is supported by the observations on melatonin efficacy to augment $CD4^+$ cells in submaxillary lymph nodes [38]. However, expression of MT1 melatonin receptor was found in all thymic lymphocyte subpopulations ($CD4^+$,$CD8^+$, doubled positive, doubled negative, and B cells), indicating possible effects of melatonin on all these cells [101, 111]. Human monocytes express melatonin receptors depending on their state of maturation, *in vitro* monocyte differentiation affecting negatively melatonin receptor expression [112].

Besides membrane receptors, nuclear receptors for melatonin occur in human and murine immunocompetent cells. Specific binding of melatonin is detectable in purified cell nuclei from spleen and thymus of rats [113]. Melatonin could be the natural ligand for nuclear orphan receptors RZR/ROR. The effect of melatonin on cytokine production in human peripheral blood mononuclear cells depends on the activation of nuclear rather than membrane receptors [114].

As already mentioned, melatonin is a potent antioxidant. In addition, melatonin could affect centrally the release of hormone in the hypothalamic-hypophyseal unit [76] as well as the activity of autonomic pathways to the lymphoid organs [115].

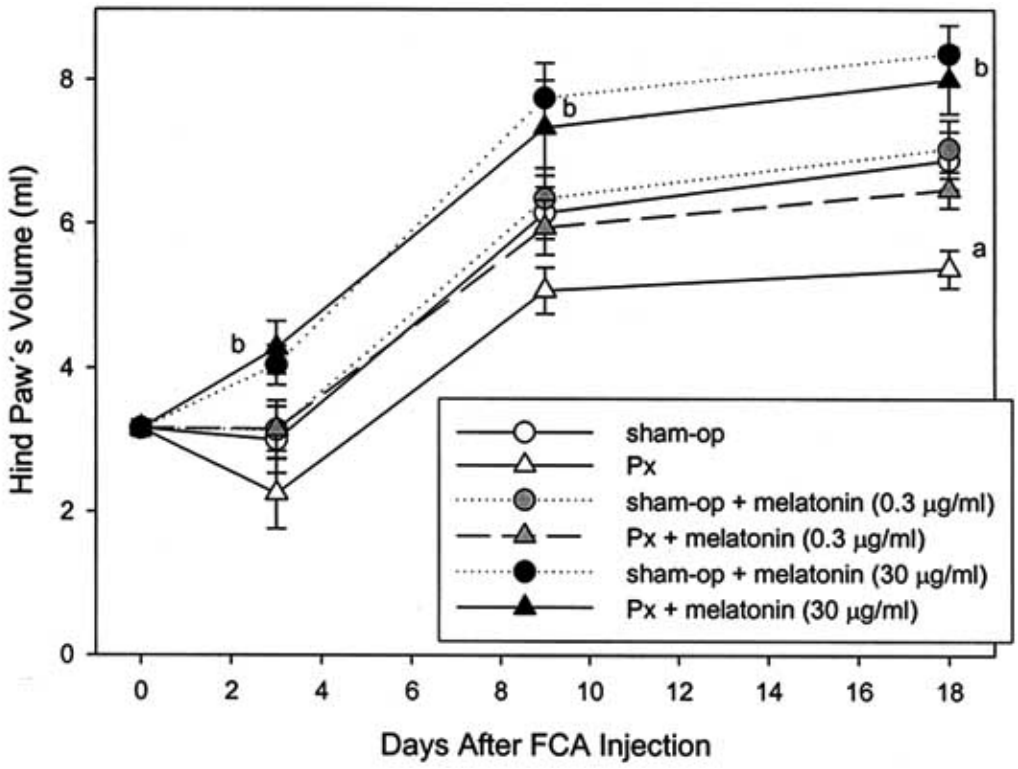

Fig. (2). Effect of pinealectomy (Px) and melatonin on inflammation of hind paws in Wistar rats. Groups of 8 rats were studied on days 0, 3, 9 and 18 after the s.c. injection of Freund's complete adjuvant. Rats were pinealectomized or sham-operated 7 days earlier. Melatonin was administered in drinking water at two concentrations (0.3 and 30 μg/ml) from day 0. Assessment of arthritis development was made by plethysmography [33]. Shown are the means ± SEM. Letters indicate significant differences in a one-way ANOVA followed by a Student-Newman-Keuls test, as follows: (a) $p < 0.05$ vs. the remaining groups; (b) $p < 0.05$ vs. the corresponding group not receiving melatonin.

Our studies on melatonin role in arthritis have been mainly addressed to examine the participation of melatonin in regulation of circadian rhythmicity of immune parameters in rats [38, 116]. Pretreatment for 11 days with a pharmacological dose of melatonin (100 μg) administered s.c. at the end of the light phase of daily photoperiod affected some aspects of the early phase of the immune response elicited by FCA injection, at the preclinical phase of disease. Cell proliferation in rat submaxillary lymph nodes and spleen during the immune reaction (as assessed by ODC activity) exhibited a pineal-dependent diurnal rhythmicity, as it was reduced by pinealectomy or pineal sympathetic denervation [117, 118]. This effect was counteracted by a pharmacological melatonin dose (100 μg/day). Exogenous melatonin also restored the reduced amplitude in diurnal rhythms of lymph node or splenic tyrosine hydroxylase (TH) activity and lymph node acetylcholine synthesis [117, 118].

Further examination of melatonin activity on circadian rhythmicity of cell proliferation in submaxillary lymph nodes and spleen at the clinical phase of arthritis was conducted in young and old Sprague-Dawley rats [33]. Pineal melatonin content was measured, as well as the efficacy of melatonin treatment to recover modified circadian rhythmicity of submaxillary lymph node and splenic ODC and TH activities and of lymph node ^{3}H-acetylcholine synthesis. After seventeen daily injections of 10 or 100 μg of melatonin at the evening, the treatment restored the inflammatory response in old rats (assessed plethysmographically in hind paws) to the level found in young animals. In young rats, an inflammation-promoting effect of 100 μg melatonin could be demonstrated. As a consequence of the immune reaction, submaxillary lymph node and splenic lymph cell proliferation augmented significantly, with acrophases of 24-hour rhythms at the afternoon for lymph nodes or in the morning for spleen. Mesor and amplitude of ODC rhythm were lowest in old rats, while melatonin injection generally augmented its amplitude. Lymph node and splenic TH activity attained maximal values at early night, while maxima in lymph node. ^{3}H-acetylcholine synthesis occurred at the afternoon. Amplitude and mesor of these rhythms were lowest in old rats, an effect generally counteracted by melatonin treatment. The results were compatible with an age-dependent, significant depression of pineal melatonin synthesis during adjuvant-induced arthritis and with decreased amplitude of circadian rhythms in immune cell proliferation and autonomic activity in lymph nodes and spleen at the clinical phase of the disease. This picture was generally counteracted by melatonin injection, mainly in old rats [33].

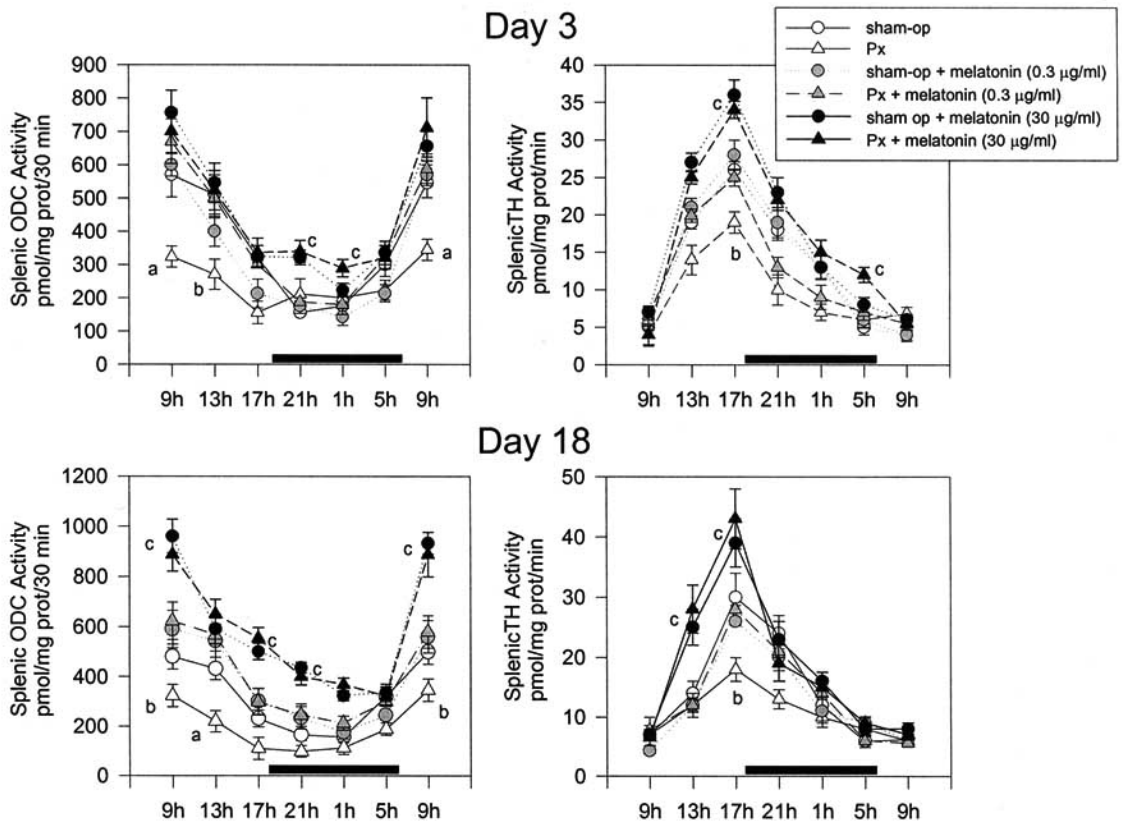

Fig. (3). Effect of pinealectomy (Px) and melatonin on 24-hour changes in splenic ornithine decarboxylase (ODC) and tyrosine hydroxylase (TH) activities. Groups of 8 rats were studied on days 3 and 18 after the s.c. injection of Freund's complete adjuvant. Rats were pinealectomized or sham-operated 7 days earlier. Melatonin was administered in drinking water at two concentrations (0.3 and 30 μg/ml) from day 0. Shown are the means ± SEM. ODC and TH were measured as described elsewhere [33]. Letters indicate significant differences in a one-way ANOVA followed by a Student-Newman-Keuls test, as follows: (a) $p < 0.01$ vs. the remaining groups; (b) $p < 0.05$ vs. the remaining groups; (c) $p < 0.05$ vs. rats receiving none or 0.3 μg/ml melatonin.

A number of studies were carried out to examine the participation of melatonin in altered 24-h rhythms of serum ACTH, GH, prolactin, LH and insulin in rats at the preclinical phase of Freund's adjuvant arthritis [119]. The data indicated that several early changes in levels and 24-h rhythms of circulating ACTH, prolactin and LH in FCA-injected rats were sensitive to treatment with melatonin (100 μg). An effect of melatonin treatment on 24-h variations in hypothalamic serotonin (5-HT) and DA turnover during the preclinical phase of Freund's adjuvant arthritis was also apparent [120]. FCA injection suppressed circadian rhythmicity of 5-HT turnover in the anterior hypothalamus, an effect prevented by the previous injection of melatonin. Melatonin decreased 5-HT turnover rate in the anterior hypothalamus. Melatonin also prevented the changes in 5-HT turnover of medial hypothalamus evoked by Freund's adjuvant. As far as hypothalamic DA turnover is concerned, the preventing effect of melatonin was less clear, sometimes synergizing with the mycobacterial adjuvant to modify the normal 24-h pattern detected in hypothalamic regions [120].

It must be noted that the pharmacological effect of melatonin on the immune response may not always be beneficial, particularly in young subjects. In autoimmune arthritis developed in mice with type II rat collagen melatonin s.c. administered at a 1 mg/kg dose induced a more severe arthritis. Accordingly, pinealectomy in two strains of mice immunized with rat type II collagen reduced severity of the arthritis as shown by a slower onset of the disease, a less severe course of the disease (reduced clinical scores) and reduced serum levels of anti-collagen II antibodies [121, 122]. We demonstrated, by using a 100 μg dose of melatonin, an inflammation-promoting effect in young rats injected with FCA [33]. Therefore, high levels of melatonin in young animals may stimulate the immune system and cause exacerbation of both autoimmune collagen II and mycobacterial arthritis.

In the experiments shown in Fig. (**2**) and (**3**) we wished to examine inflammatory and immune responses in pinealectomized and sham-operated rats injected with FCA and receiving low, within the physiological range, melatonin

dose or a high pharmacological dose in the drinking water. Physiological circulating levels of melatonin at midnight in rats are about 90 pg/ml in rats [123], while the melatonin levels achieved within 15 min after the systemic administration of a 100 μg-dose are about 30 or 200 ng/ml plasma [124], with a half-life of about 20 min [123].

In our study melatonin was dissolved in ethanol and was added to the drinking water at a concentration of 0.3 or 30 μg/ml; the final ethanol concentration was 0.01% for both melatonin-treated and control rats. As reported elsewhere, adult rats drank about 20 ml/day with 90-95% of this total daily water taken up during the dark period [125]. Thus melatonin doses were approximately 6 and 600 μg/day. Plasma values in sham-operated and pinealectomized rats after both melatonin doses, assessed at 0200 h are depicted in Fig. (**1**).

Fig. (**2**) depicts the effect of the two melatonin doses on inflammation of hind paws in pinealectomized or sham-operated rats injected with FCA, when assessed plethysmographically. Pinealectomized rats exhibited a significantly less pronounced inflammatory response, which was restored to normal by the low melatonin dose. The high melatonin dose, which resulted in a 50-60-fold increase in plasma melatonin (Fig. (**1**) augmented the inflammatory response.

Fig. (**3**) shows the changes in splenic ODC and TH activity at day 3 and 18 after FCA injection in pinealectomized or sham-operated rats receiving the two melatonin doses. It can be concluded that a physiological dose of melatonin was effective to counteract the impaired response of lymph node ODC seen in pinealectomized rats, whereas in pharmacological amounts melatonin exacerbated the immune response and presynaptic noradrenergic activity in the spleen.

Recent data indicate that rheumatoid arthritis patients have increased nocturnal plasma levels of melatonin and that their synovial macrophages respond to melatonin with an increased production of IL-12 and nitric oxide [126, 127]. These results and those of Fig. (**2**) and (**3**) underline the potential contraindication of using pharmacological amounts of melatonin in arthritic patients.

Recent data support the notion that melatonin has beneficial effects on bone and cartilage destruction in arthritis. Destruction of cartilage and bone in rheumatoid arthritis is produced by the degradation of connective tissue by matrix metalloproteinases and by the stimulation of osteoclastogenesis by activated $CD4^+$ T cells [128]. Osteoclastogenesis is controlled by growth factors and cytokines produced in bone marrow microenvironment and by the action of systemic hormones, like parathyroid hormone, estradiol or growth hormone.

Another candidate for hormonal modulation of bone cells is melatonin [129]. Melatonin is present in high quantities in bone marrow [130], where precursors of bone cells are located. Melatonin dose-dependently augmented proteins that are incorporated into the bone matrix, like procollagen type I c-peptide [131, 132]. Osteoprotegerin, an osteoblastic protein that inhibits the differentiation of osteoclasts is also augmented by melatonin *in vitro* [133]. In a recent study an inhibitory effect of melatonin on differentiation of osteoclastic cells was uncovered [134]. Melatonin may act directly on osteoclastic cells by suppressing their differentiation as well as by impairing osteoclast activity through its free radical scavenger properties [129]. This explains the bone protecting effect of melatonin in ovariectomized rats [135, 136] particularly when treated with estradiol [137]. Whether melatonin can be used as a novel mode of therapy for to prevent bone loss in rheumatoid arthritis deserves to be studied.

ACKNOWLEDGEMENTS

Work in authors' laboratories was supported in part by DGES, Spain, Agencia Nacional de Promoción Científica y Tecnológica, Argentina, the University of Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina, Fundación Bunge y Born, Buenos Aires and Fundación Antorchas, Buenos Aires.

ABBREVIATIONS

5-HT	=	Serotonin
cAMP	=	Cyclic adenosine 3′,5′-monophosphate
cGMP	=	Cyclic guanosine 3′,5′-monophosphate
CNS	=	Central nervous system
Con A	=	Concanavalin A
CRH	=	Corticotrophin-releasing hormone
DA	=	Dopamine
FCA	=	Freund's complete mycobacterial adjuvant
GH	=	Growth hormone
GHRH	=	GH-releasing hormone
IFN	=	Interferon
IL	=	Interleukin
LH	=	luteinizing hormone
LPS	=	Lipopolysaccharide
MHC	=	Major histocompatibility complex
NE	=	Norepinephrine
NK	=	Natural killer
ODC	=	Ornithine decarboxylase
SCN	=	Suprachiasmatic nuclei
Th	=	T helper
TH	=	Tyrosine hydroxylase
TNF	=	Tumor necrosis factor
TRH	=	Thyrotropin-releasing hormone
TSH	=	Thyrotropin
ZT	=	Zeitgeber time

REFERENCE

[1] Reginster, J.Y. (2002) *Rheumatology,* (Oxford), **41**(Suppl. 1), 3-6.

[2] Goodson, N. and Symmons, D. (2002) *Ann. Rheum. Dis.,* **61**(11), 955-956.
[3] Youssef, W.I. and Tavill, A.S. (2002) *J. Clin. Gastroenterol.,* **35**(4), 345-349.
[4] Bowman, S.J. (2002) *Scand. J. Rheumatol.,* **31**(5), 251-259.
[5] Bendele, A.M. (2001) *J. Musculoskel. Neuron Interact.,* **1**(4), 377-385.
[6] Whitehouse, M.W. (1988) In: Handbook of Animal Models for the Rheumatic Diseases; R.A. Greenwald; H S Diamad Eds; CRC Press: New York, pp. 3-16.
[7] Calvino, B.; Crepon-Bernard, M.O. and Le Bars, D. (1987) *Behav. Brain Res.,* **24**(1), 11-29.
[8] Pearson, C.M. (1956) *Proc. Soc. Exp. Biol. Med.,* **91**, 95-103.
[9] Holoshitz, J.; Matiau, J. and Cohen, I. (1984) *J. Clin. Invest.,* **73**(1), 211-215.
[10] Knight, B.; Katz, D.R.; Isenberg, D.A.; Ibrahim, M.A.; Le Page, S.; Hutchings, P.; Schwartz, R.S. and Cooke, A. (1992) *Clin. Exp. Immunol.,* **90**(3), 459-465.
[11] Stenzel-Poore, M.; Vale, W.W. and Rivier, C. (1993) *Endocrinology,* **132**(3), 1313-1318.
[12] Tanaka, H.; Ueta, Y.; Yamashita, U.; Kannan, H. and Yamashita, H. (1996) *Brain Res. Bull.,* **39**(1), 33-37.
[13] Dantzer, R. (2001) *Ann. N. Y. Acad. Sci.,* **933**, 222-234.
[14] Johnson, R.W. (2002) *Vet. Immunol. Immunopathol.,* **87**(3-4), 443-450.
[15] Larson, S.J. (2002) *J. Gen. Psychol.,* **129**(4), 401-414.
[16] Feldmann, M.; Brennan, F.M.; Foxwell, B.M. and Maini, R.N. (2001) *Curr. Dir. Autoimmun.,* **3**, 188-199.
[17] Lundkvist, G.B.; Robertson, B.; Mhlanga, J.D.; Rottenberg, M.E. and Kristensson, K. (1998) *Neuroreport*, **9**(6), 1059-1063.
[18] Lundkvist, G.B.; Andersson, A.; Robertson, B.; Rottenberg, M.E. and Kristensson, K. (1999) *Brain Res.,* **849**(1-2), 231-234.
[19] Boggio, V.; Castrillon, P.; Pérez Lloret, S.; Riccio, P.; Esquifino, A.I.; Cardinali, D.P. and Cutrera, R.A. (2003) *Neuro. Signals*, **12**(2), 89-94.
[20] Ohdo, S.; Koyanagi, S.; Suyama, H.; Higuchi, S. and Aramaki, H. (2001) *Nat. Med.,* **7**(3), 356-360.
[21] Esquifino, A.I.; Castrillón, P.; Chacon, F.; Cutrera, R.A. and Cardinali, D.P. (2001) *Brain Res.,* **888**(2), 227-234.
[22] Garcia Bonacho, M.; Cardinali, D.P.; Castrillon, P.; Cutrera, R.A. and Esquifino, A.I. (2001) *Exp. Gerontol.,* **36**(2), 267-282.
[23] Castrillon, P.O.; Esquifino, A.I.; Varas, A.; Zapata, A.; Cutrera, R.A. and Cardinali, D.P. (2000) *J. Neuroendocrinol.,* **12**(8), 758-765.
[24] Cardinali, D.P.; Brusco, L.I.; Selgas, L. and Esquifino, A.I. (1998) *Brain Res.,* **789**(2), 283-292.
[25] Petrovsky, N. and Harrison, L.C. (1998) *Int. Rev. Immunol.,* **16**(5-6), 635-649.
[26] Haus, E. and Smolensky, M.H. (1999) *Chronobiol. Int.,* **16**(5), 581-622.
[27] Pelegri, C.; Vilaplana, J.; Castellote, C.; Rabanal, M.; Franch, A. and Castell, M. (2003) *Am. J. Physiol. Cell Physiol.,* **284**(1), C67-C76.
[28] Neidhart, M. and Larson, D. (1990) *J. Neuroimmunol.,* **26**(2), 97-105.
[29] Scalabrino, G.; Ferioli, M.E.; Modena, D. and Fraschini, F. (1982) *Endocrinology*, **111**(6), 2132-2134.
[30] Cardinali, D.; Della Maggiore, V.; Selgas, L. and Esquifino, A. (1996) *Brain Res.,* **711**(1-2), 153-162.
[31] Esquifino, A.; Selgas, L.; Arce, A.; Della Maggiore, V. and Cardinali, D. (1996) *Brain Behav. Immunity,* **10**(2), 92-102.
[32] Cardinali, D.P. and Esquifino, A.I. (1998) *Biomed. Rev.,* **9**(1), 47-59.
[33] Cardinali, D.P.; Brusco, L.I.; Garcia, B.M. and Esquifino, A.I. (1998) *Neuroendocrinology*, **67**(5), 349-362.
[34] Cardinali, D.P.; Brusco, L.I.; Cutrera, R.A.; Castrillon, P. and Esquifino, A.I. (1999) *Biol. Signals Recept.,* **8**(1-2), 41-48.
[35] Neidhart, M. (1996) *Experientia*, **52**(9), 900-908.
[36] Selgas, L.; Pazo, D.; Arce, A.; Esquifino, A.I. and Cardinali, D.P. (1998) *Biol. Signals Recept.,* **7**(1), 15-24.
[37] Esquifino, A.I.; Selgas, L.; Vara, E.; Arce, A. and Cardinali, D.P. (1999) *Biol. Signals Recept.,* **8**(3), 178-190.
[38] Castrillón, P.; Cardinali, D.P.; Pazo, D.; Cutrera, R.A. and Esquifino, A.I. (2001) *J. Neuroendocrinol.,* **13**(3), 288-295.
[39] Garcia Bonacho, M.; Esquifino, A.I.; Castrillon, P.; Reyes Toso, C. and Cardinali, D.P. (1999) *Life Sci.,* **66**(20), 1969-1977.
[40] Cano, P.; Cardinali, D.P.; Castrillón, P.; Reyes Toso, C. and Esquifino, A.I. (2001) *BMC Physiology,* **1**(1), 14.
[41] Cardinali, D.P. (1981) *Endocr. Rev.,* **2**(3), 327-346.
[42] Arendt, J. (2003) *J. Neuroendocrinol.,* **15**(4), 427-431.
[43] Borjigin, J.; Li, X. and Snyder, S.H. (1999) *Annu. Rev. Pharmacol. Toxicol.,* **39**, 53-65.
[44] Karsch, F.J.; Malpaux, B.; Wayne, N.L. and Robinson, J.E. (1988) *Reprod. Nutr. Dev.,* **28**(2B), 459-472.
[45] Pitrosky, B.; Masson-Pevet, M.; Kirsch, R.; Vivien-Roels, B.; Canguilhem, B. and Pevet, P. (1991) *J. Pineal Res.,* **11**(3-4), 149-155.
[46] Malpaux, B.; Thiery, J.C. and Chemineau, P. (1999) *Reprod. Nutr. Dev.,* **39**(3), 355-366.
[47] Cardinali, D.P.; Lynch, H.J. and Wurtman, R.J. (1972) *Endocrinology,* **91**(5), 1213-1218.
[48] Tetsuo, M.; Markey, S.P. and Kopin, I.J. (1980) *Life Sci.,* **27**(2), 105-109.
[49] Lewy, A.J.; Tetsuo, M.; Markey, S.P.; Goodwin, F.K. and Kopin, I.J. (1980) *J. Clin. Endocrinol. Metab.,* **50**(1), 204-205.
[50] Dawson, D. and Armstrong, S.M. (1996) *Pharmacol. Ther.,* **69**(1), 15-36.
[51] Cardinali, D.P. and Pevet, P. (1998) *Sleep Med. Rev.,* **2**(3), 175-190.
[52] Karasek, M. (1999) *Neuroendocrinol. Lett.,* **20**(3-4), 179-188.
[53] Monti, J.M. and Cardinali, D.P. (2000) *Biol. Signals Recept.,* **9**(6), 328-339.
[54] Cagnacci, A.; Arangino, S.; Angiolucci, M.; Melis, G.B.; Facchinetti, F.; Malmusi, S. and Volpe, A. (2001) *Clin. Endocrinol.* (*Oxf.*), **54**(2), 261-266.
[55] Maestroni, G.J. (2001) *Expert. Opin. Investig. Drugs,* **10**(3), 467-476.
[56] Stehle, J.H.; von Gall, C. and Korf, H.W. (2003) *J. Neuroendocrinol.,* **15**(4), 383-389.
[57] Buijs, R.M.; Van Eden, C.G.; Goncharuk, V.D. and Kalsbeek, A. (2003) *J. Endocrinol.,* **177**(1), 17-26.
[58] Cardinali, D.P.; Vacas, M.I. and Boyer, E.E. (1979) *Endocrinology*, **105**(2), 437-441.
[59] Dubocovich, M.L. and Hensler, J.G. (1986) *Br. J. Pharmacol.,* **88**(1), 51-61.
[60] Vakkuri, O.; Lamsa, E.; Rahkamaa, E.; Ruotsalainen, H. and Leppaluoto, J. (1984) *Anal. Biochem.,* **142**(2), 284-289.
[61] Krause, D.N. and Dubocovich, M.L. (1990) *Trends Neurosci.,* **13**(11), 464-470.
[62] Morgan, P.J.; Barrett, P.; Howell, H.E. and Helliwell, R. (1994) *Neurochem. Int.,* **24**(2), 101-146.
[63] Sugden, D. (1994) *Neurochem. Int.,* **24**(2), 147-157.
[64] Dubocovich, M.L. (1988) *FASEB J.,* **2**(12), 2765-2773.
[65] Dubocovich, M.L. (1995) *Trends Pharmacol. Sci.,* **16**(2), 50-56.
[66] Weaver, D.R.; Rivkees, S.A. and Reppert, S.M. (1989) *J. Neurosci.,* **9**(7), 2581-2590.
[67] Reppert, S.M.; Weaver, D.R.; Rivkees, S.A. and Stopa, E.G. (1988) *Science*, **242**(4874), 78-81.
[68] Reppert, S.M.; Weaver, D.R.; Cassone, V.M.; Godson, C. and Kolakowski, L.F.J. (1995) *Neuron*, **15**(5), 1003-1015.
[69] Reppert, S.M.; Weaver, D.R. and Ebisawa, T. (1994) *Neuron*, **13**(5), 1177-1185.
[70] Reppert, S.M.; Godson, C.; Mahle, C.D.; Weaver, D.R.; Slaugenhaupt, S.A. and Gusella, J.F. (1995) *Proc. Natl. Acad. Sci. USA,* **92**(19), 8734-8738.
[71] Dubocovich, M.L.; Cardinali, D.P.; Delagrange, P.; Krause, D.N.; Strosberg, D.; Sugden, D. and Yocca, F.D. (2000) In: The IUPHAR Compendium of Receptor Characterization and Classification, 2nd. Edition; IUPHAR Ed;.IUPHAR Media: London, pp. 271-277.
[72] Slaugenhaupt, S.A.; Roca, A.L.; Liebert, C.B.; Altherr, M.R.; Gusella, J.F. and Reppert, S.M. (1995) *Genomics*, **27**(2), 355-357.
[73] Molinari, E.J.; North, P.C. and Dubocovich, M.L. (1996) *Eur. J. Pharmacol.,* **301**(1-3), 159-168.
[74] Masana, M.I. and Dubocovich, M.L. (2001) *Sci. STKE,* 2001(107), E39.
[75] Witt-Enderby, P.A.; Bennett, J.; Jarzynka, M.J.; Firestine, S. and Melan, M.A. (2003) *Life Sci.,* **72**(20), 2183-2198.
[76] Cardinali, D.P.; Golombek, D.A.; Rosenstein, R.E.; Cutrera, R.A. and Esquifino, A.I. (1997) *J. Pineal Res.,* **23**(1), 32-39.
[77] Reiter, R.J.; Tan, D.X.; Manchester, L.C. and Qi, W. (2001) *Cell Biochem. Biophys.,* **34**(2), 237-256.

[78] Reiter, R.J.; Tan, D.X. and Allegra, M. (2002) *Neuroendocrinol. Lett.,* **23** (Suppl. 1), 3-8.
[79] Okatani, Y.; Wakatsuki, A.; Reiter, R.J. and Miyahara, Y. (2002) *Neurobiol. Aging,* **23**(4), 639-644.
[80] Liebmann, P.M.; Wolfler, A.; Felsner, P.; Hofer, D. and Schauenstein, K. (1997) *Int. Arch. Allergy Immunol.,* **112**(3), 203-211.
[81] Fraschini, F.; Demartini, G.; Esposti, D. and Scaglione, F. (1998) *Biol. Signals,* **7**(1), 61-72.
[82] Skwarlo-Sonta, K. (1999) *Neuroendocrinol. Lett.,* **20**(3-4), 151-156.
[83] Beskonakli, E.; Palaoglu, S.; Aksaray, S.; Alanoglu, G.; Turhan, T. and Taskin, Y. (2001) *Neurosurg. Rev.,* **24**(1), 26-30.
[84] Martins, E.; Ligeiro De Oliveira, A.P.; Fialho De Araujo, A.M.; Tavares, D.L.; Cipolla-Neto, J. and Costa Rosa, L.F. (2001) *J. Pineal Res.,* **31**(4), 363-369.
[85] Skwarlo-Sonta, K. (2002) *Neuroendocrinol. Lett.,* **23** (Suppl. 1), 61-66.
[86] Pioli, C.; Caroleo, M.C.; Nistico, G. and Doria, G. (1993) *Int. J. Immunopharmacol.,* **15**(4), 463-468.
[87] Konakchieva, R.; Kyurkchiev, S.; Kehayov, I.; Taushanova, P. and Kanchev, L. (1995) *J. Neuroimmunol.*, **63**(2), 125-132.
[88] Wajs, E.; Kutoh, E. and Gupta, D. (1995) *Eur. J. Endocrinol.,* **133**(6), 754-760.
[89] Drazen, D.L.; Bilu, D.; Bilbo, S.D. and Nelson, R.J. (2001) *Am. J. Physiol. Regul. Integr. Comp. Physiol.,* **280**(5), R1476-R1482.
[90] Giordano, M. and Palermo, M.S. (1991*) J. Pineal Res.,* **10**(3), 117-121.
[91] Poon, A.M.; Liu, Z.M.; Pang, C.S.; Brown, G.M. and Pang, S.F. (1994) *Biol. Signals,* **3**(2), 107-117.
[92] Bonilla, E.; Rodon, C.; Valero, N.; Pons, H.; Chacin-Bonilla, L.; Garcia, T.J.; Rodriguez, Z.; Medina-Leendertz, S. and Anez, F. (2001) *Trans. R. Soc. Trop. Med. Hyg.,* **95**(2), 207-210.
[93] Negrette, B.; Bonilla, E.; Valero, N.; Pons, H.; Garcia, T.J.; Chacin-Bonilla, L.; Medina-Leendertz, S. and Anez, F. (2001) *Neurochem. Res.,* **26**(7), 767-770.
[94] del Gobbo, V.; Libri, V.; Villani, N.; Calio, R. and Nistico, G. (1989) *Int. J. Immunopharmacol.,* **11**(5), 567-573.
[95] Currier, N.L.; Sun, L.Z. and Miller, S.C. (2000) *J. Neuroimmunol.,* **104**(2), 101-108.
[96] Morrey, K.M.; McLachlan, J.A.; Serkin, C.D. and Bakouche, O. (1994) *J. Immunol.,* **153**(6), 2671-2680.
[97] Lissoni, P.; Barni, S.; Brivio, F.; Rossini, F.; Fumagalli, L.; Ardizzoia, A. and Tancini, G. (1995) *Oncology*, **52**(5), 360-362.
[98] Maestroni, G.J.; Conti, A. and Pierpaoli, W. (1988) *Ann. N. Y. Acad. Sci.,* **521**, 140-148.
[99] Maestroni, G.J. (1995) *J. Pineal Res.,* **18**(2), 84-89.
[100] Akbulut, K.G.; Gonul, B. and Akbulut, H. (2001) *Immunol. Invest.,* **30**(1), 17-20.
[101] Yu, Q.; Miller, S.C. and Osmond, D.G. (2000) *J. Pineal Res.,* **29**(2), 86-93.
[102] Raghavendra, V.; Singh, V.; Kulkarni, S.K. and Agrewala, J.N. (2001) *Mol. Cell Biochem.,* **221**(1-2), 57-62.
[103] Arzt, E.S.; Fernandez-Castelo, S.; Finocchiaro, L.M.; Criscuolo, M.E.; Diaz, A.; Finkielman, S. and Nahmod, V.E. (1988) *J. Clin. Immunol.,* **8**(6), 513-520.
[104] Lewinski, A.; Zelazowski, P.; Sewerynek, E.; Zerek-Melen, G.; Szkudlinski, M. and Zelazowska, E. (1989) *J. Pineal Res.,* **7**(2), 153-164.
[105] Persengiev, S.P. and Kyurkchiev, S. (1993) *Int. J. Biochem.,* **25**(3), 441-444.
[106] Cuzzocrea, S. and Reiter, R.J. (2002) *Curr. Top. Med. Chem.,* **2**(2), 153-165.
[107] Reiter, R.J.; Tan, D.X. and Burkhardt, S. (2002) *Mech. Ageing Dev.,* **123**(8), 1007-1019.
[108] Guerrero, J.M. and Reiter, R.J. (2002) *Curr. Top. Med. Chem.,* **2**(2), 167-179.
[109] Sasaki, M.; Jordan, P.; Joh, T.; Itoh, M.; Jenkins, M.; Pavlick, K.; Minagar, A. and Alexander, S.J. (2002) *BMC Gastroenterol.,* **2**(1), 9.
[110] Garcia-Perganeda, A.; Pozo, D.; Guerrero, J.M. and Calvo, J.R. (1997) *J. Immunol.,* **159**(8), 3774-3781.
[111] Pozo, D.; Delgado, M.; Fernandez-Santos, J.M.; Calvo, J.R.; Gomariz, R.P.; Martin-Lacave, I.; Ortiz, G.G. and Guerrero, J.M. (1997) *FASEB J.,* **11**(6), 466-473.
[112] Barjavel, M.J.; Mamdouh, Z.; Raghbate, N. and Bakouche, O. (1998) *J. Immunol.,* **160**(3), 1191-1197.
[113] RafiiElIdrissi, M.; Calvo, J.R.; Harmouch, A.; GarciaMaurino, S. and Guerrero, J.M. (1998) *J. Neuroimmunol.,* **86**, 190-197.
[114] Garcia-Maurino, S.; Gonzalez-Haba, M.G.; Calvo, J.R.; Goberna, R. and Guerrero, J.M. (1998) *J. Neuroimmunol.,* **92**(1-2), 76-84.
[115] Brusco, L.I.; Garcia-Bonacho, M.; Esquifino, A.I. and Cardinali, D.P. (1998) *J. Auton. Nerv. Syst.,* **74**(1), 49-61.
[116] Esquifino, A.I. and Cardinali, D.P. (1999) *Neuroendocrinol. Lett.,* **20**(3-4), 163-166.
[117] Cardinali, D.P.; Cutrera, R.A.; Castrillon, P. and Esquifino, A.I. (1996) *Neuroimmunomodulation*, **3**(2-3), 102-111.
[118] Cardinali, D.P.; Cutrera, R.A.; Garcia Bonacho, M. and Esquifino, A.I. (1997) *J. Pineal Res.,* **22**(4), 210-220.
[119] Esquifino, A.I.; Castrillon, P.; Garcia-Bonacho, M.; Vara, E. and Cardinali, D.P. (1999*) J. Pineal Res.,* **27**(1), 15-23.
[120] Pazo, D.; Cardinali, D.P.; Garcia Bonacho, M.; Reyes Toso, C. and Esquifino, A.I. (2000) *Biol. Rhythm Res.,* **32**(2), 202-211.
[121] Hansson, I.; Holmdahl, R. and Mattsson, R. (1993) *Clin. Exp. Immunol.,* **92**(3), 432-436.
[122] Hansson, I.; Holmdahl, R. and Mattsson, R. (1992) *J. Neuroimmunol.,* **39**(1-2), 23-30.
[123] Chan, M.Y.; Pang, S.F.; Tang, P.L. and Brown, G.M. (1984) *J. Pineal Res.,* **1**(3), 227-236.
[124] Raynaud, F.; Mauviard, F.; Geoffriau, M.; Claustrat, B. and Pevet, P. (1993) *Biol. Signals,* **2**(6), 358-366.
[125] Rasmussen, D.D.; Boldt, B.M.; Wilkinson, C.W.; Yellon, S.M. and Matsumoto, A.M. (1999) *Endocrinology*, **140**(2), 1009-1012.
[126] Maestroni, G.J.; Sulli, A.; Pizzorni, C.; Villaggio, B. and Cutolo, M. (2002) *Ann. N. Y. Acad. Sci.,* **966**, 271-275.
[127] Sulli, A.; Maestroni, G.J.; Villaggio, B.; Hertens, E.; Craviotto, C.; Pizzorni, C.; Briata, M.; Seriolo, B. and Cutolo, M. (2002) *Ann. N. Y. Acad. Sci.,* **966**, 276-283.
[128] Lee, D.M. and Weinblatt, M.E. (2001) *Lancet*, **358**(9285), 903-911.
[129] Cardinali, D.P.; Ladizesky, M.G.; Boggio, V.; Cutrera, R.A. and Mautalen, C. (2003) *J. Pineal Res.,* **34**(2), 81-87.
[130] Conti, A.; Conconi, S.; Hertens, E.; Skwarlo-Sonta, K.; Markowska, M. and Maestroni, J.M. (2000) *J. Pineal Res.,* **28**(4), 193-202.
[131] Nakade, O.; Koyama, H.; Ariji, H.; Yajima, A. and Kaku, T. (1999) *J. Pineal Res.,* **27**(2), 106-110.
[132] Roth, J.A.; Kim, B.G.; Lin, W.L. and Cho, M.I. (1999) *J. Biol. Chem.,* **274**(31), 22041-22047.
[133] Koyama, H.; Nakade, O.; Takada, Y.; Kaku, T. and Lau, K.H. (2002) *J. Bone Miner. Res.,* **17**(7), 1219-1229.
[134] Suzuki, N. and Hattori, A. (2002) *J. Pineal Res.,* **33**(4), 253-258.
[135] Ladizesky, M.G.; Cutrera, R.A.; Boggio, V.; Somoza, J.; Centrella, J.M.; Mautalen, C. and Cardinali, D.P. (2001) *Life Sci.,* **70**(5), 557-565.
[136] Ostrowska, Z.; Kos-Kudla, B.; Marek, B.; Kajdaniuk, D.; Staszewicz, P.; Szapska, B. and Strzelczyk, J. (2002) *Neuroendocrinol. Lett.,* **23** (Suppl. 1), 104-109.
[137] Ladizesky, M.G.; Boggio, V.; Albornoz, L.E.; Castrillon, P.O.; Mautalen, C. and Cardinali, D.P. (2003) *J. Pineal Res.,* **34**(2), 143-151.