Melatonin and tryptophan as therapeutic agents against the impairment of the sleep-wake cycle and immunosenescence due to aging in *Streptopelia risoria*

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Submitted: June 13, 2007 *Accepted: July 21, 2007*

Key words: **tryptophan; serotonin; melatonin; activity/rest rhythms; cell viability; phagocytic process; aging; bird**

Neuroendocrinol Lett 2007; **28**(6):757–760 **PMID:** 18063930 NEL280607A01

Abstract

All organisms present circadian rhythm in most of their physiological functions, and among them there stand out sleep, motor activity, immune function, the secretion of melatonin, and the production and release of numerous neurotransmitters, in particular of serotonin because of its relationship with the aforementioned factors. Aging changes these rhythms, altering sleep quality and contributing to immunosenescence. Treatment with exogenously administered melatonin or tryptophan may restore these impaired functions due to aging. In our animal model (*Streptopelia risoria*), both the hormone and the amino acid acted on the activity-rest rhythms, modulating the circulating levels of melatonin and serotonin, and increased the cell viability and resistance to induced oxidative stress of blood heterophils, at the same time as enhancing the phagocytic function and neutralizing the superoxide anions deriving from this immune function. Also, in the old individuals, the treatments with melatonin and tryptophan at the concentrations and times of administration considered suitable improved nocturnal rest besides reverting the immunosuppressory and oxidative effects accompanying phagocytosis at these advanced ages.

ORIGINAL ARTICLE

INTRODUCTION

It is known that the hormone melatonin is a key factor in the relationship between the sleep-wake cycle and the immune system. Nevertheless, it is also well known that age modifies them profoundly, diminishing the amount and quality of sleep and the capacity of the immune response, factors that are accompanied by major changes in melatonin secretion. Melatonin is used in the treatment of age-related problems of insomnia (Cajochen *et al.*, 2006). In alterations of sleep, melatonin is not involved by itself, but also acts through the mechanism of the organism's internal clock. Melatonin therapy has been able to correct alterations in the sleep-wake patterns of the elderly, although it also corrects problems of insomnia in younger individuals (Cubero *et al.*, 2006a). Also, exogenous

administration of tryptophan increases serotonin levels in the brain, and, being the precursor of melatonin, one must also presume that it influences the synthesis of this hormone. The administration of tryptophan in the diet increases serotonin's availability in the brain as well as the delta potential in the electroencephalogram and NREM (Garau *et al*., 2006). In recent years, there has been an extension of the use of tryptophan in the treatment of depression and sleep alterations (Cubero *et al*., 2006a,b). One hence deduces that improving the contribution of tryptophan or administering melatonin according to the requirements of the circadian cycle in elderly is probably translated into an improved adjustment of their circadian cycles, which in turn implies an improvement in their state of health.

There are now specific data on the intimate relationship of the sleep-wake cycle and the immune system. Thus, there exists evidence of connections between the circadian rhythms of the immune system and the neuroendocrine and thermoregulatory systems on the one hand with those of the sleeping brain on the other, one of the essential functions proposed for sleep being immunological restoration (Everson *et al*., 2005).

Human studies of the differences in the circadian factors that determine immunity, sleep, and the changes that occur with age, involve a series of methodological problems that limit their possibilities of obtaining data. They are also costly in terms of effort and means. Studies on other mammals are difficult to extrapolate to man since most mammals are nocturnally active, as well as being multiphasic (Tobler, 1995). For this reason, the work described in the present thesis was on the ringdove (*Streptopelia risoria*), which is a diurnal and monophasic animal as is man, and hence an appropriate experimental model for the analysis of the alterations undergone by circadian cycles with age. Also, under safe conditions, as is the case with these birds in captivity, they sleep with both hemispheres simultaneously, with both eyes closed. This gives rise to what is denominated "bihemispheric sleep", a phenomenon that also occurs in man.

In previous studies on the ringdove (*Streptopelia risoria*) we have observed a positive correlation between the phagocytic function and the serum concentrations of melatonin (Rodriguez *et al*., 2001). In *in vitro* studies, we have found a stimulating effect on the function of phagocytic cells after their incubation with physiological and pharmacological concentrations of the hormone (Terrón *et al*., 2002). Also, recently, we have observed that the circadian rhythm of melatonin is lost with age, with the physiological concentrations in old animals being lower than in young animals (Terrón *et al*., 2005).

Thus, the aim of this research was to evaluate the functional connection between tryptophan and melatonin in their action on the activity-rest and non-specific immune response rhythms, evaluating the latter through the phagocytic function and oxidative metabolism of heterophils from young and old ringdove (*Streptopelia risoria*).

RESULTS AND DISCUSSION

The circadian rhythms undergo important changes with aging, causing the disorders that appear in old age (Pandi-Perumal *et al*., 2005; Cajochen *et al*., 2006). There thus appear misfits in the maintenance of nocturnal sleep and diurnal wakefulness, sleep becomes fragile and its continuity difficult to maintain. In addition there appear many other physiological disorders, as is the case with the immune function.

Aging is associated with numerous changes in the morphology, physiology, and biochemistry of the pineal gland which lead to a significant reduction in the nocturnal levels of melatonin and serotonin. The production and secretion into the blood of melatonin is under circadian control, with maximum production during the nocturnal period of the light/dark cycle. It is known that, although the production of melatonin at a 24-hour rhythm is very clear in young animals, including humans, this cycle deteriorates with age. One of the causes is the significant decline of serotonin levels in the pineal, since the synthesis of melatonin depends critically on the amount of this neurotransmitter present. Various studies point to an intimate relationship of the secretion of melatonin and serotonin with the sleep-wake cycle or the activity-rest rhythm (Pandi-Perumal *et al*., 2002), because serotonin increases the proportion of slow wave sleep (Jouvet, 1999), and behaves as a regulatory neurotransmitter for waking (Ursin, 2000), whereas melatonin acts as a link between the circadian pacemaker and the sleep-wakefulness generating mechanism (Lavie, 2001). For this reason, both elements need to be studied in any approach to understanding the deterioration of the circadian rhythm that takes place as a result of aging. In this sense, it was observed in the ringdove (*Streptopelia risoria*) that there were daily oscillations of the melatonin and serotonin levels in young and old animals, with a significant reduction in the serum levels and amplitudes of the oscillations of both secretions with advanced age. There were negative correlations of melatonin and positive correlations of serotonin with the animals' activity-rest rhythms, the old animals presenting greater levels of nocturnal activity (Paredes *et al*., 2006).

As it has been established that there is a causal relationship between the reduction of melatonin and serotonin and the activity-rest rhythm, and knowing that melatonin therapy has an action of circadian adjustment on sleep (Lewy *et al*., 2006), melatonin and its precursor, the amino acid tryptophan, were administered at different doses and times of administration to investigate their effects on the activity-rest rhythm of young and old ringdove (Paredes *et al*., 2007a,b). It was observed that the doses of 0.25 and 2.5 mg melatonin per kg weight administered to young and old ringdove, respectively, one hour before lights-off, and of 300 mg tryptophan per kg weight to old ringdove one hour after lights-on, were the most appropriate therapeutically in terms of noctur-

nal rest, affecting least the diurnal activity. These doses and times of administration led to increased diurnal and nocturnal serum levels of melatonin, and of serotonin in the case of the treatment with tryptophan. Also, in the case of the treatment with melatonin, the amplitude of the rhythms in the group of old ringdove were similar to or even greater than in the young animals, and in the case of the tryptophan treatment the amplitude in the old animals was greater than that of their controls (Paredes *et al*., 2007a,b). This confirms on the one hand that exogenous melatonin acts as a regulatory element of sleep, as well as improving the temporal organization of the aging circadian system of the ringdove (Karasek, 2004), and on the other that a tryptophan rich diet increases the available amount of melatonin and serotonin, with the consequent implication that this has for the activity-rest rhythms, as we had previously suggested in nursing infants and in very young, sexually immature, birds (Cubero *et al*., 2005; 2006a,b, 2007).

The studies performed in this research have also confirmed that the old ringdove present a reduction in heterophil function. This could be at least in part due to the reduction in the amplitude and circulating levels of the daily melatonin rhythm (Paredes *et al*., 2006; 2007c,d). In this sense, the focus was on the changes related to age in the levels of melatonin, and the possible role that the administration of the hormone might have in managing the changes in phagocytic activity (phagocytosis and oxidative metabolism) during aging. Melatonin administered to old ringdove increased the differences between the nocturnal and diurnal serum levels of the hormone, at the same time as enhancing phagocytosis and reducing superoxide anion levels in the heterophils. Also, the results showed that, in old animals, with the melatonin administration there was a clear positive correlation between the levels of the hormone and the values of the phagocytosis index observed at the acrophase and nadir of the rhythm of the circulating indole (Paredes *et al*., 2007d). This is indicative that, as the levels of melatonin increase in the plasma of the old animals, there was also an increase in their blood heterophils' capacity to phagocytose latex beads. These findings are coherent with previous results showing the existence of a positive melatonin-phagocytosis correlation in young and old ringdove (Rodriguez *et al*., 1999; Terrón *et al*., 2005), as well as with the increased phagocytic activity of the heterophils of old animals that have been treated with tryptophan (Paredes *et al*., 2007c), being higher in this latter case the circulating levels of not only melatonin, but of serotonin as well, as was described above (Paredes *et al*., 2007a,b). The study therefore confirmed in this bird species that the administration of melatonin and tryptophan has a stimulatory effect on the phagocytic function, at the same time as neutralizing the oxidative stress that derives from it. These effects were less in the old animals, possibly because of the aforementioned reduction decline in the circulating levels of melatonin characteristic of their aging (Paredes *et al*., 2006). The increase observed in both the serum melatonin levels and in the heterophil phagocytic activity of the old animals when they were administered orally the hormone itself or its precursor, the amino acid tryptophan, is of especial interest since the melatonin deficiency of old age is related to suppression of the immune function (Karasek and Reiter, 2002). Also, both treatments had a protective effect against the oxidative stress induced by high hydrogen peroxide concentrations in cells from old animals, in agreement with numerous studies that have shown the antioxidant power of melatonin and serotonin (Paredes *et al*., 2007c,d).

CONCLUSIONS

In sum, although aging is a multifactorial process, the age-related decline in melatonin and serotonin secretion seems to be one of the most important factors. The direct consequences of their loss with age are related to problems in the capacity to achieve sleep, perturbations of the circadian rhythm, lessened antioxidant protection, depressed immune function, and other disorders (Karasek and Reiter, 2002). An ever growing body of research indicates the existence of a close relationship between the 24-hour melatonin rhythm and age-related changes in the physiology of the immune system and in the activity-rest rhythms. Considering that melatonin acts by sending the organism information on its temporal organization, this hormone, either directly or through its creation by the conversion of its precursor, the amino acid tryptophan, could be an important pharmacological agent to attenuate age-related changes in circadian organization, the immune system, sleep, and other disorders that accompany aging. The way is thus signposted for new studies to allow a recommendation of melatonin, or diets rich in tryptophan, as therapeutic agents to counteract what has been called the "melatonin deficient state" (Wurtman, 2000; Karasek and Reiter, 2002) that typically appears in old age. The findings obtained in the research work support this proposal.

ACKNOWLEDGEMENTS

This research was supported by grants from the Spanish Ministry of Science and Technology (BFI2002-04583-FC02-01) and Consejería de Infraestructuras y Desarrollo Tecnológico (Junta de Extremadura, 3PR05A053). S. D. Paredes was the beneficiary of a grant from Consejería de Infraestructuras y Desarrollo Tecnológico – Fondo Social Europeo (Junta de Extremadura, FIC02A049). The authors would like to express their thanks to Dr. María Pilar Terrón, Dr. Javier Cubero, Dr. Vicente Valero, Dr. José Antonio Pariente, Ms. Ana María Marchena, Ms. Elena Circujano, Mr. Ricardo Megías Cebrino, and Ms. Ana Royano for their technical assistance; and to Professor Russel J. Reiter for helpful comments on the research.

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