Preservation of Slow Wave Sleep as a Neuroprotective Strategy in Aging.

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ABSTRACT

Preservation of normal sleep, proper nutrition and physical exercise are key elements in maintaining healthy aging. Their deficiency predisposes to fragility, a pathophysiological condition that leads to greater vulnerability to diseases and adverse effects, resulting from the loss of homeostasis and a decreased functional reserve. Melatonin, a molecule of extraordinary phylogenetic conservation present in all known aerobic organisms, is effective both as a chronobiotic and a cytoprotective agent in aging. The late afternoon increase of melatonin "opens the sleep doors" every night and its therapeutic use as a guardian of slow wave sleep has been demonstrated. There is a growing use of hypnotics such as benzodiazepines (BZP) and Z drugs, which have shown a deleterious effect on slow wave sleep and, due to their tolerance and dependence, are a serious problem of public health. Thirty years ago, we demonstrated the interaction of melatonin with central BZP receptors and in 1997 we published the first demonstration of the reduction of BZP consumption in melatonin-treated elderly subjects. The approval by the EMA of melatonin as a drug in 2007 has allowed obtaining pharmacoepidemiologic information on this topic. Several studies have found that more than half of patients treated with BZP and who had started treatment with melatonin abandoned BZP consumption. In another study in 9 European countries it was concluded that campaigns to reduce BZP consumption fail if there is no availability and reimbursement of the melatonin received in replacement. The data support the use of melatonin in patients who chronically use BZP or Z-drugs. Melatonin has no addictive and tolerant effects and thus becomes the treatment of choice for the preservation of slow wave sleep in the elderly.

INTRODUCTION

The Greeks called the sleep "the brother of death", because they thought that in the sleeping man the soul temporarily abandoned the body and wandered the world, that is, brain activity ceased. They were wrong; today we know that sleep is a process: a) active, neural activity being in many brain areas greater than during wakefulness; b) heterogeneous, composed of different states that occur cyclically, and c) rhythmic, its rhythmicity being relatively independent of external conditions [1].

The activity of the thalamic-cortical circuit is the determinant of the three functional stages: wakefulness, slow wave sleep and rapid eye movement (REM) sleep, in which we can find the brain activity in a 24-hour period. Each of these stages is characterized by the level of thalamic "gate" that allows or not to pass up the sensory information. REM sleep and wakefulness are characterized by an "open gate" that allows sensory information to reach the cortex

(exteroceptive information in wakefulness, interoceptive information in REM sleep). During slow wave sleep, the gate is closed and there is minimal input of information attaining the cerebral cortex [1].

A common mistake is to identify sleep as a CNS-only phenomenon. In fact, it is a complete physiological program, different from wakefulness, and which comprises two very different physiological states of organs and systems (slow wave sleep and REM sleep). It is as if we live in three different bodies (wakefulness, slow wave sleep and REM sleep) that must necessarily happen in harmony to ensure the state of health. The physiological characteristics of the three stages are summarized in Figure 1.

A 76-year-old man (a feasible life expectancy today) who sleeps 8 hours/day has lived 50 years in the physiological state of wakefulness, 20 years in slow wave sleep and 6 years in REM sleep. However, it should be noted that, having modern 24/7 Society a reduction of sleep hours by 25% over the past 40 years, the above calculation now changes to a 56 year of wakefulness, 15 years of slow wave sleep, and 5 years of REM sleep. We thus live in a private sleep society, and where the sympathetic catabolic configuration of wakefulness has become prevalent at the expense of the parasympathetic anabolic configuration of slow wave sleep. In the elderly an age-related reduction in slow wave sleep further adds to the unbalanced of sleep with obvious consequences for health, such as obesity, high blood pressure and diabetes, and neurodegenerative diseases (Figure 1) [1].

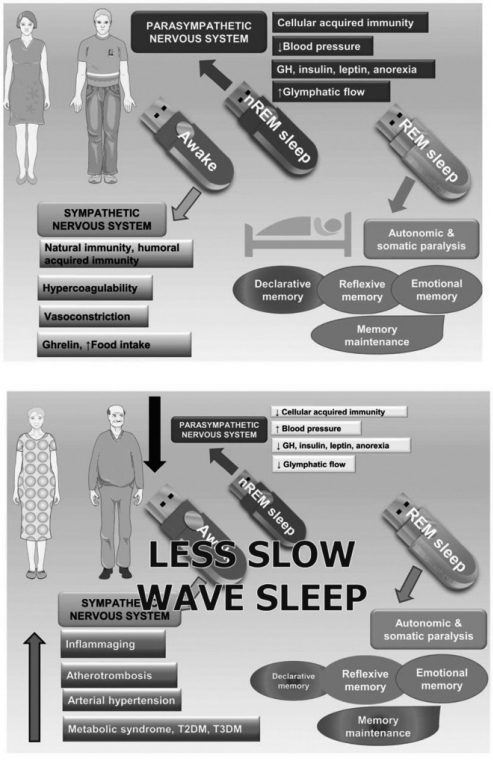

Figure 1. The 3 body configurations in a 24-hour cycle

One of the most interesting recent findings in sleep physiology in recent years has been the description of the glymphatic system, a tubing dependent system in glial cells. During slow wave sleep there is a marked increase in cerebrospinal fluid (CSF) exchange due to a 60% increase in space between nerve cells [2]. In young people, the CSF enters the cerebral parenchyma through periarterial pathways, washes the solutes of the interstitial space, and dumps them through the veins. With aging, the glymphatic function is reduced, because astrocytes become reactive and aquaporin 4 is activated depolarizing the vascular ends of the parenchymal glial processes. In Alzheimer's disease, the perivascular space of the penetrating arteries is subject to the accumulation of pathological peptides such as β-amyloid. There is strong evidence on that the accumulation of β-amyloid caused the deterioration of the glymphatic system since the perivascular pathways are blocked by protein aggregates such as β-amyloid [2].

Aging of Sleep

The prevalence of primary insomnia varies from 1% to 10% of the general population and up to 25-30% in the elderly, for whom the treatment of insomnia is a clear medical necessity. The direct and indirect costs of insomnia represent a substantial social economic burden. Benzodiazepines (BZP) and other BZP receptor agonists (Z-drugs like zolpidem, zaleplon, zopiclone) are the most commonly prescribed drugs

for the treatment of insomnia in the elderly [3]. Several meta-analyses pondering the risks and benefits of these therapeutic options in older patients have reported statistically significant improvements in sleep but have also reported a statistically significant risk of life-threatening adverse events. In fact, these drugs are only approved by regulatory agencies for treatment for older adults no more than a few weeks in length due to safety reasons.

Z drugs are used, unlike BZPs, exclusively for the treatment of insomnia and are assumed to have a lower tendency to induce physical dependence and addiction than BZP [5]. However, their safety remains a matter of concern. Indeed, both types of drugs produce tolerance and addiction. Adverse effects have been reported in more than 40% of users of both types of drugs with no difference between BZPs and Z drugs. In several countries, excessive consumption of BZP and Z drugs is a public health problem.

There is therefore a need for education programs that warn on these consequences of self-medication and for more elaborate control systems to prevent illegal sale without an archived prescription. In Europe, health authorities have initiated policies and recommendations to reduce BZP and Z-drug use.

What is melatonin

The light-dark variation in the synthesis of melatonin by pinealocytes is the essential fact that explains the involvement of the pineal gland in the physiology of biological rhythms [6]. The role of melatonin is twofold: on the one hand it "opens the doors of sleep" by inhibiting the late wake-up activity driven by the central circadian pacemaker, the suprachiasmatic nuclei of the hypothalamus (NSQ) [7]. In turn, melatonin is the "hormone of darkness", a chemical code of the duration of the night, and has an established in the transmission of light information to the neuroendocrine system. Melatonin represents a "hand" of the biological clock in the sense that it responds to signals from the NSQ, the temporal variation of the melatonin rhythm indicating the state of the clock, both in terms of phase (time in the internal clock in relation to the external time) and amplitude [8].

Melatonin is the prototype of "chronobiotics", drugs used to synchronize and increase the amplitude of circadian rhythms [9]. A synthetic analogue of melatonin (tasimelteon, Hetlioz®, Vanda Pharmaceutical) was approved by the Food and Drug Administration (FDA) in the US in 2013 for use in the circadian sleep disorder with a different rhythm of 24 hours occurring in blind individuals. In the Argentina, melatonin was introduced as a drug for insomnia in 1995 and there are today melatonin analogs used for this purpose in the USA (ramelteon, RozeremR, Takeda) as well as for the treatment of depression (agomelatine, ValdoxanR; Servier) approved by the European Medicines Agency (EMA) in Europe. In 2007 a slow release form of melatonin (2mg, Circadin®, Neurim) was approved as a drug by EMA.

In the early 1970s, working in the Laboratory of Neuroendocrine Regulation of the Massachusetts Institute of Technology in collaboration with Richard Wurtman and Harry Lynch. we did the first studies on the binding of melatonin to plasma proteins in

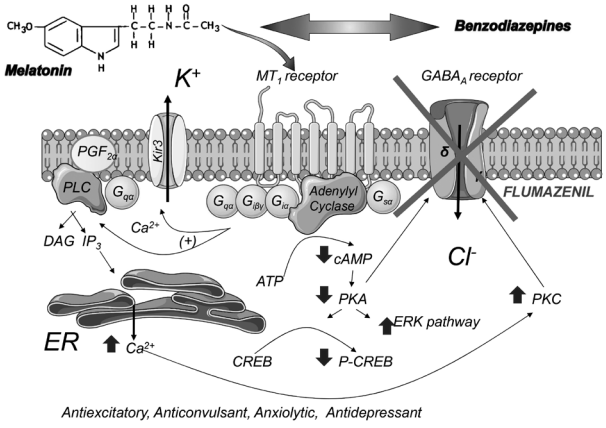

Figure 2. Intracellular signals involved in the modulation of GABA type A receptor by melatonin MT1 receptors. BZPs interact with the GABAergic receptor. This is the basis of the anti-excitatory, anticonvulsant, anti-anxiety and antidepressant effects of melatonin.

the plasma, identifying albumin in such a role [10]. Also at that time, and in collaboration with Markuu Hyyppa we evaluated the uptake and metabolism of 3H-melatonin when administered intracisternally to rats [11]. For these studies we used a primitive method of radioactive melatonin synthesis: 3H-labeled acetic anhydride (of a very low specific activity) was reacted with methoxytryptamine. Although indirect evidence of a saturable neural mechanism of uptake was obtained (i.e., unlabeled melatonin competed with the uptake of the labeled one) we were far from being able to achieve the description of the melatonin receptor with a radioisotope of such a low specific activity.

In '80 an observation of Aaron Lerner, the discoverer of melatonin, started to rise interest: the drowsiness produced by the melatonin when Lerner administered it to himself and to his patients [12]. At that time, our laboratory in Buenos Aires had acquired considerable experience in the determination of receptors by methods of high affinity binding and we were expecting the introduction in the market of tritiated melatonin of specific high activity for the detection of possible receptor sites. In 1977, one of the leading suppliers of radioactive material, New England Nuclear, introduced tritiated melatonin of specific high activity (30 Ci / mmol) and once we had that material we shortly identified the brain receptors for melatonin in areas of the bovine and rat brains [13; 14]. In other studies, we verified changes in receptor concentration correlated with circulating levels of melatonin and ambient light status [15]. Soon, other researchers confirmed our results but by 1981 New England Nuclear decided for technical stability problems to remove its high specific activity 3H-melatonin from the market, and our research on the subject stopped.

By 1983, there were significant advances in autoradiographic and immunohistochemical receptor detection techniques by the introduction of an iodinated ligand of melatonin, 2-125I-melatonin, and the knowledge in the field of melatonin receptors exploded, culminating with receptor cloning in the 1990s. Based on their kinetic properties, specificity and localization different melatonin receptors have been identified in both the CNS and the periphery. The MT1 and MT2 receptors [16], all belonging to the membrane receptor superfamily associated with G proteins, have been cloned. These receptors mediate melatonin inhibition of adenylate cyclase (and in the case of the MT2 receptor, guanylate cyclase) and participate in the action of melatonin on the phase and amplitude of the circadian rhythms. Due to its

THE BEHAVIORAL ACTIVITY OF MELATONIN IS INHIBITED BY BENZODIAZEPINE ANTAGONISM

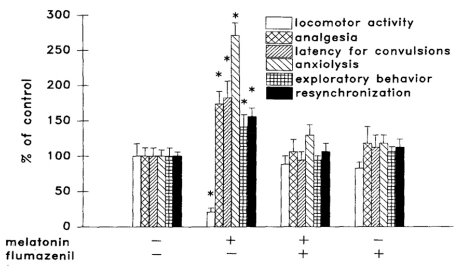

Figure 3. Blocking by flumazenil, a central BZP antagonist, of the effect of melatonin on various behaviors in rat, mouse and hamster. Data of [24].

liposolubility melatonin crosses the membranes and is associated with cytoplasmic proteins such as calmodulin and tubulin, causing important changes in the cytoskeleton [17]. Melatonin also accesses the cell nucleus where receptor sites have also been described. The nuclear receptor for melatonin belongs to the superfamily of orphan receptors RZR / ROR and participates in immunomodulation [18].

Both in the cytoplasm and in the cell nucleus melatonin has important antioxidant and scavenger effects on free radicals, which are largely independent on receptors [18]. These effects are exerted in three ways: (a) Melatonin has scavenger activity. (b) Melatonin is metabolized to compounds with high antioxidant activity. (c) Melatonin is an indirect antioxidant, stimulating the synthesis of antioxidant enzymes and inhibiting that of prooxidant enzymes. Several anti-

apoptotic and cytoprotective effects of melatonin are exerted under conditions of ischemia (not related to free radicals) and can be attributable to a mitochondrial membrane stabilizing activity [19].

Basic studies on the interaction of melatonin and BZP

GABA is the main inhibitory neurotransmitter in the supraspinal portions of the CNS. It is present in cerebral circuit interneurons, in projection neurons in some brain areas, in retinal horizontal cells and, particularly, in most NSQ neurons [20]. By the early 1980s there was no clear idea of the neuronal systems affected by melatonin in the CNS but GABA emerged as a possible candidate. With the premise that to prove that a neurotransmitter was involved in the action of melatonin, we set two requirements: (a) the neurotransmitter should show dynamic changes because of melatonin injection; (b) the functional obliteration of the neurotransmitter should significantly modify the effect of melatonin. It should be noted that there were data indicating that monoaminergic pathways were not important for the chronobiotic effect of melatonin since intraventricular injection of 6-hydroxydopamine and / or 5,7 dihydroxytryptamine, suppressing the levels of catecholamines and indoleamines, did not alter the action of melatonin on the circadian rhythms.

We devoted considerable efforts to examine the involvement of GABAergic neurons in the brain effects of melatonin [21]. GABA type A receptors inhibit neuronal firing by increasing the conductance to Cl-, an effect allosterically modified by BZP. We demonstrated diurnal changes in the number of high affinity receptors for GABA and BZP in rat brain that were altered with pinealectomy and restored by the administration of melatonin. Since the measurements of the content showed a poor evaluation of the transmitter dynamics, we studied the circadian rhythms of GABA turnover in the cerebral cortex, the basal hypothalamus, the cerebellum and the pineal gland of hamsters maintained under different lighting

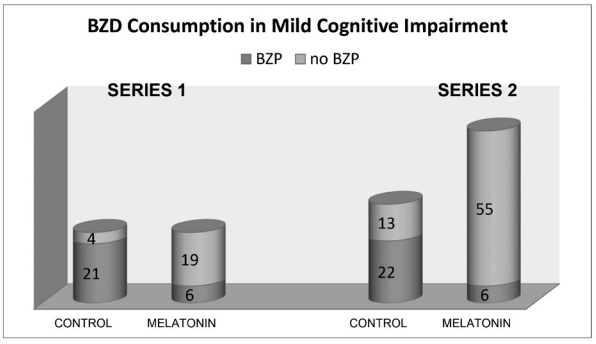

Figure 4. In two series of patients with mild cognitive impairment we were able to verify the effectiveness of melatonin (3-24 mg / day) to reduce BZP consumption. For details see [32,33].

conditions. GABA turnover in the cerebral cortex, hypothalamus, cerebellum and pineal gland exhibited significant phase relations, showing maximum values towards the first half of the night [22]

That changes in GABA in the hamster brain were associated with concomitant changes in GABA type A receptor activation was demonstrated by different experiments in which we studied the physiologically relevant phenomenon of the GABA A receptor, i.e., the Cl- ionophore [23]. We could determine that melatonin significantly affected this brain neurotransmission system. Other research groups established the participation in melatonin action of MT1 receptors and the intracellular signals triggered by them (Figure 2).

The second criterion that we considered necessary to characterize GABA as a specific mediator of melatonin was that the functional obliteration of the neurotransmitter should significantly modify the effect of melatonin. Diego Golombek demonstrated in our laboratory the dependence of melatonin activity on various behavioral parameters of the integrity of GABAergic mechanisms [24]. Among these parameters we studied the locomotor activity of the hamster, the analgesia in the mouse (today the basis for the use of melatonin in central hyperalgesia such as fibromyalgia, headaches and irritable bowel), the anticonvulsant activity (the basis of the use of melatonin

as an anticonvulsant) and the anxiolytic effect (used today, as we will see, in clinical practice to reduce BZP consumption) (Figure 3). These studies were the first to identify that the central action of melatonin in the GABAergic and BZP system may explain the chronobiotic activity of the molecule [25].

Clinical studies on the interaction of melatonin and BZP

With the introduction of melatonin as a drug for the treatment of insomnia in older adults in Argentina, the first studies were carried out on elderly patients admitted to the Centro Médico Ingeniero A. Rocca, Italian Hospital of Buenos Aires. This first study was a short-term pilot study designed to assess the efficacy and tolerance of melatonin in the treatment of sleep disorders in older adults [26]. We examined 41 patients (28 women, average age 74 years) grouped in: (a) 22 patients with sleep disorders; (b) 9 patients with sleep disorders and symptoms of depression; and (c) 10 patients with sleep disorders and dementia. All patients received 3 mg of melatonin in gelatin capsules orally 30 min before bed for 21 days. Sleep quality and daytime alertness were assessed globally through structured clinical consultations and sleep schedules completed by patients (or those caring for patients with dementia). Beginning on the 2nd or 3rd day of treatment, melatonin significantly improved sleep quality and decreased the number of awakenings in patients with sleep disorders with or without associated depression. The estimation of the function of the following day (i.e. morning and daytime alert) was significantly improved only in patients who exclusively showed sleep disorders. Although patients with dementia did not show significant improvement in sleep quality, evening agitation decreased significantly in 70% of them. Four (31%) of the 13 patients with primary insomnia who received BZP as a concomitant treatment reduced the dose (50-75% of the initial dose) and 4 (31%) discontinued the use of hypnotic agents. Of the 7 patients with depression and 7 with Alzheimer's disease who received BZP concomitantly, 2 (29%) in each group reduced BZP use by more than 50%. There were no reported side effects attributable to treatment with melatonin [26]. This confirmed the suspicion we had a long time ago: melatonin could be effective in removing patients from BZP dependence.

In the same year Dagan et al. published a case report on the efficacy of 1 mg of controlled release melatonin to discontinue the use of BZP in a 43-year-old woman who had suffered from insomnia for the past 11 years [27]. That same research group published in 1999 a double-blind, placebo-controlled study including 34 outpatients aged 40-90 years with primary insomnia who were taking BZP and who had low levels of melatonin production [28]. Fourteen of the 18 subjects who had received melatonin discontinued BZP therapy, whereas only 4 out of 16 discontinued BZP therapy in the placebo group.

Another open study from our research group supported the efficacy of fast-release melatonin to decrease BZP use. We examined the safety and efficacy of melatonin in 22 women with insomnia to whom we administered 3 mg of melatonin orally daily for 6 months, 30 min before expected sleep time [29]. Twenty of the 22 patients received BZP and continued their intake during treatment. At the end of 6 months there were no changes in serum prolactin, FSH, TSH or estradiol levels nor was there any indication of hematological or biochemical alteration in routine analysis. Melatonin significantly increased sleep quality and duration and decreased sleep latency and the number of wake episodes during sleep. It also improved alertness the next day. Urinary excretion of the melatonin metabolite 6-sulfatoximelatonin was correlated with age in this group of patients. Thirteen of the 20 patients taking BZP discontinued their use and in another 4 patients the BZP dose was reduced to 25-66% of the initial dose [29].

In collaboration with Dr. Ignacio Brusco we studied different aspects of the application of melatonin in patients with Alzheimer's disease [30; 31]. We could verify its beneficial effect on sleep disorders

and sundowning as well as on the evolution of the disease. But this therapeutic action was much clearer in mild cognitive impairment, a heterogeneous syndrome etiologically defined by cognitive deficit as the prodrome of Alzheimer's dementia. In our laboratory we have reported two retrospective analyzes of outpatients with mild cognitive impairment who had received 3-24 mg of melatonin daily. or. before bedtime for 15 to 60 months [32; 33]. There was a significant improvement in cognitive and emotional performance and sleep / wake cycles in both groups. As shown in Figure 4, comparison of the medication profile of patients with mild cognitive impairment indicated that 21 of 25 patients in the control group of series 1 and 22 of 35 patients in the control group of series 2 required BZP while that only 6 of 25 patients in series 1 and 6 of 61 patients in series 2 treated with melatonin required BZP as treatment. None of the patients treated with melatonin evolved to Alzheimer's disease. So far, 7 double-blind, placebo-controlled trials (n= 721) in the literature indicated increased sleep quality and cognitive performance by melatonin in minimal cognitive impairment patients.

What is the current situation regarding these chronobiological effects of melatonin on sleep that we observed in our initial clinical studies? In relation to sleep, several studies were generally followed by indication of a significant sleep-regulatory effect of melatonin. In a meta-analysis including 19 studies involving 1683 subjects melatonin demonstrated significant efficacy in reducing sleep latency and increasing total sleep time [34]. Longer-term trials and the use of higher doses of melatonin demonstrated greater effects. Sleep quality improved significantly in subjects taking melatonin [34].

Another relevant fact has been the approval of Circadin (a prolonged release of 2 mg of melatonin) as a prescription drug for the treatment of insomnia in the elderly by EMA in 2007. Melatonin thus acquired a status that allows its incorporation into the vademecum of several European countries. Both FDA and EMA emphasized that with melatonin or melatonin analogs there is no evidence of dependence, withdrawal syndrome, rebound insomnia or negative influence on alertness during the day.

A retrospective study of the melatonin prescription data from a German longitudinal database included all patients who used Circadin during the period April 2008 to February 2009. Of the 512 eligible patients, 112 (22%) were users of BZP/Z drugs. Approximately one-third of patients discontinued treatment with these drugs after the administration of melatonin [35].

Another pharmacoepidemiologic study aimed to analyze and evaluate the impact of market availability of melatonin in campaigns to reduce BZPZ drug use in several European countries. In their conclusion it is emphasized that the campaigns failed when they were not associated with the availability and reimbursement of melatonin [36].

A post marketing surveillance study of Circadin was conducted in Germany. The effect of 3 weeks of treatment on sleep in 597 patients was examined. Most patients (77%) who used traditional hypnotics prior to melatonin treatment had stopped using them and only 5.6% of untreated patients previously started such drugs after discontinuation of melatonin [37]. An important advantage of the use of melatonin as a chronobiotic is that it has a very safe profile, it is generally very well tolerated and in some studies has been given to patients in very large doses and for long periods of time without any potentiality of abuse.

Conclusion

A large proportion of insomniac patients on BZP treatment fail to achieve complete and sustained recovery and have residual symptoms that make relapse or recurrence of the disease more likely. Given the impact on the quality of life produced by insomnia, it is necessary to pay more attention when evaluating the effect of treatment on the daily functioning of patients. In this regard, most safety concerns with

the use of hypnotic BZP/Z-type drugs do not apply to melatonin, a fact recognized by the British Psychopharmacology Association consensus statement on evidence-based treatment of insomnia, parasomnias and circadian rhythm disorders, which recommended melatonin as first-line therapy in insomnia patients elder than 55 years of age [38]. Melatonin thus becomes the treatment of choice for reducing dependence on BZP in the treatment of insomnia in older adults.

Acknowledgements

Studies in author' laboratory were supported by grants PICT 2007 01045 and 2012 0984 from the Agencia Nacional de Promoción Científica y Tecnológica, Argentina.

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