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Physiological and metabolic functions of melatonin

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Melatonin is a lipophilic hormone, mainly produced and secreted at night by the pineal gland. Melatonin synthesis is under the control of postganglionic sympathetic fibers that innervates the pineal gland. Melatonin acts via high affinity G protein-coupled membrane receptors. To date, three different receptor subtypes have been identified in mammals: MT_1 (Mel 1a) and MT_2 (Mel 1b) and a putative binding site called MT_3. The chronobiotic properties of the hormone for resynchronization of sleep and circadian rhythms disturbances has been demonstrated both in animal models or in clinical trials. Several other physiological effects of melatonin in different peripheral tissues have been described in the past years. In this way, it has been demonstrated that the hormone is involved in the regulation of seasonal reproduction, body weight and energy balance. This contribution has been focused to review some of the physiological functions of melatonin as well as the role of the hormone in the regulation of energy balance and its possible involvement in the development of obesity.

Key words: Obesity, Energy homeostasis, Melatonin.

Melatonin (N-acetyl-5-methoxytryptamine) is a lipophilic molecule discovered in 1958 by LERNER and colleagues (57) as a hormone that promotes the aggregation of melanin granules in the dermal melanophore cells of frogs in order to lighten their skin color (48). In mammals, the hormone is mainly produced and secreted at night by the pineal gland, although other production sites have also been described such as the retina and the gut which do not contribute significantly to blood levels in mammals, but may be of local importance (7).

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Melatonin synthesis

Whatever species considered, melatonin is synthesized during the dark phase. In humans, as well as in other diurnal species, plasma levels of melatonin are high during sleep, whereas in nocturnal species (ie, the majority of laboratory animals) they peak during the active period. The duration and magnitude of the hormone secretion by the pineal gland, is directly related to the lenght of darkness period so the hormone acts as a neuroendocrine mediator of the photoperiod (82). Light has an acute suppressing effect on melatonin synthesis. The photoperiod information are conveyed from the retina to the suprachiasmatic nuclei. These nuclei, located in the hypothalamus, are the biological circadian clock in mammals. Photoperiod cues then reach the pineal gland through a polysynaptic pathways terminating in sympathetic innervation from the superior cervical ganglia (65). The synthesis of melatonin is therefore mainly controlled by the release of norepinephrine from the sympathetic nerves. The first step for the synthesis of melatonin is the uptake of the dietary amino acid tryptophan from the circulation into the gland. This uptake of tryptophan into the brain depends on transport mechanisms of the blood-brain barrier, which are in turn influenced by the presence of other neutral amino acids that compete for the transport system.

Thus, dietary conditions may affect the transport of tryptophan into the brain and consequently affect the synthesis of melatonin (7). A recent study performed in rats in which folate-deficiency was induced showed a decreased secretion of melatonin as the urinary excretion products of melatonin metabolism were diminished (38).

Tryptophan is then converted to 5-hydroxytryptophan by the enzyme tryptophan hydroxylase and then to 5-hydroxytryptamine (serotonin) by the enzyme 5-hydroxytryptophan decarboxylase. Subsequently, serotonin is acetylated by ararylalkylamine N-acetyltransferase (AA-NAT) to N-acetylserotonin (NAS), which is the rate-limiting step in melatonin biosynthesis. Finally NAS is O-methylated to melatonin by hydroxyindole-O-methyltransferase. Inside the pinealocyte the regulation of melatonin synthesis is very complex (97).

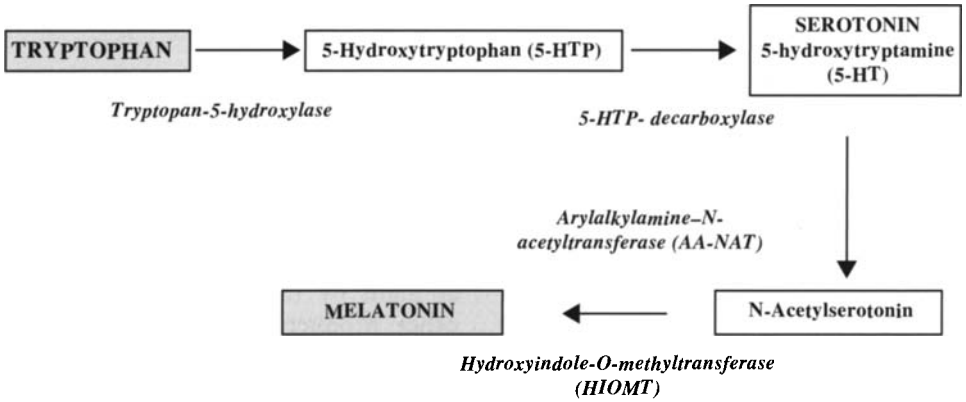

Fig. 1. *Schematic representation of the different steps in the synthesis of melatonin from tryptophan as the precursor.*

Melatonin receptors and binding sites

The discovery of melatonin binding sites has been achieved mainly because of the synthesis of 2-[^{125}I]-iodomelatonin in 1984 by VAKKURI *et al.*, (101). It has now been used by several groups to identify and characterize melatonin binding sites in a range of tissues and species using both *in vitro* autoradiography and homogenate binding (67). Using this radioligand both high- and low- affinity binding sites have been described and named ML_1 (Ki=10-300 pM) and ML_2 (Ki=1-10 nM) (32). One of the particularity of the melatonin binding sites is their very low density of expression. In rats the highest is in the pars tuberalis (about 70 fmol/mg protein) but the mean is generally around 5 fmol/mg protein which is approximately 100 fold lower than the serotonin receptors (41). This is probably the reason why the first melatonin receptor was cloned with difficulty ten years after by expression cloning using mRNA from *Xenopus laevis* immortalized melanophore (35) but it was not found in mammals. Two other melatonin receptors, Mel_{1a} and Mel_{1b}, were then cloned in human (87, 88) and referenced as MT_1 and MT_2 by the Nomenclature Committee of IUPHAR, respectively (34). These two receptors are G-protein coupled receptors and show 55% overall homology at the amino acid level. Both receptors inhibit adenylcyclase via a pertussis toxin-sensitive Gi protein. In addition, MT_1 receptor activates calcium mobilization through a pertussis-toxin insensitive Gq/11 protein and MT_2 receptor inhibits cGMP (20, 76). Both receptors display some different signalling pathways (62) and respond differently to short-term exposure to agonists (42). The expression of melatonin receptors in the rat suprachiasmatic nucleus is regulated by the exposure to the hormone. In this way, the receptors are down-regulated during night period and up-regulated during light (41). The administration of exogenous melatonin reverses this effect (40).

The tissue distribution of the human MT_1 and MT_2 receptors has been mainly studied at the brain level. They are expressed in the suprachiasmatic nucleus, the cerebellum, the cortex, the hippocampus, the hypothalamus and the cerebral arteries (87, 64, 105, 4, 93). The peripheral distribution in human tissues has been poorly studied despite the numerous binding sites described in other species (67). MT_1 is expressed in the retina, in adipose and in the kidney tissue (96, 30, 39, 20) whereas MT_2 is only expressed in adipose tissue and kidney (30, 20). In primate MT_1 is also expressed in the adrenal gland (100).

MT_2 receptor subtype is not expressed in sheep (68) and is not functional in Siberian and Syrian hamsters because of two nonsense mutations within the coding region in the MT_2 coding gene (105). Mice with targeted disruption of the MT_1 or MT_2 or both receptor subtypes have no obvious phenotype with the exception of the MT_1 in which the acute neuronal inhibition of the suprachiasmatic by melatonin is abolished whereas the phase shifting effect is only modestly altered (60, 47). This observation was in favour of an implication of the MT_2 receptor subtype in the chronobiotic effect of melatonin.

Another melatonin binding site named *MT3* which has a lower affinity for melatonin (about 10 nM) has been recently purified in hamster and identified as the human homologue of the cytoplasmic quinone reductase 2 (73). Melatonin nuclear binding sites have also been reported in the liver (1, 43) and melatonin has also been described as ligand for the ROR/RZR family of orphan nuclear

receptors (14) but the direct binding to these receptors has not been repeated (43, 15).

The pharmacological characterisation of melatonin receptors in the central nervous system (31) has stimulated the search of melatonin receptor agonist and antagonist in order to understand the mechanism of action of the hormone and to characterize the physiological role of each receptor subtype. In this way, many new molecules have been synthesized during the last years, but only a few ligands display selectivity for one subtype. Most of them are MT_2 selective (33, 59, 103, 6). Recently, the first MT_1 selective ligands have been reported (29). The selective MT_2 melatonin ligands have yet lead to the identification of some specific functions of MT_1 and MT_2 receptor subtypes and studies on cells expressing only one of the subtypes have given information on the signaling (62).

The most important site for the melatonin metabolism is within the liver. The hormone undergoes a 6-hydroxylation followed by a sulphate or glucuronide conjugation. The amount of each of these compounds formed depend on the species. In humans, the major urinary metabolite is 6-sulfatoxymelatonin. The measurement of its level provides a simple and reliable assessment of melatonin secretion (53). The absolute bioavailability of commonly used dosages of oral melatonin in healthy human is rather poor and the serum half-life is short (30-60 min) (3, 28).

Melatonin: pharmacology and therapeutic properties

The research on this molecule has provided basic physiological information on fundamental rhythmic function from micro-organisms to human. Our organism must work in harmony with the environment (photoperiod, seasonal factors and outside temperature). When the organism biological clock is not in phase with its environment (jet-lag, shift workers) the different circadian rhyhms such as melatonin, sleep-wake cycle, temperature, hormones, could desynchronize and in some case may lead to the development of pathologies. In this way cardiovascular diseases and metabolic disorders have been reported to be more frequent in shift workers (51, 50). All these rhythms are under the control of the suprachiamastic nuclei since lesions of these nuclei, at least in rodents, completely abolish circadian rhythmicity in locomotor activity, drinking, feeding, corticosterone and melatonin plasma levels and sleep-wake cycle (90, 66). Melatonin is not essential for the circadian organisation of these rhythms. Indeed pinealectomy has no effect on rodent circadian rhythms and rats that have undergone pinealectomy adjust more rapidly to light-dark cycle shift than intact rats (81). It is more the presence of melatonin at an inappropriate time, (i.e. during the day in the resynchronisation process after jet-lag or in shift workers) which is deleterious. In normal conditions melatonin probably consolidate the whole rhythmic organisation by it's chronobiotic properties. These properties have been extensively studied with success both in animal models of circadian rhythm sleep disorders or in human subjects which presented circadian sleep disorders (blind subjects, shift workers, Delayed Sleep Phase Syndrome) (5, 37, 8, 25, 92). The chronobiotic effect of melatonin is observed only if melatonin is given at appropriate schedule. A phase response curve has been determined in humans by Lewy and coworkers (58). Melatonin

administration in the morning causes phase-delays and in the afternoon phase-advances. In fact, in humans, the chronobiotic activity is the only activity that have been confirmed with appropriate clinical trials (randomized, doubled-blind, placebo-controlled trials).

In humans there is a close temporal relationship between high plasma levels of melatonin and sleep propensity rhythm. For these reasons melatonin has been claimed to have hypnotic properties. In fact appropriate clinical trials in healthy volunteers have shown that exogenous melatonin accelerate sleepiness probably via thermoregulatory mechanisms (23). Acute melatonin treatment facilitates sleep when given in a situation favorable to sleep (before bedtime, after sleep deprivation). However, although melatonin administration induces a behaviour normally associated with night in humans, it has no effect on sleep in rats, a nocturnal animal which is active during the night period when plasma levels of melatonin are highest (45, 55). Melatonin could also, when given at the appropriate time in phase-shift condition, induce phase shifts in the circadian clock such that the circadian phase of increase propensity occur at a new desired time (23).

The role of melatonin in other diseases of the central nervous system has not been clearly defined and require appropriate clinical trials to be demonstrated. In the majority of cases abnormal plasma levels of melatonin were observed in some of these pathologies which may be only a consequence and not the cause.

Melatonin is also involved in a wide range of other physiological functions but melatonin or melatonin ligands have yet to prove their efficiency in animal models relevant to human pathologies before clinical trials (27). More generally, melatonin has been described during the last ten years as a powerful antioxidant (83) and this property would explain the protective

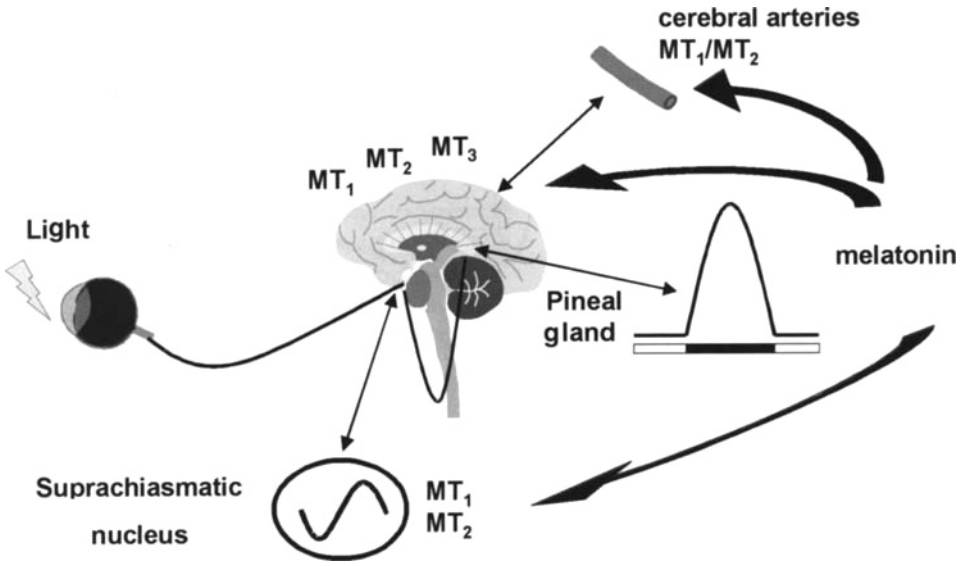

Fig. 2. *Schematic representation of the different targets of melatonin at the brain level.*

effects observed in different animal models of ischemia (85, 84). Nevertheless, if melatonin has some antioxidant properties, in the majority of cases this effect was observed at very high concentrations (1 µM – 100 mM) far from the physiological concentrations (10 – 400 pM) (94, 46). Moreover some studies have shown *in vitro* a prooxidant effect of melatonin (74, 107) and *in vivo* a deleterious effect of melatonin (99).

The duration of melatonin secretion is correlated to the length of the photoperiod and is therefore responsible for the information of seasonal changes in the organism. This hormonal signal triggers in photoperiodic animals some change in different functions (reproduction, metabolism, pelage color) in order to adapt to the environmental conditions. These mechanisms have been extensively studied mainly in hamsters (10). When these animals are maintained under constant light/dark cycle, melatonin treatments have the same effects than short day exposure i.e. gonadal regression and body weight change. This latter parameter is of particular interest because it is accompanied by modification of energy expenditure, food intake and adipose tissue distribution. In non seasonal species like mice, rats and even humans melatonin could also have some effects on energy homeostasis and body weight regulation. These melatonin effects are developed in the last part of this review.

Melatonin and energy balance

Obesity is a chronic imbalance between food intake and energy expenditure (61). The prevalence of obesity has been rising in the last years and now affects up to 30 % of adult population in different countries. Obesity is associated with high prevalence of cardiovascular disease, diabetes and cancer as well as other physiopathological conditions with high economic costs and health relevance. The sci-

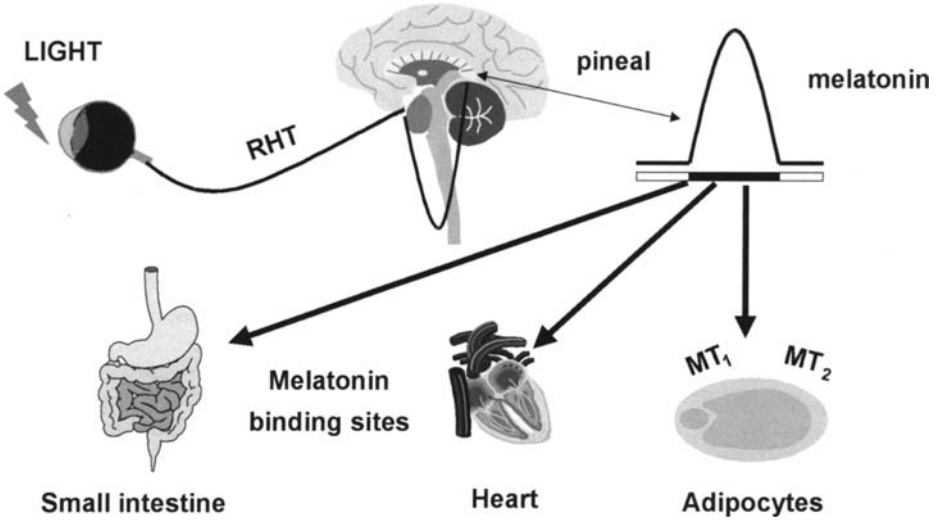

Fig. 3. *Schematic representation of the different targets of melatonin at the peripheral level.* RHT, retinohypoythalamic tract.

entific community has made a big effort in the last years in order to elucidate the origin and causes of obesity in wich neuroendocrine and genetic factors are involved in addition to eating disorders and lifestyle influences (19, 54). Although a number of treatment are available, sustained weight loss is rare, indicating the need for effective new therapeutic approaches.

Different studies performed in hibernating animals have shown that characteristic seasonal changes in fat mass observed in these animals depend on the photoperiod through the nocturnal release of melatonin. This ability to manipulate energy balance through photoperiod or melatonin in these animals provides new opportunities to discover new control mechanisms involved in the central control of energy homosteasis and body weight regulation (68, 13).

The mechanism of action of melatonin has not yet been totally understood but interesting results have been obtained during the last years showing that melatonin could act both at the central level and at the periphery.

Melatonin may affect white and brown adipose tissues via its innervation through the sympathethic nervous from brain to fat and thereby modulate adiposity. The suprachiasmatic nucleus, the organism circadian clock, is one of the nuclei which project through the sympathetic nervous system to both white and brown adipose tissues (9, 12). The suprachiasmatic nuclei express both MT_1 and MT_2 receptor subtypes and recently MT_1 receptors have been identified on the neurons of these nuclei which project to the white adipose tissue (96). Moreover microinfusions of short day-like melatonin signal into the suprachiasmatic nuclei in Siberian hamster trigger short-day-like responses including decrease in body fat (11). In Siberian hamster short days increase the sympathetic drive on white adipose tissue therefore an increase of norepinephrine turnover and consequently an increase of lipolysis which lead to the decrease of adiposity (108). The mechanism may be opposite or different in Syrian hamsters which increase their fat mass when submitted to short days (10) . Melatonin may inhibit the sympathetic nervous system drive on white adipose tissue (13). This decrease, which is observed in different animal models of obesity as in obese subjects, is generally associated with an increase in fat cell number.

In hamster MT_1 receptor subtypes are present in other hypothalamic nuclei such as the paraventricular nucleus and the dorsomedial nucleus which are known to be involved in the regulation of body weight (49,96). These hypothalamic nuclei receive different afferent signals fom neurons implicated in the regulation of food intake such as neuropetide Y (NPY), cocaine and anphetamine related transcript (CART) or proopiomelanocortin (POMC) neurons (79, 18). Melatonin may also modulate the activity of these nuclei and neuropeptides release or neuropeptide receptor expression (2).

Melatonin plays also an important role in the regulation of seasonal thermoregulation including torpor and hibernation, being involved in the circadian thermoregulatory adjustements of body temperature in different animals (91, 44). In winter many animals balance immune functions with competing physiological demands such as thermoregulation to survive. The patterns of secretion of melatonin induce several adaptations in order to conserve energy (16). The hormone acts by sending signals to the preoptic area of the hypothalamus adjusting the set point

of body temperature according to the metabolic rate of the animal. Thus, melatonin acts as a mediator of the information about energy balance in the organism (91). In Syrian hamsters exposed to short photoperiod and low temperature, treatment with a melatonin antagonist decreased the total hibernation duration. This decrease was due to a marked reduction in the number and duration of hypothermic bouts (77). In the garden doormouse, treatment with a melatonin antagonist prevented the body weight gain induced by increase duration of the night. Non-shivering thermogenesis was involved in this effect (56). Recently, other authors have demonstrated that the administration of exogenous melatonin and the presence of constant light (altered photoperiod) increased carbohydrate metabolism of rat liver and decreased hepatic lipolysis. Both manipulations induced changes in liver and kidney energy metabolism suggesting the implication of melatonin in mammalian weight regulation (71).

In addition, a direct effect of the hormone on different peripheral tissues could not be discarded due to the presence of melatonin receptors in different tissues such as white and brown adipose tissue or gastrointestinal tract from different species including human (56, 20, 109, 78). Furthermore melatonin is able to modify in vitro adipocyte function. In isolated rat epididymal adipocytes melatonin at high concentration (0.1-1 mM) inhibited basal lipolysis and insulin-stimulated lipogenesis (72). In another study, melatonin (1 nM-1 μM) inhibited isoproterenol-induced lipolysis in rat inguinal adipocytes but had no effect on epididymal adipocytes despite the expression of MT_1 and MT_2 melatonin receptor subtypes on both fat pads (109). These findings support the idea that melatonin could regulate the release of fatty acid in a site-specific manner. In the human brown adipose cell line, PAZ6, which express both MT_1 and MT_2 receptor subtypes, melatonin (10 nM) decreased Glut4 protein levels and glucose uptake (20). In isolated brown adipocytes from Siberian hamsters melatonin inhibited the expression of the mitochondrial genome through an unknown mechanism (80).

Moreover two studies have shown that exogenous melatonin can modulate the release of leptine and ghrelin, two neuropeptides secreted either by the adipocytes or the stomach and involved in the control of energy balance. In rats, exogenous melatonin decreased plasma leptin levels whereas pinealectomy increased them (24). In another study, plasma ghrelin levels were reduced by exogenous melatonin (70).

Taking into account all those evidences, it is possible that melatonin acts on body weight regulation, through the activation of central and peripheral receptors. The activation of these receptors could result in changes in metabolic rate via sympathetic nervous activity, altered feeding behaviour or directly modulate adipocyte metabolism.

In the last few years, the effect of melatonin or melatonin antagonist treatments have also been evaluated in rats. Rats are traditionally considered as non-photoperiodic animals. Nevertheless different studies have shown that at least the body weight could be reduced by short photoperiod exposure and that this effect could be mimicked by melatonin treatments or prevented by pinealectomy (27). In constant photoperiod (14h:10h light/dark cycle) daily melatonin administration in drinking water to middle-aged rats decreased their body weight, intra-abdominal adiposity and plasma leptin

without effect on food intake. The same treatment had no effect in young rats (106). In Sprague Dawley rats submitted to high fat diet treatment with a melatonin antagonist twice daily before and at the end of the dark phase reduced body weight gain without effect on cumulative food intake. In Zucker rats, a genetic model deficient in leptin receptors, the same treatment had no effect (26). The fact that in these different studies melatonin and melatonin antagonist have the same effects, ie a reduction of body weight gain, shows that the mechanisms are complicated.

Some aspects of human pathologies like seasonal affective disorders (SAD) could be compared to the state of prehibernating animals, suggesting that melatonin could be of importance in human obesity related to altered circadian rythms. These patients present atypical depressive symptoms such as increased appetite, carbohydrate craving and mass gain (89). Phototherapy has been demonstrated as an effective treatment of SAD. This is the reason why phototherapy has been evaluated in four overweight women. The result of this investigation indicated that phototherapy affects the melatonin-serotonin system and the carbohydrate regulation resulting in a loss of body weight in the women analyzed (22). Abnormalities of the melatonin circadian rhythm has also been reported. Ferrari and colleagues in 1990, demonstrated the existence of an internal desynchronizated hormonal rhythm of several plasma hormones such as adrenocorticotropine hormone (ACTH), cortisol and melatonin in women with eating disorders as anorexia nervosa and obesity (36). Another study carried out in obese women with gynoid or android type of adipose tissue distribution, melatonin rhythmicity was suppressed, suggested a role for melatonin in the hypothalamic-pituitary-ovary axis disturbances observed in obese women of post-menopausal age (75). In night-eating syndrome, characterized by morning anorexia, evening hyperphagia and insomnia, the nocturnal rised in plasma melatonin and leptin levels were attenuated (17). As in the other studies in humans, it is difficult to interprete these results. Indeed the modification of the circadian profile of melatonin could be the reason of the overweight or more probably the consequence as suggested in a recent study on the daytime fasting. Plasma melatonin levels were measured before and at the end of the Ramadan period. The nocturnal peak of melatonin was diminished and may have been delayed (21).

Future perspectives

The fact that melatonin exerts its actions in physiological or pharmacological concentrations together with the widely expression of its receptor in the organism (52) make melatonin a hormone with several therapeutic possibilities (59,26).

Moreover the ability to manipulate energy balance through photoperiod and melatonin in animal models give new opportunities to identify potential mechanisms involved in the central control of energy homeostasis and body weight (68). Indeed, this fact could help to open new research fields in the development of novel anti-obesity drugs. One problem is that obesity is a multifactorial disease and an enhanced food intake is not the only factor involved. So, in order to achieve and maintain weight loss any drug therapy should probably be directed to multiple targets. Melatonin receptor subtypes may be one these targets. New compounds able to act as specific melatonin

agonist or antagonist will help to better understand the mechanisms of action of the hormone in the regulation of different physiological functions in laboratory animals. Then appropriate clinical trials have to be performed in order to verify the hypothesis in humans.

J. BARRENETXE, P. DELAGRANGE y J. A. MARTÍNEZ. *Papel funcional y metabólico de la melatonina* (minirrevisión). J. Physiol. Biochem., **60** (1), 61-72, 2004.

La melatonina es una hormona producida fundamentalmente durante la noche por la glándula pineal. La síntesis de melatonina está controlada por las fibras simpáticas postglanglionares que inervan la glándula pineal. La melatonina actúa a través de receptores de membrana de alta afinidad acoplados a proteína G. Hasta la fecha, tres diferentes subtipos de receptores han sido identificados en mamíferos: MT1 (Mel 1a), MT2 (Mel 1b) y un posible sitio de unión denominado MT_3. Las propiedades cronobióticas de esta hormona se han descrito tanto en modelos animales como en ensayos con humanos, participando en la sincronización del sueño y los ritmos circadianos. También se han observado en los últimos años otros efectos fisiológicos de la melatonina en diferentes tejidos periféricos. Así, se ha comprobado que esta hormona está implicada en la regulación de la reproducción estacional y en el control tanto del peso corporal como del balance energético. Esta revisión supone una actualización de las funciones fisiológicas de la melatonina así como el papel de esta hormona en la regulación de la homeostasis energética y su posible implicación en la obesidad.

Palabras clave: Obesidad, Melatonina, Homeostasis energética.

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