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Role of melatonin on diabetes-related metabolic disorders

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Abstract

Melatonin is a circulating hormone that is mainly released from the pineal gland. It is best known as a regulator of seasonal and circadian rhythms, its levels being high during the night and low during the day. Interestingly, insulin levels are also adapted to day/night changes through melatonin-dependent synchronization. This regulation may be explained by the inhibiting action of melatonin on insulin release, which is transmitted through both the pertussis-toxin-sensitive membrane receptors MT_1 and MT_2 and the second messengers 3′,5′-cyclic adenosine monophosphate, 3′,5′-cyclic guanosine monophosphate and inositol 1,4,5-trisphosphate. Melatonin may influence diabetes and associated metabolic disturbances not only by regulating insulin secretion, but also by providing protection against reactive oxygen species, since pancreatic β-cells are very susceptible to oxidative stress because they possess only low-antioxidative capacity. On the other hand, in several genetic association studies, single nucleotide polymorphisms of the human MT_2 receptor have been described as being causally linked to an elevated risk of developing type 2 diabetes. This suggests that these individuals may be more sensitive to the actions of melatonin, thereby leading to impaired insulin secretion. Therefore, blocking the melatonin-induced inhibition of insulin secretion may be a novel therapeutic avenue for type 2 diabetes.

Key words: Melatonin; Circadian rhythm; Diabetes; Insulin secretion; Pancreatic β-cell; Melatonin receptor

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INTRODUCTION

From a physiological perspective, all living organisms have several common features, including a high level of robustness against external and internal perturbations. Robustness is one of the fundamental organizational principles of biological systems, and the robust design of biological systems mediates adaptation, survival and reproduction. Metabolic diseases are viewed as a breakdown of robustness in biological systems, with the disease becoming persistent if the damage cannot be repaired. Consequently, the concept of robustness can be defined as 'continuous maintenance of physiological functions' despite external and internal perturbations.

Although the human genome has remained unchanged over the last 10 000 years, our lifestyle has progressively diverged from that of our ancestors. Socially, we are people of the twenty-first century, but genetically, we remain similar to our early ancestors. In conjunction with this discordance between our ancient, genetically-determined biology and the nutritional, cultural and activity patterns in contem-

porary Western populations, many diseases have emerged. In fact, life style changes, such as nocturnality and overly rich diets, may increase the risk of metabolic diseases including diabetes and obesity[1]. Likewise, disorders of circadian rhythms have been reported as correlating with the development of metabolic diseases[2,3] and promoting glucose intolerance in humans[4]. Therefore, it is possible that the control of seasonal and circadian rhythms may be important in the prevention of diabetes.

CHRONOBIOLOGY, METABOLIC CONTROL AND DISEASE

It was realised as far back as the eighteenth century that organisms, ranging from unicellular to multicellular, exhibit inherent rhythms. Such rhythmicity plays an important role in the temporal control of a wide range of biological processes in the body, the most notable of which is metabolism[3].

The most important and well-known biological rhythm is the circadian rhythm, which is defined as the roughly 24 h cycle that characterises virtually all organisms on Earth. It is an adaptation to the periodicity at which our planet moves around its axis, which determines day length. In addition to circadian rhythms, there are ultradian rhythms, which are shorter than 24 h, and infradian rhythms, which extend beyond 24 h. To be considered a circadian rhythm, three major criteria must be fulfilled: (1) it should persist under constant external conditions, i.e. be endogenously generated; (2) it should be temperature-insensitive; and (3) it can be reset by an external stimulus, i.e. entrainment.

Tremendous advances have been made in recent years in the understanding of how circadian rhythms are controlled[2]. A complex of transcriptional factors referred to as circadian locomotor output cycles kaput (CLOCK) and brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT)-like 1 (BMAL1) controls the Period (PER) genes. This offsets oscillating feedback loops of transcription and translation, which generate waves of gene expression with a periodicity of 24 h. While this machinery is endogenously generated, it is entrained by external stimuli, of which light is perhaps the most critical one. It is also sensitive to signals from metabolism, e.g. cellular redox state has been shown to affect CLOCK activity[5]. The system is hierarchical, the suprachiasmatic nucleus in the hypothalamus being the 'master clock', with additional clock activities in numerous peripheral tissues. In fact, there is some evidence for a circadian rhythm in pancreatic islets[6]. The peripheral clocks are all thought to signal back to the 'master clock' in the suprachiasmatic nucleus.

Given the intimate relationship between circadian rhythms and metabolism, a link between the disruption of circadian rhythm and metabolic perturbation has been considered[2]. Indeed, the metabolic syndrome is more prevalent in shift workers[3], known to exhibit disturbances of the circadian rhythm, and sleep-deprivation has been associated with both obesity and type 2 diabetes[7]. Moreover, when the circadian rhythm is experimentally misaligned in humans, a profound effect on both plasma insulin and glucose levels, promoting glucose intolerance, is observed[4].

SYNTHESIS AND FUNCTION OF MELATONIN

Melatonin is an integral part of the homeostatic mechanism in the body. It signals whether light or dark prevails. Melatonin, like the neurotransmitter serotonin, is an indoleamine. It is converted in two steps from the amino acid tryptophan into serotonin (5-hydroxytryptamine, 5-HT), and then acetylated by arylalkylamine *N*-acetyltransferase (AA-NAT), the rate-limiting step in melatonin biosynthesis, before finally being converted into melatonin by hydroxyindole-*O*-methyltransferase (HIOMT)[8].

The indoleamine is mainly secreted by endocrine cells (pinealocytes) in the pineal gland, which is located in the midline of the brain, just above the posterior commissure at the dorsal edge of the third ventricle. Melatonin remains detectable after pinealectomy in some species[9], and subsequent investigations have revealed that the hormone is also produced by neurendocrine cells in the retina, Harderian glands, gastrointestinal tract and pancreas[10]. Melatonin is also produced by numerous non-endocrine cells, e.g., immune cells. Hence, while the pineal gland quantitatively accounts for the circulating pool of the hormone, substantial local synthesis also occurs in retinal and peripheral tissues such as the gastrointestinal tract.

From a physiological perspective, the most well-known role of melatonin is that as a chronobiotic factor or *zeitgeber*, adjusting the timing or reinforcing oscillations of the biological clock, i.e., entrainment[11]. As such, it is thought to participate in the control of seasonal as well as circadian rhythms. This is based on the fact that the secretion of melatonin reflects ambient light and normally exhibits a tightly regulated diurnal pattern. For this reason, melatonin is sometimes called 'the hormone of darkness'. Disruptions may occur in individuals deprived of light, e.g., shift workers or travellers across time zones. On a daily basis, melatonin has a small modulatory effect on the pacemarker activity of the circadian clock in the suprachiasmatic nucleus. On a seasonal basis, the varying lengths of the peaks and troughs of the circulating levels of melatonin follow the changes in the duration of daylight. The seasonal regulation of the nocturnal secretory duration is the primary cue regulating the reproductive function in mammals that breed seasonally.

Melatonin also affects the cardiovascular system[12] and interacts with the immune system[13]. It has also been implicated in metabolic control[14]. Given that the sites of melatonin production are widespread, its effects may be both endocrine, *via* melatonin released from the pineal gland, and paracrine/autocrine, *via* melatonin released in the vicinity of its target tissues[15]. An interesting feature of melatonin is its capacity to act as an antioxidant, owing to its chemical structure. However, melatonin does not undergo redox cycling, i.e., repeated oxidation and reduction, but is a ter-

minal or suicidal antioxidant instead[16].

From a pharmacological view, the phase-advancing effects of melatonin have frequently been exploited[17], with the indoleamine proven to be effective in the treatment of insomnia[18,19] and efficient in limiting jet lag when travelling across time zones[20]. Therefore, the administration of pharmacological doses of melatonin promotes both phase advancement and resynchronisation of the biological clock.

MELATONIN RHYTHM AND INSULIN SECRETION: AN ANTAGONISTIC RELATIONSHIP

There is favourable evidence that the circadian rhythm of melatonin influences insulin secretion and the endocrine pancreas[21,22]. Most studies conclude that the pineal gland has a suppressive effect on the activity of the β-cell, because melatonin lowers insulin levels in rats[23-25] and these effects are in agreement with a reduction in glucose tolerance[26,27]. Based on these findings, and the realization that an increased insulin level exerts an inhibitory effect on the pineal gland and melatonin[28,29], a functional antagonism between insulin and melatonin has to be assumed. This fact is even more striking when taking into account that high levels of insulin have always been measured when melatonin concentration was reduced, i.e., during the day; contrary to the situation of low levels of insulin along with high melatonin and glucose levels during the night[30]. In accordance with these results are rat studies which proved that the synthesis of melatonin declines with increasing age, whereas the synthesis of insulin and leptin increases[23], and that melatonin is able to stop the age-related insulin increase[25]. Complementary to these findings are publications reporting that melatonin levels are reduced in diabetic hamsters[28,29,31]. On the other hand, there is evidence for a diabetes-preventing effect of melatonin, whereas pinealectomy increases the risk[32,33]. Likewise, further data demonstrate that melatonin directly influences both glucose metabolism and insulin secretion from the β-cell[34-37].

That insulin secretion is controlled by circadian mechanisms is supported by studies of humans with circadian misalignment, who are reported to show profound perturbations of glucose and insulin levels[4]. The concept is supported by the assumption that there is a circadian clock in pancreatic islets[6]. Moreover, there are indications that the diurnal secretion of melatonin is altered in diabetes, particularly when neuropathy is evident[38]. Peschke *et al*.[22] reported reduced circulating melatonin levels and elevated insulin levels in type 2 diabetic patients, with a statistically significant negative correlation between both molecules. Similarly, nocturnal melatonin levels are reduced in the Goto-Kakizaki (GK) rat, a model of type 2 diabetes[22]. Also, the amounts of mRNA of the melatonin synthesizing enzymes, such as HIOMT, are altered under diabetic conditions. In addition, the concentrations of all precursors of melatonin, including tryptophan and serotonin, are diminished in the pineal glands of diabetic GK rats, and the pineal glands of diabetic GK rats contain less noradrenaline and produce less melatonin in reaction to noradrenaline *in vitro*[39]. Confusingly, animal models of type 1 diabetes, i.e., streptozotocin- and alloxan-treated rodents, exhibit either elevated[40] or decreased[41] levels of melatonin. These observations suggest a functional interrelationship between melatonin and insulin, and may indicate a reduction of melatonin in the genesis of diabetes[22]. In this context, novel results have reported that melatonin-enhanced insulin receptor kinase activity increased insulin receptor substrate 1 (IRS1) phosphorylation, thus suggesting the potential existence of a signalling pathway cross-talk between melatonin and insulin[42]. Furthermore, melatonin also increased the activity of phosphatidylinositol 3-kinase (PI-3-kinase), whereas 3',5'-cyclic adenosine monophosphate-activated protein kinase (AMPK), another important glucose transport stimulatory mediator (*via* an insulin-independent pathway), was not influenced by melatonin application[43]. Therefore, melatonin stimulates glucose transport to skeletal muscle cells through the IRS1/PI-3-kinase pathway, which implies, at the molecular level, a putative role in glucose homeostasis and possibly in diabetes[43]. Additionally, it was speculated that aging and the exposure to light at night, both of which lower melatonin levels, may contribute to the incidence and/or development of diabetes[43].

INSULIN SECRETION IN PANCREATIC β-CELLS IS ORGANIZED BY A CIRCADIAN RHYTHM

Various investigators have postulated oscillations of insulin secretion within a range of seconds, to periods of between 9 and 14 min under both *in vivo* and *in vitro* conditions[44-46]. Furthermore, in clonal pancreatic β-cells, with periods of 5 to 8 min, a rhythm was superimposed with 15- to 20-min interval fluctuations[47]. The current opinion is that they are generated by a pacemarker located within the pancreas. These observations have been made on decentralized islets of dogs[48], mice[49], rats[50] as well as those of humans[44]. Thus, in man, a circadian rhythm of enhanced insulin secretion during the day, and a decrease during the night has been described[30]. In this case, insulin and melatonin plasma concentrations change in an opposing manner during the 24-h period, i.e., melatonin peaks when insulin is at a low level, and *vice versa*. Further information on the circadian rhythms of insulin secretion was obtained from isolated rat pancreatic islets, maintained in an *in vitro* perifusion system[6]. In this case, a circadian pattern was also observed, with periods between 22 and 26 h. Adding melatonin as a *zeitgeber* during analysis of the phase responses in insulin secretion resulted in circadian phase shifts. After melatonin application, the circadian period was maintained, but the amplitude was enhanced. From this experiment, it was concluded that an endogenous oscillator is located within the pancreatic islets of the rat which regulates the insulin secretion of β-cells in a circadian fa-

shion. Additionally, important investigations in rat insulinoma cells INS1 have shown that an overnight pre-treatment with melatonin produced a marked increase in insulin secretion, 3',5'-cyclic adenosine monophosphate (cAMP)-response element (CRE)-mediated gene expression and insulin-promoter-driven luciferase gene expression in response to glucagon-like peptide 1 (GLP1) or forskolin[36]. However, prolonged exposure of INS1 cells to melatonin application (12 h) caused sensitization of cAMP-mediated responses to forskolin and GLP1. This phenomenon may represent the first evidence of a specific physiological role for melatonin-induced sensitization of pancreatic β-cells with respect to cAMP signalling[36]. On the other hand, an inappropriate time schedule for the administration of melatonin may induce supraphysiological concentrations of melatonin, thus resulting on a desensitization of melatonin receptors. Lengthy exposure to melatonin might mimic 'artificial darkness', thereby causing physiological disturbances, e.g., to glucose metabolism[51], whereas pinealectomy, which leads to melatonin depletion, appears to decrease insulin sensitivity, as well as GLUT4 gene expression[52].

MELATONIN RECEPTORS IN β-CELLS

If melatonin has direct effects on insulin secretion, its receptors should be present in islets of Langerhans, preferably β-cells. This indeed appears to be the case, as inferred from studies using the non-hydrolysable guanosine-5'-trisphosphate (GTP) analogue guanosine 5'-*O*-(3-thiotrisphosphate) and the melatonin antagonist luzindole[34], both of which block the effects of melatonin on insulin secretion from neonatal rat islets. Likewise, using molecular techniques, it was demonstrated that a melatonin receptor mRNA identical to that cloned from the rat brain is expressed in pancreas tissue of newborn rats[53]. The specificity of the single amplification product was confirmed by restriction analysis and nested PCR, indicating that it corresponds to the predicted MT_1 receptor. A possible co-expression of the MT_2 receptor in the pancreatic tissue was initially excluded[54]. Thus the results indicate that a melatonin receptor, most likely the MT_1 receptor, was located in the pancreatic islets of neonate rats and that the pancreatic islets are targets for receptor-mediated melatonin influences[34,36,37].

Since molecular results concerning the detection of a melatonin receptor was collected on whole pancreatic tissue only, it was therefore crucial to institute a cell system that allowed detection at the level of a single β-cell. To examine this aspect, a glucose-responsive, insulin-producing insulinoma cell line INS1, isolated from rats, was used. Comparable to the results of islets, the competitive receptor antagonist luzindole diminished the insulin-decreasing effect of melatonin. Moreover, PCR experiments using specific primers for the rat melatonin receptor MT_1 showed that this melatonin receptor mRNA is also expressed in the INS1 cells[35,36]. Evidence was exclusively found for expression of the MT_1 receptor in the pancreatic β-cell model INS1, in the pancreatic islet and in the whole rat pancreas. In contrast, phase-shifting effects on the insulin rhythm in isolated islets of rats after application of melatonin indicated expression of a putative MT_2 receptor on the pancreatic β-cell[6]. By using the recently developed technique of fluorescence-dye-coupled real-time RT-PCR, rat pancreatic tissue, isolated islets and INS1 cells were examined for melatonin receptor transcript expression. Experiments succeeded in amplifying MT_1 as well as MT_2 mRNA-derived PCR products, which were verified by gel electrophoresis and restriction analysis[55]. A quantitative comparison of MT_1 versus MT_2 receptor expression for islet-derived transcripts indicated that the MT_2 transcript concentration is much lower (86-fold) in this tissue compared to MT_1 mRNA level. This low level expression possibly explains the lack of conclusive results for the existence of the MT_2 receptor in earlier studies[34,35]. Recently, molecular and immunocytochemical investigations have established the presence of the melatonin membrane receptors MT_1 and MT_2 in human pancreatic tissue and, notably, also in the islets of Langerhans[56]. On the other hand, an upregulation of the expression of melatonin receptors in type 2 diabetic patients was also observed in immunocytochemical investigations[21]. In addition, the transcription factors RORα, RZRβ and RORγ were detected in human pancreatic tissue and islets. In correlation with membrane melatonin receptors, data indicated increased mRNA expression levels of RORα, RZRβ, and RORγ in type 2 diabetic patients. Thus, the data demonstrate the existence of the melatonin membrane receptors MT_1 and MT_2, as well as mRNA expression of nuclear orphan receptors in human pancreatic tissue, with upregulated expression levels in type 2 diabetic patients[21]. These data on nuclear melatonin receptors are still preliminary, but complement the results on the better characterized membrane receptors MT_1 and MT_2, without claiming to imply connections to specific functions for insulin secretion within the islet.

SIGNAL TRANSDUCTION OF MELATONIN RECEPTORS IN β-CELLS

It is widely accepted that melatonin exerts some of its biological effects through specific, high-affinity, pertussis toxin-sensitive, inhibitory G protein (Gi)-coupled receptors[53,54]. Several physiological studies have focused on MT_1 receptor-mediated effects on insulin secretion. These studies employed either rat pancreatic islets[34,37] or the rat insulinoma β-cell line INS1 and receptor antagonists like luzindole[35,36], and confirmed the inhibitory effects of melatonin on cAMP-stimulated insulin secretion, which are mediated via Gi protein-coupled MT_1 receptors. However, the intracellular signalling of melatonin in pancreatic β-cells is not limited to the cAMP signalling pathway. In fact, an interplay between the cAMP or 3',5'-cyclic guanosine monophosphate (cGMP) cascades seems possible in the light of the recent discovery of the second melatonin receptor isoform, MT_2, and the fact that cGMP-dependent protein kinase G (PKG) is highly expressed in rat islets[57] and

insulin-secreting β-cells lines[58]. Recently, Stumpf *et al*[59,60] shed some light on the cGMP signalling pathway in rat INS1 cells, since they showed that melatonin inhibits the second messenger cGMP and suppresses insulin secretion through MT_2 receptor activation. However, as cGMP is synthesized via the action of the membrane and, particularly, the soluble nitric oxide (NO)-dependent guanylate ciclases[55], NO synthase-transmitted effects have to be considered.

On the other hand, there is evidence for the involvement of the inositol 1,4,5-trisphosphate (IP_3) system in the signalling cascade of melatonin in a growing number of cell types. In contrast to the uniform cAMP-dimishing effect of melatonin, both IP_3-increasing[61,62] as well as IP_3-decreasing[63,64] effects of melatonin have been described in different cell types. Previous studies in INS1 insulinoma cells indicated a dose-dependent stimulation of IP_3 release by melatonin, while the competitive melatonin receptor antagonist luzindole was able to completely abolish such IP_3-liberating effects of melatonin, thus giving strong evidence for the involvement of melatonin receptors[65]. Furthermore, it was also shown that such a melatonin-induced IP_3 liberation was able to release Ca^{2+} from intracellular stores[65], a mechanism that is commonly accepted as a trigger for insulin secretion. Despite *in vitro* expressed melatonin receptors exhibiting differential abilities to stimulate phospholipase C (PLC) through Gq proteins[66,67] and the MT_1 receptor having been shown to couple with Gq proteins in an agonist-dependent and guanine nucleotide-sensitive manner in HEK293 cells[68], the melatonin-dependent stimulation of PLC through Gq-coupled MT_1 receptor can only be hypothesized in pancreatic β-cells. In this regard, it has been reported that stimulation of INS1 cells with melatonin provokes the release of IP_3[65,69], and when Gi coupling is blocked by pertussis toxin, a stimulatory effect of melatonin is revealed[69]. In conclusion, it was found that the melatonin receptors on β-cells are coupled to three parallel signalling pathways, with different influences on insulin secretion. In terms of insulin release, the insulin-inhibiting action of melatonin is transmitted by the dominantly expressed MT_1 receptor through attenuation of Gi-coupled adenylate cyclase activity, thereby negatively modulating incretin-induced rises in cAMP. Likewise, it was recently detected that melatonin inhibits the cGMP signalling pathway and, consequently, insulin secretion, possibly in a MT_2 receptor-mediated fashion. Meanwhile, melatonin-dependent IP_3 release may play a role in the short-term support of other IP_3-releasing agents, like acetylcholine, or may be related to the activation of protein kinase C (PKC) or the long-term regulation of β-cell functions with enhancing effects on insulin secretion.

MELATONIN MODULATES DIABETES-RELATED ALTERATIONS

Hyperglycemia is the backbone of the pathophysiology of diabetes, leading to the development of complications like diabetic neuropathy or vascular diseases, through many intertwined cellular pathways which have been shown to coalesce into a common fate, i.e. oxidative stress. Vascular diseases are major long-term complications in patients with diabetes. For instance, vascular production of both excessive reactive oxygen species (ROS) and excessive reactive nitrogen species (RNS) may contribute to endothelial dysfunction during diabetes, as well as modification of low density lipoproteins induced by high glucose concentrations[70]. In addition to their ability to inflict direct damage on macromolecules, ROS and RNS activate a number of cellular stress-sensitive pathways, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and p38 mitogen-activated protein kinase (MAPK) pathways, which play a key role in the development of not only the late complications in type 1 and type 2 diabetes, but also the insulin resistance and impaired insulin secretion seen in type 2 diabetes [71]. Pancreatic β-cells are very susceptible to oxidative changes because they possess only low antioxidative capacity[72]. In this sense, it has been suggested that antioxidant treatment might be an important therapeutic option for preventing vascular complications in diabetes[73]. Since melatonin provides both *in vivo* and *in vitro* protection at the level of cell membranes, mitochondria and nucleus, due to its free-radical scavenging and antioxidant properties[74], the relationship between melatonin and the impaired antioxidant status in diabetes has become a topic of great interest in the last few years[75,76].

For some decades, alloxan and streptozotocin have been widely used to induce diabetes in animals. Both compounds lead to selective destruction of pancreatic β-cells, as they rapidly accumulate in β-cells, where they induce radical-generating reactions. Alloxan, once inside the cells, produces ROS, specially superoxide anions and hydrogen peroxide, thereby consuming reduced glutathione (GSH) and further weakening the cellular antioxidant defence system[77]. There is no question that melatonin, due to its well-established importance as a free-radical scavenger, protects against alloxan- and streptozotocin-induced diabetes. Thus, it was postulated that melatonin may protect against alloxan-induced diabetes in mice[78] and attenuate diabetes-induced alterations in the GSH redox state and in the hydroxyl radical levels in rabbit[79]. This fact was also confirmed at the level of perifused pancreatic islets[80,81]. Furthermore, it was demonstrated that melatonin can effectively scavenge alloxan-induced production of hydroxyl radicals, and inhibit hydroxyl radical-triggered lipid peroxidation in liposomes[82]. Melatonin also reduces morphological damage of the β-cells after application of alloxan, and counteracts alloxan-mediated leakage of insulin from pancreatic β-cells[81,82]. Additionally, melatonin has been shown to restore the reduced levels of nitric oxide, glutathione peroxidase and superoxide dismutase to normalcy in alloxan-induced diabetes[83], thus highlighting the putative use of melatonin to prevent atherosclerosis and other complications arising from diabetes. On the other hand, alloxan-induced diabetes may decrease pineal melatonin synthesis in rats by reducing the activity of HIOMT, thus resulting in a drop in pineal melatonin secretion[41].

During metabolism of streptozotocin, a variety of toxic intermediates are produced. Besides alkylating agents like methyl cations and methyl radicals[84], it has been shown that ROS are produced by streptozotocin as well[85]. Moreover, streptozotocin liberates NO which has been proposed to be one of the key intermediates of its toxicity[86]. Taken together, streptozotocin-induced diabetes increases oxidative stress through generation of free radicals[87], lipid peroxidation, superoxide dismutase, protein glycosylation[88], decreased levels of catalase and glutathione peroxidase[89], as well as DNA single-strand breaks[85]. In the serum of animals with streptozotocin-induced diabetes, melatonin remarkably reduces the degree of both lipid peroxidation and protein glycosylation[88], decreases the levels of cholesterol, triglyceride, low-density lipoprotein[90], sialic acid[91] and glucose[92], as well as possibly regulating the activities of antioxidant enzymes[89]. Nevertheless, the most pronounced effect of melatonin administration was the prevention of an increase in NO levels in blood plasma during streptozotocin-induced diabetes[93], which implies that melatonin may operate as an NO scavenger and carrier. Despite this fact, another investigation concluded that the protective effects of melatonin against streptozotocin-caused β-cell damage may be related to interference with DNA damage and poly(ADP-ribose) polymerase (PARP) activation rather than through effects on NO pathways[94]. On the other hand, streptozotocin-induced diabetes reduced the nocturnal pineal melatonin content in Syrian hamsters[29], but not in rats[31], and the plasma and saliva melatonin levels in type 1 and type 2 diabetic patients[95]. Also, stretozotocin-induced diabetes resulted in lower melatonin levels in the pancreas, kidney and duodenum compared to the control, thus suggesting that the lower amplitude of melatonin in target organs induced by streptozotocin might contribute to the desynchronization of daily rhythms and might also weaken the antioxidant capacity of tissues[96].

Diabetic neuropathy is counted among the most prevalent and incapacitating complications of diabetes, and is associated with clinically significant morbidities[97,98]. As mentioned earlier, ROS are also notorious for contributing to cell and tissue dysfunction and damage in diabetic neuropathy. It is assumed that prolonged hyperglycemia, through overproduction of ROS, is likely to damage dorsal root ganglion mitochondrial DNA, thus contributing to long-term nerve dysfunction[99]. Likewise, such an overproduction of ROS ultimately leads to exhaustion of natural antioxidant pools in the vascular endothelium and Schwann cells of the sciatic nerve, which in turn may contribute to the neurovascular and metabolic deficits in diabetic neuropathy[100]. In this context, the beneficial effects of various antioxidants in experimental diabetic neuropathy have been shown in the last few years[101-103]. As a matter of fact, previous studies indicate that melatonin is neuroprotective in streptozotocin-induced rat model of diabetic neuropathy[104]. Once again, it would appear that the quenching of free radicals by melatonin might be the central mechanism for exerting neuroprotection[105,106], although other melatonin-induced neuroprotective mechanisms cannot be ruled out. Thus, recent evidence in an experimental model of diabetic neuropathy suggests that melatonin modulates neuroinflammation by inhibiting the NF-κB pathway and downstream mediators of inflammation, and protects against oxidative stress by upregulating the nuclear erythroid 2-related factor 2 (Nrf2) pathway, thereby contributing to melatonin's neuroprotective effect[107]. Apart from its abovementioned effects, melatonin has been shown to forestall many other diabetes-related complications, such as altered pain perception[108], fatty liver[109], obesity[110] and renal[76] injuries, although mechanisms underlying such beneficial effects of melatonin need to be clarified further.

ASSOCIATION BETWEEN POLYMORPHISMS OF THE *MTNR1B* LOCUS AND DIABETES

Melatonin receptor deficiency or malfunction has been related to various diseases. Changes in insulin secretion observed in MT_2 variants[111] and melatonin effects on glycogen synthesis mediated through an atypical PKC (PKCζ)-Akt-glycogen synthase kinase 3β (GSK3β) signaling pathway[112] may be interpreted in this context. Likewise, the finding that the MT_1 knockout causes insulin resistance in mice[113] seems to support the general idea of intact melatonin signaling required for avoiding type 2 diabetes, but may also be indicative of species differences between mice and humans. Recently, genome-wide association studies revealed a close link between specific single nucleotide polymorphysms (SNP) of the melatonin MT_2 receptor (*MTNR1B*) locus and a prognostic risk of type 2 diabetes[114-116]. In fact, these studies present evidence that a particular SNP (rs10830963) significantly increases the risk of type 2 diabetes in the European cohorts examined. The coding sequence of *MTNR1B* is interrupted by a single intron and the SNP rs10830963 is localised within the non-coding intron sequence, although it does not interfere with consensus sequences for transcription factors or with splicing[115]. However, this SNP is correlated with higher fasting glucose levels and a high incidence of this allele is also correlated with pathologically altered insulin secretion responses[114].

A second SNP with modulatory effects on the glucose metabolism in populations of European origin has been identified[117]. This SNP is again correlated with increasing fasting glucose levels in carriers of this allele. Nevertheless, it is not correlated with obesity or body mass index, which are type 2 diabetes risk factors. This SNP is localised in the 5' promoter region of the *MTNR1B* locus and may thus influence transcription. It was independently published that the *MTNR1B*-associated SNPs rs10830962, rs4753426 and the aforementioned rs10830963 were all significantly linked to higher fasting plasma glucose concentrations and reduced insulin release in German cohorts[118]. Moreover, the intron-localised risk allele rs10830963 is not restricted to cohorts of Caucasian origin, but also occurs in Han Chinese individuals[119]. These studies suggest that de-

fined single nucleotide base pair variations in the vicinity of the *MTNR1B* locus, or overlapping with it, are causally linked to an increased risk of developing type 2 diabetes. In fact, increased MT_2 receptor transcription in human islets from the risk genotype with an intron-localised SNP was reported, compared to transcription from the non-risk allele[114]. This observation may be viewed in a broader context with the increased MT_2 and MT_1 receptor expression observed in pancreas explants of type 2 diabetes patients[21]. Despite the fact that only one SNP is located in the 5' regulatory region of *MTNR1B*, these studies suggest that changes in β-cell MT_2 receptor expression may be responsible for the development of type 2 diabetes. It can therefore be speculated that increased melatonin receptor expression due to its coupling with Gi proteins leads to decreased second messengers (cAMP or cGMP) levels, with subsequent detrimental effects on insulin secretion[114].

CONCLUSION

Melatonin is a pleitropic, nocturnally peaking and systemically acting chronobiotic. Several generalizations can be proposed regarding melatonin. Since it readily passes all biological membranes to reach intracellular organelles, many cells can synthesize melatonin, presumably to scavenge the oxygen- and nitrogen-based reactants produced in these cells, and, moreover, its membrane receptors are widespread in mammals and mediate some of the melatonin's actions. It has been determined that the effects of melatonin on insulin secretion are mediated through the melatonin receptors (MT_1 and MT_2). By inhibiting cAMP and/or cGMP pathways, melatonin reduces insulin secretion. However, it has been shown that melatonin activates the PLC/IP_3 pathway, which mobilises Ca^{2+} from intracellular stores and, subsequently, increases insulin secretion. Meanwhile, insulin secretion, both *in vivo* and *in vitro*, exhibits a circadian rhythm, apparently generated within the islets, which is influenced by melatonin by inducing a phase shift in insulin secretion. The observation that clock genes exhibit circadian expression in pancreatic tissue could be an indicator of the generation of circadian rhythms in the pancreatic islets themselves. Also, plasma melatonin levels and AA-NAT are decreased in type 2 diabetes patients. Taken together, these results indicate a close interrelationship between insulin and melatonin, which may be significant for the genesis of diabetes. This has been recently supported by genome-wide association studies revealing a close link between SNPs of the MT_2 receptor (*MTNR1B*) locus and an increased prognostic risk of type 2 diabetes. Time will tell whether MT_2 antagonists could serve as therapeutic agents in type 2 diabetes. The option of blocking the effect of melatonin in islets is an attractive possibility, although an islet-specific attenuation of melatonin action may be required, since it can be foreseen that the systemic effects of an MT_2 blockade may be disadvantageous. Nevertheless, the discovery of the link between the MT_2 receptor and type 2 diabetes emphasises the importance of biological rhythms for metabolic regulation.

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