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Rhythmic melatonin release controlled by the SCN and its consequences for glucose homeostasis

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Abstract

The regulation of glucose homeostasis by melatonin starts in the suprachiasmatic nucleus (SCN). Here, two neurotransmitters are produced which indirectly influence melatonin synthesis by the pineal gland. GABA inhibits the sympathetic input from the paraventricular nucleus (PVN) to the pineal gland during the light period, and glutamate, in contrast, stimulate the PVN whereby the signal can be transmitted to the pineal gland resulting in melatonin synthesis. Nocturnal melatonin secretion is an output signal of the circadian clock able to convey photoperiodic, as well as circadian signals to multiple structures/organs possessing melatonin receptors within the brain or the periphery. There is probably an endogenous rhythm, generated by the SCN, responsible for the rhythmic entrainment of the pineal gland. Melatonin is a time messenger candidate to entrain all the clocks in the body, which is especially important in glucose homeostasis. It is shown that a lack of clock genes in the liver results in impaired glycogenesis, reduced hepatic glucose production and increased glucose tolerance, while a lack of melatonin signalling displays glucose intolerance and a desynchronized circadian pattern of gluconeogenesis, hallmarked by increased night time glucose levels. Plasma insulin levels show a daily rhythm as well as it follows on food intake. Ablation of the peripheral clock in the pancreas caused hyperglycemia and diabetes mellitus type 2 due to disturbed β-cell function. Is has been made clear that melatonin has an inhibitory effect on these pancreatic β-cells via its MT1 and MT2 receptors. Melatonin has its receptors not only in the periphery, but also in the brain. Stimulation of the MT1 receptor in the SCN has a stimulatory effect on the neuronal activity. Melatonin has also antioxidant and anti-inflammatory functions, which can be helpful in the treatment against obesity and diabetes through the JAK-STAT3-SOCS3 pathway. The aim of this literature study is to evaluate the effect of the SCN on melatonin release and the consequences on glucose homeostasis. In conclusion, melatonin is a promising candidate for a variety of disorders related to glucose metabolism, with or without the influence of circadian rhythms.

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1. Introduction

Over the past two decades, molecular mechanisms which determine circadian clockworks in mammals have been increasingly elucidated. It is understood that the master biological clock in the suprachiasmatic nucleus (SCN) is similar to peripheral circadian clocks (Erren & Reiter, 2015). Light is the primary environmental cue that entrains the main circadian clock in the SCN, thus allowing the internal rhythms are put at a 24 hour period on a day-to-day basis. In mammals, including humans, retinal ganglion cells perceive the ambient light and transduce this photic signal to the SCN through the retinohypothalamic tract. Through this tract, the circadian rhythm is generated within SCN neurons and this rhythm is entrained to an overt 24 hour rhythm. These coordinated outputs are conveyed to the rest of the body via behavioural, neuroendocrine, and autonomic pathways (Schibler et al., 2015). This endogenous circadian timing system is regulated very well across the 24 hour day when it is properly aligned with the sleep/wake cycle. Violations of conditions set by our biological clock, such as shift work, jet lag, sleep deprivation, or simply eating at the wrong time of the day, may have deleterious effects on health. This infringement, also known as circadian desynchronization, is associated with chronic diseases like diabetes, hypertension, cancer, and psychiatric disorders (Buijs et al., 2016). It is no surprise that the SCN regulates much of our physiology and behaviour across the 24 hour day (Roden et al., 1993). For example, the great majority of night shift workers showed no evidence for a nocturnal orientation of their endogenous clock and did not even show a discernible phase shift of the hormonal rhythms when compared with diurnal workers. Is has been established that the endogenous component in the circadian variation of certain variables may be disturbed by exogenous influences such as sleep, food intake, fluid intake, physical activity, and stress, which are so called masking effects (Knutsson, 2004).

Melatonin is an important hormone to convert environmental information (i.e. length of the dark period) to an organism and thereby synchronizing the circadian 'master clock' in the hypothalamic SCN (Morgan et al., 1994). This lipophilic hormone is produced by the pineal gland which is regulated tightly by the SCN. Rhythmic release of the neurotransmitter gamma-aminobutyric acid (GABA) from the SCN inhibits sympathetic input from the paraventricular nucleus (PVN) of the hypothalamus to the pineal gland during the light period. During the dark period, the constant inhibition of the GABAergic input disappears, which leads to secretion of melatonin (Picinato et al., 2002). This hormone is secreted at night in both diurnal and nocturnal animals. This specific timing suggests that melatonin is best described as a 'night time hormone', rather than a 'sleep hormone' (so called for its mild sedative properties in humans) (Cajochen et al., 2003).

One of the most important circadian regulators is the regulation of energy metabolism. It is very well documented that for example plasma glucose concentration, glucose tolerance and insulin sensitivity vary throughout the day and are rhythmically coordinated by circadian clocks (Kalsbeek et al., 2007). Furthermore molecular clock mechanisms play a specific role in the control of glucose metabolism and speculate on how disruption of these tissue clock may lead to the disturbances in glucose homeostasis (Kalsbeek et al., 2014). The first evidence that the SCN is involved in the daily rhythm in glucose metabolism came from the work of Nagai and Nakagawa, who showed that SCN lesions abolished the daily rhythms in plasma concentrations of glucose and insulin (Yamamoto et al., 1987). In shift workers, Hampton et al. found that after phase shift there were significantly higher postprandial glucose levels than before. Similar results were obtained for insulin (Hampton et al., 1996). Recent research concluded there is also a robust endogenous circadian rhythm in glucose control in humans (Qian & Scheer, 2016).

While neuronal and hormonal cues appear to be involved in circadian rhythms from the SCN, it is still unclear how time messaging from one central clock to billions of peripheral clocks is achieved. Melatonin is a promising time messenger candidate when trying to understand how time and timing information is provided throughout the body (Erren & Reiter, 2015). Findings in a genome wide association study indicate a link between circadian rhythm regulation, glucose homeostasis, and melatonin signalling in the pancreas; supporting the idea that uncoupling of peripheral circadian clocks may contribute to metabolic dysregulation in humans (Bouatia-Naji et al., 2009).

Two important hormones involved in glucose homeostasis are insulin and glucagon. Insulin is synthesized in pancreatic β-cells as a reaction on high blood glucose levels which is continuous monitored by Islets of Langerhans cells in the pancreas. Insulin causes glycogenesis in the liver and body cells to take up glucose from the blood. If the blood glucose levels are low, α-cells of the pancreas release glucagon whose effects on liver cells act to increase blood glucose levels. Furthermore the hepatocytes of the liver can synthesize new glucose when hepatic glycogen reserves become exhausted, this is called gluconeogenesis.

Hence the pancreas and the liver are the most important organs in the regulation of glucose homeostasis. Therefore these organs will be mainly mentioned in this review. The aim of this literature study is to evaluate the effect of the SCN on melatonin release and the consequences on glucose homeostasis.

2. Melatonin secretion by the SCN

2.1. Pathway from SCN to pineal gland

The regulation of melatonin production is probably the best known and the most studied physiological function regulated by the SCN via the autonomic nervous system. The SCN is connected to the pineal gland by a multisynaptic pathway that begins with a projection from the SCN to the PVN of the hypothalamus, then via sympathetic preganglionic neurons to the intermediolateral nucleus (IML) of the upper thoracic spinal cord (between the first and third thoracic vertebra), subsequently noradrenergic sympathetic postganglionic neurons of the superior cervical ganglion (SCG) and finally the pineal gland (Moore, 1996; Teclemariam-Mesbah et al., 1999)(*figure 1*). This pathway is well established. Some evidence has been obtained by humans with complete spinal cord injury. Their circadian rhythm in melatonin disappears, while people with a spinal cord injury below the third thoracic vertebra, which leaves the pathway intact, display a normal circadian rhythm in melatonin (Zeitzer et al., 2000). So, it is assumed that the SCN is involved in the release of pineal melatonin. It is important to clarify the respective role of the structures controlling the melatonin generating rhythm system. Since it is known that GABA from the SCN inhibits the PVN, it seems logic that removal of the influence of the SCN would result in a constant stimulation of the pineal activity, and consequently, in constantly elevated levels of melatonin release (Perreau-Lenz et al., 2003). The PVN is not solely inhibited by the SCN, it gets also stimulated by the essential neurotransmitter glutamate (*figure 2*), which results in melatonin synthesis as well. The importance of this pathway is established in an in vivo experimental setting

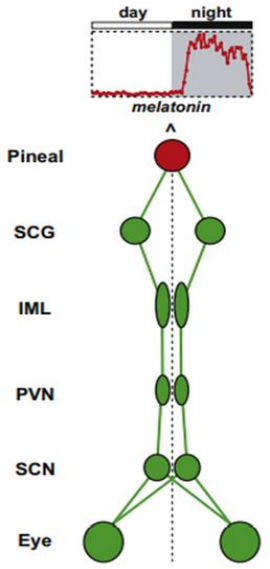

Figure 1. The neuronal circuit that controls pineal rhytmicity. SCG: superior cervical ganglion; IML: intermediolateral nucleus of the spinal cord; PVN: paraventricular nucleus of the hypothalamus; SCN: suprachiasmatic nucleus (Borjigin et al., 2012).

and after an acute and reversible shutdown of the PVN neuronal activity. Hence even if the general neuronal activity of the SCN is rather weak at night it is sufficient and, moreover, necessary to stimulate melatonin synthesis (Perreau-Lenz et al., 2004).

2.2. Nocturnal melatonin secretion

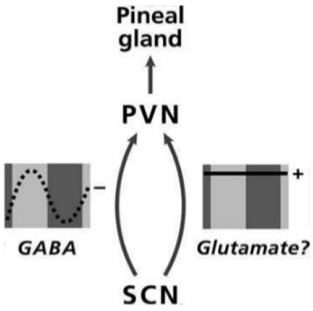

Figure 2. SCN control of pineal gland activity through the preautonomic paraventricular nucleus (PVN) neurons. We propose that the pineal gland activity rhythm is controlled by combined rhythmic inhibition (sustained by a GABA-ergic output) and a constant stimulatory SCN output (which could be sustained by a glutamatergic output) (Perreau-Lenz et al., 2003).

Pineal melatonin is tightly controlled by the SCN and can act as an entrainment signal for the circadian system (Morris et al., 2012). Therefore is interesting to find out how this melatonin secretion is related to a daily 24 hour rhythm. Melatonin has the peculiarity of being always secreted by the pineal gland at night in both diurnal and nocturnal animals (Cajochen et al., 2003). Nocturnal melatonin secretion is an output signal of the circadian clock able to convey photoperiodic, as well as circadian signals to multiple structures/organs possessing melatonin receptors within the brain or at the periphery. It causes the integration of seasonal changes in day length (photoperiodic cues) to control seasonal physiology, because the nocturnal secretion of pineal melatonin is in proportion to the duration of the night (Pevet, 2003). Circulating melatonin levels are barely influenced by sleep per se (i.e., independent of the circadian phase at which it occurs), suggesting that the nocturnal onset of melatonin secretion is not a consequence of the transition from wakefulness to sleep (Gooley et al., 2011). In addition, the daily rhythm of plasma melatonin is relatively impervious to feeding (with the exception of calorie restriction during which both the suprachiasmatic clock and melatonin rhythm are phase shifted) (Mendoza et al., 2005). These findings suggest there is an endogenous rhythm, probably generated by the SCN, which regulates the nocturnal melatonin secretion. Bilateral SCN lesions in sheep completely disrupts the rhythm of melatonin secretion and conforms the role of the SCN as an endogenous pacemaker responsible for the rhythmic entrainment of the pineal gland activity. Tessoneaud et al. found out that the anterior part of the sheep SCN is the main drive for the decline in melatonin secretion at dawn and that an excitatory input to the pineal gland may function at night (Tessonneaud et al., 1995). This seems to be the previous mentioned neurotransmitter glutamate.

2.3. Peripheral clocks

In pinealectomized animals, the long term absence of melatonin had a significant effect on shortening the circadian period of the SCN driven activity, suggesting that melatonin might be involved in fine tuning the speed of the SCN clock, which is in line with the findings mentioned above (Houdek et al., 2016). The clock mechanism is based on autonomously regulated circadian cycles in the expression of clock genes (Buhr & Takahashi, 2013). These genes mediate the generation and maintenance of circadian rhythms by a number of interlocked transcriptional and post-translational negative feedback loops. CLOCK and BMAL1 are transcription factors that act as positive regulators of circadian gene expression and activate the expression of the negative regulators of circadian gene expression: cryptochrome (CRY1 and CRY2) and period (PER1, PER2, PER3) families. CRY and PER proteins feedback and inhibit their own expression as well as the expression of other clock-controlled genes (Reppert & Weaver, 2001).

2.3.1. Liver

In the liver, it has been shown that restricted feeding shifts rhythmic patterns of clock genes and thus uncouples them from the central clock, where no changes in rhythmic patterns of clock genes are observed (Damiola et al., 2000). Over 350 circadian transcripts have been identified in the liver, 10% per which, including the core gene Per2, maintain rhythmicity in the absence of a functional hepatocyte clock. This leaves room for a role for behavioural hormonal and autonomic rhythms, in the regulation of clock gene expression rhythms in the liver (Kornmann et al., 2007). The role of these genes in hepatic glucose metabolism have been clearly shown using tissue specific knockouts. This liver specific knockout of the molecular clock mechanism seems to result in impaired glycogenesis and therefore, probably, in reduced hepatic glucose production and increased glucose tolerance (Kalsbeek et al., 2014).

2.3.2. Pancreatic islets of Langerhans

Other signals vital for glucose homeostasis are insulin and glucagon secretion by pancreatic islets. Plasma glucose concentration displays circadian variation, with the highest levels during the beginning of the active phase. Since feeding induces insulin secretion, plasma insulin levels follow the daily rhythm in food intake and may show a daily rhythm as well (Peschke & Peschke, 1998). The postprandial rise in insulin participates in the synchronization of peripheral clocks to feeding (Challet, 2015). On the other hand, autonomous circadian rhythms in pancreatic islet cells underscores the presence of a circadian control over pancreatic function. Indeed, glucose stimulated insulin release appears to be dependent on a functional clock. To understand the specific role of a pancreatic clock in vivo, Bmal1 was targeted specifically in the pancreas. This showed that conditional ablation of the pancreatic clock caused diabetes mellitus type 2 due to defective β-cell function (Marcheva et al., 2010). Disrupted clock in the endocrine α-cells resulted in a decreased glucagon release (Vieira et al., 2013). This might partly compensate for the impaired insulin release, which, of course, ultimately results in hyperglycemia, which is found in disturbed β-cells (Lamia & Evans, 2010).

2.3.3. Influence of melatonin

The absence of melatonin significantly decreased amplitude of the rhythmicity of the peripheral clocks, suggesting that the presence of melatonin rhythm may be required for the proper function of circadian oscillators (Houdek et al., 2016).

3. Influence of melatonin

It is hard to assess the independent effect of the circadian system on the 24 hour diurnal variations in glucose metabolism in humans. This is because behavioural factors, such as food intake, fasting duration, and activity level, are known to strongly impact glucose metabolism (Qian & Scheer, 2016). As melatonin has a clear rhythm during the day, it is interesting to find out its effects on peripheral organs. Melatonin transmits its action on peripheral organs through the activation of specific transmembrane receptors, namely MT1 and MT2, which are both expressed in the islet of Langerhans of rats and humans (Muhlbauer & Peschke, 2007; Peschke et al., 2007)(*figure 3*). These receptors are also involved in other organs involved in glucose regulation, such as mouse hepatocytes (Muhlbauer et al., 2009). In addition to these two classes of high-affinity G-protein-coupled melatonin receptors, there is some weaker evidence for an intracellular binding domain, namely MT3 (Slominski et al., 2012). This receptor was also characterized as the enzyme quinone reductase 2, which belongs to a group of reductases that participate in the protection against oxidative stress by preventing electron transfer reactions of quinones (Nosjean et al., 2000)(*figure 3*). The function of this “receptor” is not discovered yet.

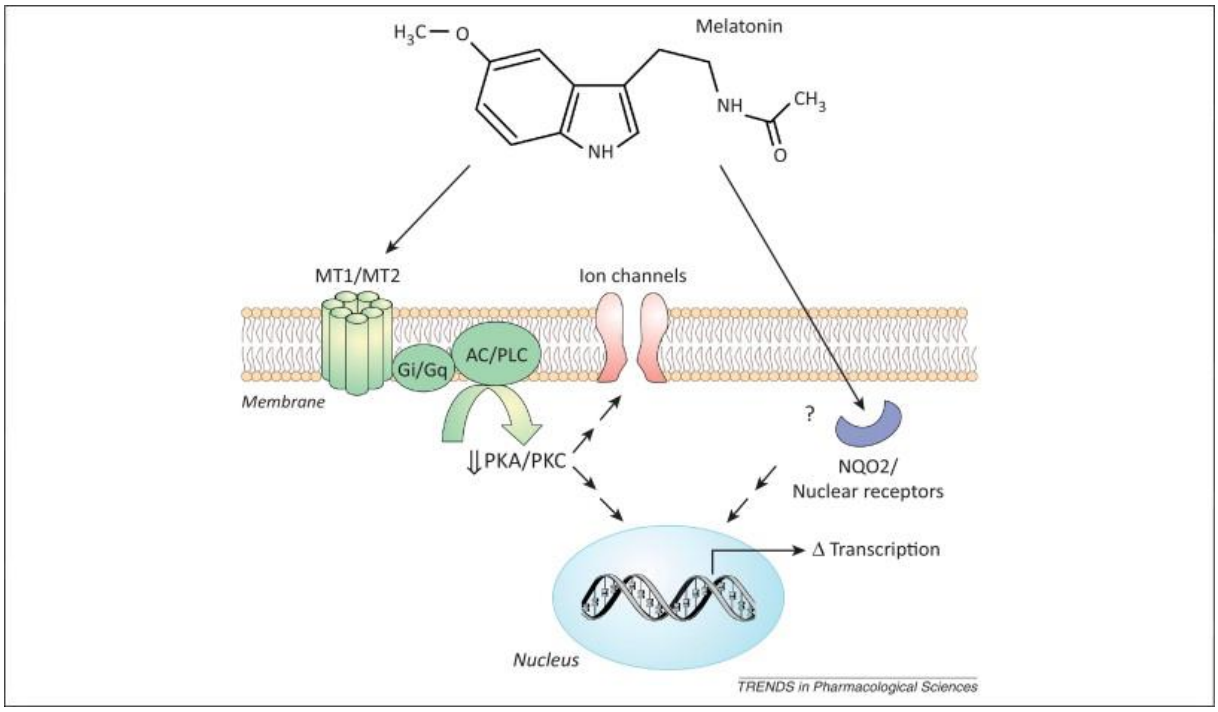

Figure 3. Melatonin signaling and modulation of the molecular clock. Melatonin interactions with the melatonin MT1 and MT2 $G\alpha_i$ and $G\alpha_q$ protein-coupled receptors leads to the inhibition of adenylate cyclase (AC) and phospholipase C (PLC) and downregulation of protein kinase A (PKA)/protein kinase C (PKC) signaling, altering ion-channel function and changes in circadian-related transcription. Melatonin also binds to the enzyme quinone reductase 2 (NQO2) and modulates the function of nuclear receptors, although the physiological significance of this binding is not yet clear (Schroeder & Colwell, 2013).

3.1. Pancreatic β-cells

Most evidence exist for the suggestion that the pineal gland has a suppressive effect on the activity of the pancreatic β-cell, because melatonin reduces insulin levels and glucose tolerance (Dhar et al., 1983; Rasmussen et al., 2001). The molecular pathways of these receptors are quite well investigated. The MT1 activation in pancreatic β-cells is linked to signalling via inhibitory G-proteins (Gi). The inhibitory effect on insulin release proceeds via modulation of adenylate cyclase (AC) activity and downregulation of the intracellular cyclic adenosine monophosphate (cAMP) level (Kemp et al., 2002). Strong evidence exists that the MT2 receptor is coupled in an inhibitory manner to the Gi-signalling pathways in pancreatic β-cells. This signalling is transmitted by the two second messengers cAMP and cyclic guanosine monophosphate (cGMP) (Muhlbauer et al., 2011). Generally, it seems to be clear that melatonin has an inhibitory effect on the cAMP-signalling pathway of the pancreatic β-cells, mediated via Giα-protein-coupled MT1 receptors (Peschke et al., 2013). This is confirmed by the finding that the unspecific competitive receptor antagonist luzindole diminished the insulin decreasing effect of melatonin (Peschke et al., 2002). As luzindole has a 15- to 25-time higher affinity for the MT2 than for the MT1 receptor, it remains unclear which specific receptor is responsible for the decreasing effect on insulin. Stumpf et al. investigated the function of the melatonin receptor MT2 only as a mediator of melatonin effects (Stumpf et al., 2008). They used the specific and selective MT2-receptor antagonist 4P-PDOT, which exhibits very high selective binding and a high affinity for the MT2 receptor (Nonno et al., 1999). Their results showed that the MT2 receptor subtype is also functionally relevant in rat pancreatic β-cells. Unfortunately, no commercial and well established selective MT1 receptor antagonist is available to investigate the melatonin effects via this specific receptor subtype (Stumpf et al., 2008). Another possibility to investigate the

effect of melatonin receptors is with use of knockout (KO) mice. MT1/MT2 double KO mice displayed significant increased plasma insulin levels, indicating that the loss of both melatonin receptors impaired the well documented inhibitive effect of melatonin on insulin. However, deletion of one of the melatonin receptors did not appear to cause a notable effect on insulin secretion, possibly because the remaining melatonin receptor was able to compensate for the loss in these two knockout lines (Bazwinsky-Wutschke et al., 2014). In contrast to above mentioned findings, Mühlbauer et al. displayed reduced mean insulin concentrations in both MT1, MT2, and MT1/MT2 KO mice compared to wild type animals (Muhlbauer et al., 2009). Though the inhibitory effect of melatonin on insulin secretion is already widely accepted.

3.2. Hepatocytes

It is already shown that clock mechanisms have some influence on the glucose metabolism functions of the hepatocytes in the liver. Furthermore, pinealectomized rats show an altered glucose homeostasis by the absence of melatonin. They display glucose intolerance and a desynchronized circadian pattern of gluconeogenesis, hallmarked by increased night time glucose levels (la Fleur et al., 2001; Lima et al., 1998). Moreover, chronic melatonin administration has been shown to improve glucose homeostasis not only in pinealectomized rats but also in rats rendered insulin resistant by diet manipulation (Kitagawa et al., 2012). MT1 and MT2 specific receptors were found to be expressed in the liver of wild type mice. Thus, a receptor KO with deleterious effects on cellular signal transduction was expected to affect the organs examined. When only the MT1 receptor was knocked out, higher mean glucose levels were determined. Remarkably, MT2 receptor KO animals proved to contain lower levels of glucose compared to wild type animals. MT1/MT2 double KO mice resulted in lowered blood glucose levels throughout much of the light period, but increased levels during part of the period of darkness (Muhlbauer et al., 2009). On the other hand, Naji et al. found solely expression of the MT1 receptor in the liver (Naji et al., 2004). Another study found lower mean peripheral blood glucose levels in MT1 KO and in the MT1/MT2 double KO mice. There was no difference in glucose expression of MT2 KO mice compared to wild type mice (Bazwinsky-Wutschke et al., 2014).

4. Involvement of leptin

There is considerable evidence that glucose homeostasis is physiologically regulated by the key metabolic hormones leptin and insulin. Leptin is an adipocyte derived hormone that regulates energy balance and neuroendocrine function by acting on specific hypothalamic pathways. Mutations that disrupt leptin or its receptor in rodents and humans result in a phenotype that includes severe obesity, insulin resistance and endocrine dysfunction. However, in most cases of obesity, such mutations are rare and leptin levels are high, suggesting the presence of leptin resistance (Ahima & Flier, 2000; Friedman & Halaas, 1998). The development of resistance to the action of both leptin and insulin, which can occur with age, obesity, and inflammation, appears to have a prime role in the pathogenesis of obesity, type 2 diabetes, and other metabolic disorders (Howard & Flier, 2006). Nevertheless, the cellular mechanism linking insulin and leptin resistance in skeletal muscle remain poorly understood (Yang et al., 2012). Adipocytes are the main source of leptin, but the hormone is also produced in other tissues (Park & Ahima, 2015). Secretion of leptin from adipocytes displays a circadian rhythm analogous to melatonin secretion (Gunduz, 2002). Remarkably, a deficiency of melatonin has been demonstrated to correlate with obesity (Bonnefont-Rousselot, 2014). Melatonin, leptin, adiponectin, and insulin may act through concurrent signalling pathways, interfering mutually with the effects which they induce in the organism. However, the chronobiological aspects of these

relationships seem to be extremely fascinating, especially in light of the fact that chronodisruption is considered to be related to obesity. Different properties of melatonin, including antioxidant and anti-inflammatory functions, definitely contribute to its anti-obesity effects (Szewczyk-Golecet al., 2015). At a molecular level, an important causal mechanism for leptin-resistant obesity may be associated with the inhibition of the leptin signalling cascade through over activity of suppressors of cytokine signalling (SOCS3) (Howard & Flier, 2006).

4.1. JAK-STAT3-SOCS3 pathway

The pathway of SOCS3 induced leptin resistance in obesity starts with the evolutionary conserved Janus kinase (JAK)-signalling transducers and activators of transcription (STAT) signalling (*figure 4*). JAK can be bound by various ligands to get activated (Brooks et al., 2014). The activated JAKs phosphorylate signature tyrosine residues in the cytoplasmic region of the receptors to create docking sites for STATs (Zhong et al.l, 1994). This phosphorylation results in the formation of parallel STAT dimers, which are stabilized by reciprocal phosphotyrosine and Src homology 2 (SH2) domain interactions. Dimerization of STATs induces their nuclear translocation, where they ultimately control the expression of their target genes (Ihle, 1996). The JAK-STAT signalling pathway transcriptionally regulates its own suppression. Suppressors of cytokine signalling (SOCS) molecules act as a negative feedback signal by inhibiting JAK and STAT activation and phosphorylation (Krebs & Hilton, 2001). Moreover, the transcriptional activity of STAT in the nucleus can also be controlled by protein inhibitors of activated STAT (PIAS), which blocks the DNA-binding activity of STAT (Shuai, 2006). Chronic activation of this JAK-STAT3-SOCS3 signalling pathway is induced by accelerated leptin as present in obesity (Kiu & Nicholson, 2012)(*figure 4*).

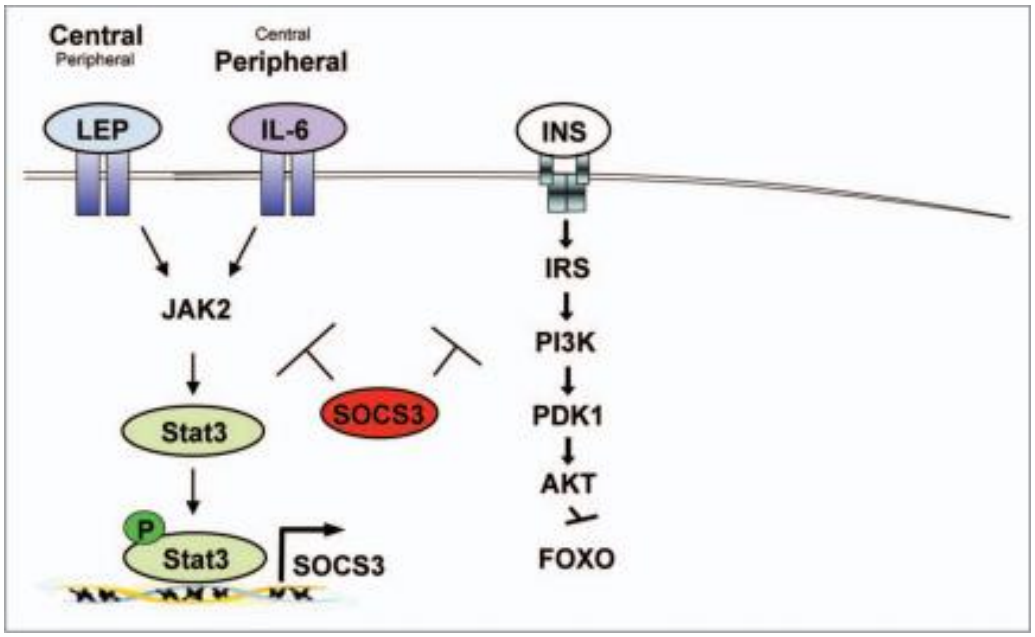

Figure 4. Chronic JAK-STAT3-SOCS3 signalling in obesity. Obesity increses circulating levels of leptin and IL-6 that in turn chronically activate intracellular JAK-STAT3 signalling. While leptin (LEP) acts predominantly in the central nervous system, IL-6 has been reported to mainly function in peripheral organs, through both factors can act vice versa. Chronic JAK-STAT3 siganlling induced by leptin and IL-6 lead to the increased expression of the negative regulator SOCS3. SOCS3 in turn not only negatively regulates leptin and IL-6 signalling but also impairs insulin (INS) action eventually leading to obesity and insulin resistance (Wunderlich et al., 2013).

4.2. Mechanism of action

SOCS3 is established as a negative regulator of leptin signalling in the hypothalamus, and plays a role in regulating leptin sensivity (Bjorbaek et al., 1999). Previous studies have also shown that SOCS3 is a negative regulator of insulin signalling in fat and liver (Shi et al., 2004). Therefore, SOCS3 is a single molecule that mediates both leptin and insulin resistance due to its ability to down regulate leptin and insulin signalling. Furthermore, SOCS3 also antagonized both leptin and insulin signalling in skeletal muscle, which is a peripheral site of glucose and fatty acid use and is one of the primary tissues responsible for insulin resistance in obesity. The suggestion is that SOCS3 within skeletal muscle is a critical factor in the development of both skeletal muscle specific and systemic insulin resistance in response to obesity, although the underlying molecular mechanisms remain elusive (Yang et al., 2012).

4.3. Inflammatory reaction

During the past decades, it became clear that inflammation has a central role in the pathogenesis of obesity and type 2 diabetes. Many studies have shown that the inflammatory signals disrupt the insulin action and mediate insulin resistance in obesity. The link between obesity and inflammation seems to be the adipose tissue itself (Khodabandehloo et al., 2016). It is well known that, in most cases, human obesity is accompanied by a leptin resistance and the compensatory hyperleptinemia. This leptin resistance state might have consequences on the activation of immune cells (Martin et al., 2008). SOCS proteins play an essential role in mediating the inflammatory responses in both immune cells and metabolic organs, such as the liver, adipose tissue, and skeletal muscle. Various factors including the activators of the JAK-STAT pathway, such as members of the IL-6 family of cytokines, have been proposed to induce SOCS proteins in different tissues (Galic et al., 2014). In obesity, inflammation leads to an upregulation of SOCS proteins, for example SOCS3 expression is increased in the liver, adipose tissue, and muscle of obese rodents (Emanuelli et al., 2001). It appears that the control of SOCS expression by cytokines is mediated in a tissue specific manner, as the increased IL-6 production from skeletal muscle myotubes appears to be the dominant factor driving the SOCS3 expression in skeletal muscle of obese humans (Rieusset et al., 2004). IL-6 is a multi-functional pro-inflammatory cytokine that is secreted by adipose cells and macrophages (Khodabandehloo et al., 2016; Makki et al., 2013). IL-6 is not critically regulating the severity of a spontaneous inflammatory disease but plays a role in the onset (Croker et al., 2012). After the initial phosphorylation of STAT3 in response to IL-6 followed by a subsequent inhibition by SOCS3, a second wave of activation leads to re-phosphorylation of STAT3. This phosphorylation might continue to be driven for many hours and is immune to inhibition by SOCS3 (Wormald et al., 2006). The results of a SOCS3 overexpression experiment suggests that the IL-6/SOCS3/STAT3 signalling pathway has a critical role in spermatogenesis. SOCS3 overexpression inhibits STAT3 phosphorylation, as well as STAT3-mediated downstream events. This study found that IL-6 directly regulates the expression of zinc finger protein Zfp637 through the SOCS3/STAT3 signalling pathway and affects the differentiation of permatogonia in vivo and in vitro. Interestingly, the expression of Zfp637 is reduced in obese mice (Huang et al., 2016). This might be an interesting clue in the research for obesity and diabetes treatment.

5. Central effects of melatonin

Melatonin signalling can feed back the master clock itself. As an example, a daily infusion of melatonin is capable of synchronizing the circadian rhythm or rest/activity and the suprachiasmatic clockwork in rats kept in constant darkness (Caldelas et al., 2005). Furthermore it is notable that a

high melatonin receptor density is found in the SCN (Hardeland & Poeggeler, 2012; Reppert, 1997). Exogenous melatonin administration to SCN slices in vitro induces an acute inhibition of the single unit neuron activity and phase shifts the circadian rhythms of neuronal firing (Shibata et al., 1989). However, this approach has not provided conclusive evidence about the specific role of MT1 and MT2 receptors in the modulation of SCN function by melatonin, because most of the melatonin receptor antagonists and agonists available lack the specificity to fully dissect the action of melatonin (Baba et al., 2013). The use of melatonin receptor knockouts has provided a clearer picture on the mechanisms by which melatonin can influence the circadian mammalian system. In a MT1 KO study, the inhibitory effect of melatonin on SCN neuronal activity was no longer present, whereas the phase-shift response to melatonin appeared to be normal (Roca et al., 1996). Furthermore, stimulation of MT1 receptors modulated cAMP responsive element (CREB) phosphorylation in the mouse SCN, since the induction of CREB phosphorylation was inhibited in MT1 KO mice (von Gall et al., 2000). In MT2 receptor KO mice the phase shifting response to melatonin was blunted while the inhibitory effect on firing of SCN neurons persisted (Liu et al., 1997). This is in accordance with the results found by Pandi-Perumal et al., who found that the MT1 receptor is localized in lots of tissues, including the SCN. The MT2 receptor is more restrictively expressed, being found mainly in the brain apart from the SCN (Pandi-Perumal et al., 2008). Melatonin has a third binding site, the MT3 receptor, or later characterized as the enzyme quinone reductase 2, which is present in the brain and in some peripheral organs as well (Nosjean et al., 2000). This “receptor” has possibly something to do with the feedback mechanism, this is not discovered yet. Expression of melatonin receptors in the SCN is a strong argument in favour of a physiological feedback on the circadian clock. Non-physiological conditions such as exogenous administration by an injection at the end of the light period phase shifts the activities of the circadian rhythm. However, the precise physiological role of melatonin remains obscure (Pevet et al., 2006).

6. Therapeutic value of melatonin

Recently, accumulating epidemiological evidence has indicated that circadian disturbances, such as shift work, late meal timing, late chronotype, social jet lag, and sleep loss are associated with increased risks of type 2 diabetes, obesity, and others (Buijs et al., 2016; Cipolla-Neto et al., 2014). The disturbance of internal circadian system induces glucose tolerance and insulin resistance, which could be restored by melatonin supplementation. Therefore, the presence of melatonin receptors on human pancreatic islets may have an impact on pharmacotherapy for type 2 diabetes (Sharma et al., 2015). These receptors are present on many more peripheral organs, where melatonin regulates their phase and period. This functional aspect makes melatonin one of the most important chronobiotics that directly participates in the organization of the circadian temporal coordination of physiological and behavioural phenomena (Cipolla-Neto et al., 2014). For this reason, supplementation of melatonin might be a good therapeutic target for shift workers. Shift work is generally associated with chronic misalignment between the endogenous circadian timing system and the behavioural cycles, including sleep/wake and fasting/feeding cycles (Roden et al., 1993). In general, shift work is defined as a wide variety of working time arrangements, including all working hours that are outside of the normal daytime hours (Knutsson, 2004). A recent study found that melatonin production, estimated with the metabolite 6-sulfatoxymelatonin (aMT6) urinary excretion, was progressively and significantly decreased during 3 consecutive days of simulated night work. The reduction occurred not only for the nocturnal production, but also for the 24 hour production. Here, the cause of this reduction does not seem to be a direct suppression of melatonin secretion by light exposure, but may reflect the circadian disruption associated with the process of re-entrainment, as

mentioned before (Dumont & Paquet, 2014). Furthermore, evidence for a causal role of circadian disruption in the increased risk of type 2 diabetes is provided by experimental studies showing that circadian disruption leads to impaired glucose control in healthy participants (Qian & Scheer, 2016). Hence, the circadian system may be a tractable target for decreasing prevalence of hyperglycemia and insulin resistance. Different animal studies suggest that melatonin supplementation may have beneficial effects on glucose homeostasis and body weight regulation under certain circumstances, which should encourage clinical trials in humans to evaluate the therapeutic potential of this hormone in diabetes. The present evidence suggests that melatonin induces insulin secretion via IP3 signalling and can improve β-cell function. If this is true, melatonin supplementation might have beneficial effects, although it is currently prescribed for sleep and circadian rhythm (Sharma et al., 2015). Nevertheless, the development of strategies to treat or prevent disorders of rhythmicity is a new challenge for medicine. Several pharmacological approaches have been suggested, but until now, it has been mostly melatonin or melatonin agonists which have demonstrated usefulness in modulating clock activities in vivo. A great number of structurally different melatonin receptor ligands have been developed, some of which are already approved and marketed as drugs (Pevet, 2016). Melatonin has also a function as an antioxidant and as an anti-inflammatory agent. Since inflammation seems to have a central role in the pathogenesis of obesity and type 2 diabetes, so it could be of interest to combine antioxidant therapeutic strategies with strategies to weight loss. As melatonin modulates several processes involved in obesity and its related metabolic alterations, it could have a therapeutic interest in the treatment of obesity (Bonnefont-Rousselot, 2014).

7. Discussion

Nocturnal melatonin secretion regulates much of our physiology. In this review the effects on glucose homeostasis were highlighted in particular. In many organs influenced by glucose or insulin, there is a proven autonomous rhythm generated by clock genes (Kalsbeek et al., 2014). The molecular machinery that drives circadian rhythms in the SCN and peripheral cells is similar. Despite this, synchrony between peripheral clocks within organs is lost without input from the SCN (Guo et al., 2006). The liver and pancreas functions are under tight control of melatonin via its receptors, which in turn is strictly regulated by the SCN. MT1 and MT2 receptors are expressed in both pancreatic β-cells as liver hepatocytes (Bazwinsky-Wutschke et al., 2014; Muhlbauer et al., 2009). Which of these receptors specifically decreases the glucose- and insulin production, and the glycogenesis is still unclear. As melatonin is only secreted when there is no light input in the retina, it is important to evaluate the effects of melatonin in the dark period, whereas most studies just measure the mean plasma glucose and insulin levels over an 24 hour period. Interestingly, the loss of blood glucose rhythm in both MT1 and MT2 receptor KO mice coincides with differences in daytime glucose levels rather than nocturnal levels (Owino et al., 2016). Mühlbauer et al. found a strong decrease in insulin levels in double KO mice, in both light phase and dark phase, which is in contradiction with the expectation that melatonin inhibits insulin production. The same group found increased glucose values throughout the circadian period in MT1 KO mice and also increased glucose levels during the period of darkness in double KO mice, which is in line with the expectation (Muhlbauer et al., 2009). The discrepancies in results can be explained by a difference in age of the animals used, as it is demonstrated there is a difference between young and old mice in glucose and insulin levels in melatonin receptor KO mice (Bazwinsky-Wutschke et al., 2014). Melatonin receptors are also present in several brain regions including the SCN, so it is able to feed back the master clock (Baba et al., 2013). Melatonin seems to have a stimulatory effect on firing of SCN neurons (Liu et al., 1997). This implicates that melatonin provides a positive feedback signal to the SCN. Melatonin secretion is

analogous to leptin secretion from adipocytes (Gunduz, 2002). Disruption in leptin signalling results in severe obesity, insulin resistance and endocrine dysfunction (Ahima & Flier, 2000). Leptin resistance in obesity is mediated through the JAK-STAT3-SOCS3 pathway, which is highly activated in obesity and diabetes in several peripheral sites, as well as in the hypothalamus (Kiu & Nicholson, 2012). Also an inflammatory reaction is associated with obesity, caused by hyperleptinemia mediated activation of the JAK-STAT3 pathway, mainly in adipocytes (Galic et al., 2014). Acuna-Castroviedjo et al. previous predicted a double role of melatonin in the body: synchronizing the organisms' function and protecting the cells from oxidative/inflammatory damage. They explain this anti-inflammatory effect of melatonin by the suggestion there are some different tissues who synthesize melatonin independently of the pineal gland (Acuna-Castroviejo et al., 2014). Furthermore, melatonin as a potent antioxidant has been reported to be a neuroprotector in animals and clinical studies (Tan et al., 2010).

In conclusion, circadian desynchronization is associated with deleterious effects on health. Melatonin is rhythmically released under control of the SCN and is extremely important for glucose homeostasis. Therefore melatonin is a promising candidate for a variety of disorders related to glucose metabolism, with or without the influence of circadian rhythms.

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