

Review

Melatonin Uptake by Cells: An Answer to Its Relationship with Glucose?

Juan C. Mayo^{1,2,*} , Arturo Aguado¹, Rafael Cernuda-Cernuda¹, Alejandro Álvarez-Artime^{1,2}, Vanesa Cepas^{1,2}, Isabel Quirós-González^{1,2}, David Hevia^{1,2} and Rosa M. Sáinz^{1,2} 

¹ Departamento de Morfología y Biología Celular, Facultad de Medicina, Universidad de Oviedo, 33006 Oviedo, Spain; arturoaguadog@gmail.com (A.A.); rcernuda@uniovi.es (R.C.-C.); alejandroalvarezartime@gmail.com (A.Á.A.); cepasvanesa@uniovi.es (V.C.); quirosisabel@uniovi.es (I.Q.-G.); heviadavid@uniovi.es (D.H.); sainzrosa@uniovi.es (R.M.S.)

² Instituto Universitario Oncológico del Principado de Asturias, Universidad de Oviedo; 33006 Oviedo, Spain

* Correspondence: mayojuan@uniovi.es; Tel.: +34-985-103-610

Received: 3 July 2018; Accepted: 6 August 2018; Published: 10 August 2018



Abstract: Melatonin, *N*-acetyl-5-methoxytryptamine, is an indole mainly synthesized from tryptophan in the pineal gland and secreted exclusively during the night in all the animals reported to date. While the pineal gland is the major source responsible for this night rise, it is not at all the exclusive production site and many other tissues and organs produce melatonin as well. Likewise, melatonin is not restricted to vertebrates, as its presence has been reported in almost all the phyla from protozoa to mammals. Melatonin displays a large set of functions including adaptation to light: dark cycles, free radical scavenging ability, antioxidant enzyme modulation, immunomodulatory actions or differentiation–proliferation regulatory effects, among others. However, in addition to those important functions, this evolutionary ‘ancient’ molecule still hides further tools with important cellular implications. The major goal of the present review is to discuss the data and experiments that have addressed the relationship between the indole and glucose. Classically, the pineal gland and a pinealectomy were associated with glucose homeostasis even before melatonin was chemically isolated. Numerous reports have provided the molecular components underlying the regulatory actions of melatonin on insulin secretion in pancreatic beta-cells, mainly involving membrane receptors MTNR1A/B, which would be partially responsible for the circadian rhythmicity of insulin in the organism. More recently, a new line of evidence has shown that glucose transporters GLUT/SLC2A are linked to melatonin uptake and its cellular internalization. Beside its binding to membrane receptors, melatonin transportation into the cytoplasm, required for its free radical scavenging abilities, still generates a great deal of debate. Thus, GLUT transporters might constitute at least one of the keys to explain the relationship between glucose and melatonin. These and other potential mechanisms responsible for such interaction are also discussed here.

Keywords: melatonin; glucose; GLUT/SLC2A; metabolism; insulin

1. Melatonin: A Universal and Ubiquitous Molecule

Melatonin, chemically *N*-acetyl-5-methoxytryptamine, was first isolated and further characterized by Lerner and co-workers in the late 1950s [1,2]. However, 40 years before, in 1917, McCord and Allen reported for the first time the ability of cow pineal extracts to lighten tadpoles when they were fed with them [3]. This clue was further exploited by Lerner and co-workers who coined the name ‘melatonin’ for this substance, a portmanteau from ‘mela’ (able to lighten) and ‘tonin’ because of its derived from serotonin. As a consequence, this could be considered the first, and yet minor, function attributed to the indole. Two years later, Axelrod and Weissbach identified the rate limiting synthetic enzymes, namely *N*-acetyl-transferase (aralkylamine-NAT, AANAT) and hydroxyindole-*O*-methyl-transferase (acetylserotonin *O*-methyl transferase or ASMT/HIOMT), which control melatonin synthesis from tryptophan in the pineal [4]. This epithalamic gland is responsible for melatonin nocturnal serum rise,

while light signals, transduced by the retina and transmitted via suprachiasmatic nuclei, constitute its major synthesis inhibitor [5,6]. Due to its association with the day: night cycle melatonin was rapidly associated with the regulation of circadian rhythms and further shown to modulate reproduction in seasonal breeding animals by adapting their physiology to external photoperiodic conditions [7,8], an evolutionarily acquired mechanism that ensures offspring survival [9,10]. Duration and intensity of light regulate melatonin production [11,12] and nocturnal production decays with age [13,14]. This fact prompted the further investigation of melatonin effects on aging and neurodegeneration [15–18]. In addition to this important function, it has also been linked to other important roles including immunomodulation or anti-cancer actions [19–24].

Melatonin production was initially thought to be restricted to the pineal gland, but pinealectomies did not completely remove it in rodents. Highly sensitive detection methods revealed that about 20% of normal levels was still present in serum or urine, although these basal levels were not modified by light–dark rhythms [25]. In 1965, Quay first showed in a pioneer study the existence of HIOMT (now ASMT) in both retina and pineal gland and, later on, the same activity was also demonstrated in the orbital Harderian gland [26]. Years later, Bubenik’s and Kvetnoy’s groups were the first to show that melatonin can be localized outside the pineal gland [27,28]. Thus, retina, cerebellum and the enterochromaffin cells, the latter as part of the APUD system (now commonly referred to as ‘diffuse endocrine system’), were the first organs/cells in which authors showed the potential local production of melatonin. Soon after that, the Harderian gland was also found to demonstrate an immunopositive reaction to melatonin [29], thus corroborating the findings mentioned above. However, in all these studies the demonstration included non-analytical detection strategies, but rather was based on the immunohistochemical detection of either synthetic enzymes (AANAT/ASMT) or melatonin itself. Even though this methodology could generate some doubts, these first findings definitively boosted the search for new sites of melatonin synthesis. The introduction of radioimmunoassay (RIA) [30] was necessary to reinforce evidence about the presence of extra-pineal melatonin, particularly after the discovery of ^{125}I -radiolabeled melatonin as a tracer [31,32]. Not only did this technique allow the quantification of serum melatonin, but it also allowed the discovery of high-affinity melatonin-binding sites in rat synaptosomal preparations [33], as well as in the brain of other species, including human suprachiasmatic nuclei [34–36].

Thanks to the use of RIA and more recently to high-performance liquid chromatography (HPLC) [30,37], the production of the indole has been accurately assayed in several tissues and organs including the retina, Harderian glands, gut, testis or skin, among many others, but these local productions do not seem to contribute to the nocturnal serum levels [38]. The extra-pineal sites of melatonin production and the corresponding references are summarized in Table 1. Among these extracellular sites of melatonin production, one of the first organs reported in which melatonin is thought to play a local key function was the retina [27]. Additionally, two other sites of production appear to be of particular importance, i.e., the gut and the skin, due to the relatively high amount and also the key physiological function played [39–42]. At least in the skin, melatonin synthesis can take a shortcut through an AANAT-independent pathway involving alternative enzymes [43].

In addition to the ubiquitous presence of melatonin in so many tissues, the indole has been identified in evolutionarily different organisms. One of the breakthrough discoveries in this field was made by Hardeland’s group, who first discovered the indole as well as its synthesis in the dinoflagellate *Lyngulodinium polyedrum* (syn *Gonyaulax polyedra*) [44]. Melatonin appears to be present at least in some bacteria (*Rodospirillum rubrum*) [45], unicellular phyla [46,47] as well as in *Saccharomyces cerevisiae* [48]. Likewise, the presence of melatonin in Arthropoda, Nematoda, nemertine worms or Gastropoda, among others, have also been shown [49–53]. More surprisingly, the discovery in fungi [53] and in vascular plants [54,55] has now attracted the attention of researchers to many other groups, and melatonin has been found in red and brown algae (Rhodophyta, Phaeophyta), green algae and land plants (Viridiplantae) [56–59]. This brings out the question of the ancient and basic role(s) that melatonin seems to play in all these organisms, likely preceding the more “modern” circadian-related actions [57,60]. The presence of melatonin in the different phyla of invertebrates is shown in Table 2.

Table 1. Extra-pineal sites of melatonin production reported to date.

Organ/Tissue	Strategy	Original Reference(s)
Retina, cerebellum	Immunohistochemical localization of aaNAT	Bubenik et al. (1974) [27]; Quay (1983) [61].
Gut (Enterochromaffin cells)	Frog skin melanophores lightening of enterochromaffin cells extracts	Raikhlin et al. (1975) [28]; Raikhlin & Kvetnoy (1976) [62]
Airway epithelium, adrenal, thyroid gland, liver, renal cortex, gallbladder, inner ear, ovary, endometrium, placenta, mast cells, NK cells, eosinophils, thymus	Immunohistochemistry	Raikhlin et al. (1975) [28]; Raikhlin & Kvetnoy (1994) [63]; Kvetnoy et al. (2001) [64]
Harderian gland	NAT/ASMT enzymatic activities, immunohistochemical localization, RIA, NAT and ASMT enzymatic activities, immunohistochemistry of melatonin	Cardinali & Wurtman (1972) [26]; Bubenik et al. (1976a y 1976b) [29,65]; Pang et al. (1977) [66]; Menéndez-Peláez et al. (1987) [67]; Bubenik, G.A. (1980) [68]
Digestive tract		
Cochlea (inner ear)	RIA, TLC (detection of radio-labelled metabolites after primary culture in medium supplemented with [¹⁴ C]5-HT) Direct enzyme-linked immunosorbent assay	Biesalski et al. (1988) [69]; López-González et al. (1997) [70]
Peripheral blood mononuclear cell (PBMCs)	HPLC, TLC, Mass Spectrometry, RIA	Finocchiaro et al. (1991) [71]
Eye	Ciliary body	HPLC (electrochemical detection), NAT/ASMT enzymatic activities
	Crystalline	RIA, HPLC (for precursors), NAT/ASMT enzymatic activities
Skin	HPLC (fluorometric detection), Mass spectrometry, NAT/ASMT activity and expression (RT-PCR)	Slominski et al. (1996; 2002) [73,74]
Testis	TLC, NAT/ASMT enzymatic activities	Tijmes et al. (1996) [75]
Ovary	HPLC (fluorometric detection), RIA, NAT/ASMT enzymatic activities	Itoh et al. (1997; 1999) [76,77].
Bone marrow	RIA, HPLC (electrochemical detection), Mass spectrometry Immunocytochemistry, NAT enzymatic activity, ASMT expression	Tan et al. (1999) [78]; Conti et al. (2000) [79]
Thymus, spleen, lung, heart, kidney, muscle, liver, stomach, gut, testis, spinal cord, brain, platelets	NAT/ASMT expression	Stefulj et al. (2001) [79]
Thymus	HPLC (fluorometric detection), NAT/ASMT enzymatic activities	Jiménez-Jorge et al. (2005) [79]
Lymphocytes	HPLC (fluorometric detection), NAT/ASMT activity and expression	Carrillo-Vico et al. (2004) [80]
Placenta	NAT/ASMT expression (RT-PCR)	Iwasaki et al. (2005) [81]
Liver, kidney, heart	ELISA, NAT/ASMT expression (RT-PCR)	Sánchez-Hidalgo et al. (2009) [81]
Mast cells	ELISA, NAT/ASMT activity and expression (RT-PCR)	Maldonado et al. (2010) [82]

Table 2. Melatonin in microorganisms and invertebrates.

Organism	Strategy for Detection	Original Reference(s)
EUBACTERIA		
<i>Rhodospirillum rubrum</i>	RIA	Manchester et al. (1995) [45]
<i>Erythrobacter longus</i>	RIA	Tilden et al. (1997) [83]
<i>Bacillus</i> sp.	UPLC-MS/MS	Jiao et al. (2016) [84]
<i>Euglena gracilis</i>	HPLC	Balzer et al. (1996) [85]
PROTISTS		
Lingulodinium (syn Gonyaulax) polyedra (Dinoflagellate)	HPLC	Poeggeler and Hardeland (1994) [86]
Saccharomyces cerevisiae (Yeast)	HPLC	Sprenger et al. (1999) [87]
<i>Euglena gracilis</i>	HPLC	Pandi-Perumal and Cardinali (2007) [87]
<i>Trypanosoma cruzi</i>	RIA	Macías et al. (1999) [47]
Other Dinoflagellates including: <i>Alexandrium</i> (sp.), <i>Ceratium horridum</i> , <i>Amphidinium carterae</i> , <i>Pyrocystis lunula</i> , <i>Noctiluca scintillans</i>	HPLC/RIA	Data obtained from abstracts or proceedings
Ciliates: <i>Tetrahymena thermophila</i>	HPLC	Kohidai et al. (2003) [88]
LOWER INVERTEBRATES		
<i>Dugesia japonica</i>	Biosynthetic enzymes	Itoh et al. (1999) [89]
<i>Caenorhabditis elegans</i>	Biosynthetic enzymes	Migliori et al. (2012) [90]
<i>Lumbricus terrestris</i>	Spectrophotometry	Subaraja and Vanisree (2016) [91]
ARTHROPODA		
<i>Locusta migratoria</i>	RIA	Vivien-Roels et al. (1984) [49]
<i>Drosophila melanogaster</i> , <i>Periplaneta americana</i>	TLC	Finocchiaro et al. (1988) [92]; Richter et al. (2000) [93]
<i>Musa autumnalis</i>	RIA	Wetterberg et al. (1987) [94]
<i>Daphnia magna</i>	ELISA/IHC	Markowska et al. (2009) [95]
Decapoda: <i>Carcinus maenas</i> , <i>Uca pugilator</i> , <i>Nephrops norvegicus</i> , <i>Procambarus</i> sp., <i>Neohelice granulata</i> , <i>Eriocheir sinensis</i>	RIA/HPLC	Vivien-Roels and Pevet (1993) [96]; Tilden et al. (1997) [97]; Aguzzi et al. (2009) [98]; Farca-Luna et al. (2010) [99]; Maciel et al. (2014) [100]; Yang et al. (2018) [101]
COELENTERATES		
<i>Renilla koellikeri</i>	RIA	Mechawar and Anctil (1997) [102]
MOLLUSCA		
<i>Aplysia californica</i>	HPLC	Abran et al. (1994) [103]
<i>Sepia officinalis</i>	RIA	Vivien-Roels and Pevet (1986) [52]

The antioxidant properties of melatonin, discovered in the early 1990s [104] has opened a new research line that has highlighted the important cytoprotective actions of the indole in almost every single organ, tissue and cell type [105], with important clinical implications in neurodegeneration, cancer or ischemia-reperfusion related pathologies. Similarly, melatonin might also show protection against stress-related challenges in plants [43]. Melatonin reaction with free radicals give different metabolites, namely cyclic 3-OH-melatonin, *N*(1)-acetyl-*N*(2)-formyl-5-methoxykynuramine (AFMK) and *N*(1)-acetyl-5-methoxykynuramine (AMK), which also show free radical-scavenging activities, thus converting melatonin in a very efficient free radical chain reaction [43]. These melatonin metabolite reactions might be of importance in protecting those tissues/organs that act as barriers in the organism, e.g., skin or gut, where melatonin is produced in significant quantities [106].

2. Vesicle Secretion or Membrane Diffusion?

2.1. Vesicles in the Pineal Gland

The pineal organ of non-mammalian vertebrates is photoreceptive and pinealocytes exhibit a very similar morphology to that of retinal photoreceptors. These cells display an outer segment containing multiple membrane discs, an intermediate part with a '9 + 0' cilium structure, an inner segment, and a basal part with basal processes. This basal part usually contains multiple secretory vesicles [107]. The outer photoreceptor-like segment become partially lost in the mammalian pinealocytes, thus leading to the lack of photoreceptive function. However, while these pinealocytes evolved toward a non-sensory structure, they also show numerous vesicles, frequently associated to synaptic ribbons or rosette-like structures, indicating high secretory function [108]. There is a controversy about the exact content of such vesicles. Some of them are also known as Golgi dense-core vesicles (DCR) while others show a more classic homogeneous electro-lucent content. The number of vesicles are subjected to opposite circadian rhythmicity [109] and it is altered by either superior cervical gangliectomy or melatonin treatment itself [110]. Interestingly, some authors have reported that DCR contain neither melatonin nor serotonin (5 hydroxy-tryptamine, 5-HT) and the electron-dense material observed within DCR would rather correspond to pineal neuropeptides [108,111]. On the contrary, Juillard and Collin [112], based on fluorescence histochemical methods, determined that 5-HT fluorescence corresponded with DCV. The pinealocytes can, therefore, be considered part of the diffuse NE system, as the secretion products are released either into the pineal recess blood vessels or even directly into the CVF at the third ventricle [113]. Both serotonin and melatonin display important regulating functions on their own secretion. Serotonin itself, through 5-HT₂ receptors, may contribute to the optimal secretion of melatonin, as has been shown in cultured pinealocytes [114,115]. This would explain why serotonin always precedes a melatonin peak during the dark phase in all the species studied [114]. A similar role has been found for glutamate, which also seems to accumulate inside the pinealocyte vesicles [116]. More importantly, this would indicate that these paracrine/autocrine mediators would modulate the release of melatonin, thus indicating a more complex regulatory mechanism than simple diffusion, which depends exclusively on the relative concentrations at both sides of the membranes, must also be involved. Nevertheless, the coexistence of diffusion through cell membranes with other transporter or carrier-like systems should not be completely discarded.

2.2. Chemical Features of Melatonin and Membrane Diffusion

According to PubChem database (all the chemical information has been consulted at <https://pubchem.ncbi.nlm.gov>), melatonin solubility in ethanol is very high (182 g/L) while the solubility in water varies from roughly 2 g/L (20 °C) to 3.5 g/L, a feature confirmed by Shida and co-workers, who were able to solubilize melatonin at 5 mM [117]. Strictly from the chemical point of view, this should be considered a moderate to high hydro-solubility and, more importantly, this would make unnecessary the involvement of serum proteins as blood carriers for the indole. Contrary to this hypothesis, Li and Wang have recently reported that melatonin binds to human serum albumin in a

1:1 stoichiometry [118]. As a reference, serotonin solubility in water is 10-fold higher (>25 g/L) [118]. As a reference, serotonin's solubility in water is 10-fold higher (>25 g/L) while a cholesterol-derived steroid such as testosterone is much lower (33 mg/L). This high testosterone lipophilicity makes these substances thermodynamically compatible with rapid lipid bilayer diffusion [119]. Like the rest of the steroid hormones, testosterone is supposed to be continuously liberated through the lipid bilayer in a gradient-favored manner. Interestingly, its release, which is finely regulated by LH in Leydig cells, might involve additional secretory mechanisms. Likewise, the same principle would apply to melatonin, which is thought to be released by diffusion, but it is under the control of different stimuli (see above). Nevertheless, considering the relative indole lipophilicity, the diffusion rate should be rather low when compared to androgens or estrogens. The question remains as to how much of this membrane diffusion has been really investigated throughout the literature. Surprisingly, the answer is that not so many studies have been focused on this issue.

2.3. Melatonin and Interactions with Lipid Membranes

For many years, most authors have assumed that melatonin, due to some of the physical features mentioned above, moves across biological membranes through passive diffusion. Additionally, the ubiquity of melatonin's actions, the potential synthesis in different subcellular compartments—i.e., mitochondria and chloroplasts—or the number of different sites described have been used as strong arguments to endorse membrane diffusion as the major mechanism of release. Yet, few experimental demonstrations have been found within the scientific literature.

Using optical absorption after dialysis, Lamy-Freund's group studied the interaction of the indole with lipid bilayers, determining that melatonin crosses asolectin vesicles [120] and, using fluorescence and electron spin resonance spectroscopy (ESR), they further showed association between melatonin and lipids [121]. Nonetheless, rather than crossing, these and other studies have shown that the indole preferentially associates with the polar head groups [122,123], thus creating a sort of melatonin-rich membrane domain, particularly if the concentration used is high [124]. Since much of the melatonin would be tightly associated to the polar region, the speed for crossing a multilayer membrane would be low [122]. This has also been corroborated with *in vivo* studies by Venegas and colleagues [125], who reported melatonin accumulation in membranes, cytosol, mitochondria and nuclei. However, while an increasing dose led a 10-fold increase in membranes, mitochondrial and nuclear levels reached saturation, indicating that passive diffusion would not be the exclusive mechanism of melatonin transport. The presence of high concentrations of melatonin within or tightly associated to the lipid bilayer might explain the efficiency of the indole in reducing lipid peroxidation and preserving membrane fluidity [126–128]. Contrary to these studies, Yu and co-workers, using direct amperometric measurements, have shown the efflux of melatonin from human embryonic kidney cells, reaching a fast equilibrium at both sides of membranes [129]. It can be deduced from this bulk of data that more research is needed to clarify the real behaviour of melatonin within the cell membranes, but it appears clear that the indole, as it occurs with steroids, might use different strategies to move across plasma (or organelle) membranes.

3. An Alternative View: Protein-Facilitated Transport

As mentioned above, melatonin can interact with lipid bilayers, although currently there is no consensus about how this interaction occurs. The question remains as to what potential alternative mechanisms exist for such a rather low passage through the bilayers are taking place. A potential melatonin uptake by cells is not a new concept. Forty years ago, Bubenik et al., using immunohistochemical detection of the indole in retina and Harderian glands, observed a great increase after melatonin application. Therefore, they suggested a potential uptake mechanism and/or receptors in these organs [130], which was further assessed by other studies. In this context, compounds with structural similarities to melatonin, such as tryptophan or serotonin (or other monoamines), can be translocated by different members of the solute carriers (SLCs) superfamily, including SLC3, SLC7,

SLC16 and SLC36 for tryptophan or SLC6, SCL18 and SCL22 for serotonin/monoamines, among others. These carriers are present at either the plasma membrane or the membranes of organelles such as vesicles and mitochondria, as has been recently suggested for melatonin [131–133].

The classic view of melatonin behavior with lipid bilayers has nevertheless left only a few studies focused on protein-mediated carriers or active transporters, as most of them were centered on the role of melatonin membrane receptors. However, the situation recently changed when Hevia and colleagues [134] challenged the classical idea by studying melatonin uptake in normal as well as in cancer cells. Using a specifically developed HPLC method [37] and a highly accurate estimation of the cell volume [135], these authors reported that both intracellular concentrations and kinetics adjust to a transporter-assisted mechanism rather than to a simple passive diffusion, a finding that was consolidated by studying different cell lines. Accordingly, no equilibration at both membrane sides was observed, contrary to what had been suggested by others [120], thus demonstrating that the indole might cross membranes through facilitated transport and not exclusively by passive diffusion. The carrier involved was later attributed to a member of the SCL2A/GLUT transporters subfamily [136]. The classic glucose transporter GLUT1/SLC2A1 would be the prototype member of this subfamily. However, it has only been very recently that the X-ray crystallographic structure of human GLUT1 has been elucidated [137] and until now the homologue XylE bacterial transporter has been employed as the reference protein model. With both docking models, it was predicted that melatonin binding was thermodynamically favorable. Moreover, as a physiological approach, the assays performed on melatonin uptake by erythrocytes, which only express GLUT1, showed that indole uptake was greatly enhanced by adding glucose to the growth medium [136], as it could be deduced if GLUT1 played a role in such internalization. Furthermore, overexpression of GLUT1, but not structurally-related GLUT4, leads to an increase in melatonin uptake in prostate cancer cells (Hevia et al., personal communication). Even though many other cell types and SLC2A carriers should also be investigated, this research field should provide additional data that may be of physiological importance.

Interestingly, melatonin has not been the only metabolite associated to GLUT1. It is well documented that, in addition to glucose, GLUT1/SLC2A1 is also in charge of transporting the oxidized form of ascorbic acid, i.e., DHA, into cells [138]. This might be the main role of GLUT1 in the erythrocytes of those species unable to synthesize vitamin C, a function that requires stomatin as a regulatory partner [139]. By similarities, it could be deduced that melatonin uptake through GLUT1/SLC2A1 transporter might also involve additional association with other regulatory factors. Finally, it is noteworthy to mention that the involvement of GLUT/SLC2A transporters in melatonin membrane transport can provide additional insights underlying the specific relationship between melatonin and glucose. However, it is not known whether it is GLUT1 itself or rather its association to other membrane proteins (e.g., membrane receptors) that would deserve further attention.

GLUT1 has not been the only transporter recently linked to melatonin membrane translocation. Similarly, Huo et al. [140] have described the possible involvement of PEPT1/2 oligopeptide transporters (proton-coupled SCL15A1/2 family) in the uptake of melatonin and its sulfation derivatives. SLC15A1 displays two isoforms in the rat; interestingly, while one is highly expressed in the small intestine, mainly participating in the absorption of protein digestion products [141], isoform 2 is restricted to pinealocytes, where it exhibits a striking circadian rhythmicity in its expression, with a clear 100-fold upregulation during the dark phase, which might underlie into the mechanism of melatonin secretion [142]. The study also reported the localization of PEPT1 in the mitochondria, therefore shedding some light on melatonin's translocation into this organelle, although no transition peptide for directing this protein to mitochondria was described. Again, as it occurs with previous work, authors here showed a kinetics for the uptake that is incompatible with passive diffusion [140]. So, the questions that remain to be answered are whether these two transport systems, as it appears, can be compatible with each other and whether facilitated transport would provide a faster and, perhaps under specific physiological circumstances, an additional way to internalize melatonin into cells and/or organelles. The wide expression if any of these two transporters provide an answer to

the pleiotropic role of melatonin in different cell types and tissues/organs, including metabolic and glucose-related effects.

4. Melatonin and Glucose: An Ancient Relationship?

4.1. Glucose Effect on Melatonin Secretion

Milcou and coworkers reported a relationship between the pineal and glucose homeostasis [143], but these pioneering studies used pineal extracts, since melatonin had not been yet isolated. Years later, one of the first associations between melatonin and diet glucose was made by Wetterberg's laboratory. First, using 13 participants, his group showed that water-supplemented, short-term (2 days) fasting led to a significant decrease in nocturnal melatonin levels, an effect prevented by glucose intake during the fasting period [144]. This is similar to what occurred to the inhibition of the pituitary-testicular axis under the same type of fasting [145]. Authors tried to explain this reduction based on the dependence of glucose delivery on pinealocytes to function properly. Later on, the same group found that obesity does not alter the pattern of melatonin secretion [146]. Interestingly, other early evidence found that glucose itself may affect AANAT activity [143,147] but in some of these pioneering studies they used pineal extracts, since melatonin had not been yet isolated. Collectively, all this early evidence point out that glucose could modulate melatonin secretion.

As described below, the antagonism between melatonin and insulin is well documented. But beyond those regulatory effects of the indole on insulin secretion, it has also been reported that streptozotocin-treated, diabetic rats display an elevated AANAT activity and consequently an increase in melatonin levels in the pineal gland [148]. Contrary to this, AANAT activity and melatonin content in the retina are both reduced in the retina of streptozotocin-treated rats [149], but none of the studies approached the involvement of glucose levels on this effect. This is also the case in type 2 diabetes patients, who have reduced melatonin secretion [150], and also a reduced night pineal melatonin synthesis is observed in type 2 diabetic Goto-Kakizaki rats [150,151]. Similarly, reduced night melatonin production has also been found in women with metabolic syndrome [152]. Cano and co-workers have also found a reduced melatonin content in high-fat fed rats, concomitant with hyperglycemia [153]. When the melatonin rhythm is compared between fasted and hyperglycemic rats, there is a shift in the night-peak pattern [154]. Again, all these studies demonstrate a relation between glucose levels and melatonin secretion, but the molecular insights underlying these effects are still unclear.

4.2. Melatonin and Insulin

Conversely, in addition to the glucose effects on melatonin secretion, the indole itself is involved in controlling glucose homeostasis [155] (reviewed by [156,157]). In fasted rats, a rhythm in plasma glucose has been well described, with a peak in glucose starting at the dark phase, similar to what occurs at dawn in humans, and this evidence persisted even in hyperglycemia or with a normal pattern of glucose feeding. Not surprisingly, a circadian rhythmicity was reported for insulin secretion in rats and also in human subjects, with a nadir at midnight and peaking between noon and 6 pm [154,158]. Bailey et al. and Gorray et al. [159,160] were the first groups to demonstrate that pinealectomy results in a significant increase in insulin secretion, an effect that was confirmed using pineal incubation media. Further studies from different laboratories showed that pinealectomy increases glucose levels as well as glucose intolerance in rats, an effect prevented by melatonin administration [161,162]. Overall, as described above, most authors, therefore, agree that a physiological antagonism between melatonin and insulin occurs. Reciprocally, increased insulin plays a role on melatonin secretion from the pineal gland but while most studies showed an inhibitory effect [163], a few reports have shown a stimulatory effect under some circumstances [155].

This physiological function of melatonin appears to be mainly mediated by membrane receptor signaling. These receptors are highly expressed in pancreatic islets [164] and both MTNR1A/B

membrane receptors have been involved not only in regulating insulin production [165–167] but also in glucagon and somatostatin [40,168]. In vivo data were confirmed by using cell culture models, in which melatonin directly modulates insulin secretion from pancreatic β -cells, an action directly mediated by membrane receptors [169–171]. Interestingly, some polymorphisms, particularly in MTNR1B, have been associated with a higher risk in type 2 diabetes, thus reinforcing the role of this G-coupled membrane receptors on insulin synthesis regulation [172–174]. However, whether this is due as has been suggested to an excess of melatonin signaling [175] or rather, on the contrary, to a defective receptor function is still a matter of debate [176].

Other molecular mechanisms of action have also been related to the melatonin–insulin axis. Hence, recent studies point out the involvement of redox-related pathways, namely NADPH oxidase [177]. Similarly, the direct or indirect free radical-scavenging action of melatonin might also play a role in this context, as the indole protects glucotoxicity-mediated pancreatic islets cell death through either its own signaling or an antioxidant pathway [178–180]. More recently, insulin-like growth factor binding proteins, such as IGFBP3, which can modulate insulin signaling, have been linked to melatonin actions in other tissues [181] so they should not be ruled out in playing a role in melatonin-induced glucose homeostasis.

All this strong evidence and compelling data have led some authors to propose melatonin as a potential therapeutic agent in diabetes [182,183], although the timing of administration might have different outcomes [184]. A summary of this evidence is shown in Table 3.

Table 3. Summary of the evidences/links between glucose or insulin and melatonin.

Evidence/Finding Reported	Type of Assay/Molecular Mechanism Demonstrated	Reference(s)
Glucose Influence on Melatonin Secretion		
Short-term fasting inhibits melatonin secretion (<i>H. sapiens</i>)	N/A	Röjdmarm & Wetterberg (1989) [144]
Glucose affects AANAT activity (<i>R. norvegicus</i>)	Enzyme activity	Welker & Vollrath (1984) [147]
Streptozotocin increases pineal AANAT (<i>R. norvegicus</i>)	Enzyme activity	Peschke et al. (2008) [148]
Streptozotocin decreases retinal AANAT (<i>R. norvegicus</i>)	Enzyme activity, melatonin content (RIA)	Buonfiglio et al. (2011) [149]
Type 2 diabetes patients display reduced night melatonin	Melatonin content (RIA); urine 6-sulfatoxymelatonin (RIA)	Peschke et al. (2006) [150]; McMullan et al. (2013) [150]
Type 2 diabetic Goto-Kakizaki rats display reduced night pineal melatonin	Plasma and pineal melatonin and precursors content (RIA)	Peschke et al. (2006) [150]; Frese et al. (2009) [151]
Metabolic syndrome women show lower night melatonin	Melatonin content (RIA)	Corbalán-Tutau et al. (2014) [152]
Hyperglycemia (high fat diet, <i>R. norvegicus</i>) shifts melatonin peak	Melatonin content (RIA)	Cano et al. (2008) [153]
Induced diabetes reduces night pineal melatonin content (<i>R. norvegicus</i>)	Melatonin content (RIA)	Champney et al. (1983) [163]
Melatonin and Insulin Secretion		
Circadian rhythm in insulin secretion (<i>R. norvegicus</i> , <i>H. sapiens</i>)	RIA, Immunoreaction	Rigas et al. (1968) [185]; Gagliardino y Henández (1971) [186]; Boden et al. (1996) [187]
Correlation between melatonin and insulin levels	RIA	Bizot-Spinard et al. (1998) [154]; Peschke et al. (2013) [155]
Pinealectomy-increase in insulin secretion	N/A	Bailey et al. (1974) [159]; Gorry et al. (1979) [160]
Pinealectomy increases glucose intolerance	Immunoreaction	Díaz y Blázquez (1986) [161]
Presence of melatonin receptors in pancreatic cells	Western blotting/IHC	Nagorny et al. (2011) [164]; Zibolka et al. (2018) [188]
Melatonin inhibits insulin secretion from pancreatic beta-cells	MTNR1B receptor-mediated/cGMP Raf-1/ERK mediated/NADPH oxidase	Stumpf et al. (2008) [169]; Mühlbauer et al. (2011) [165]; Li et al. (2018) [171]; Simoes et al. (2016) [177]
Melatonin influences somatostatin and glucagon	MTNR1A/B receptor-mediated	Bähr et al. (2011) [40]; Zibolka et al. (2015) [168]
Melatonin protects against glucotoxicity	Prevents ER stress	Park et al. 2014 [178]
MTNR1 polymorphisms and type 2 diabetes association	Genetic polymorphisms/altered MTNR1 signalling pathway	Bouatia-Naji et al. (2009) [189]; Sparso et al. 2009 [174]; Mssig et al. (2010) [172]; Tam et al. (2010) [173]; Tuomi et al. 2016 [175]; Mulder 2017 [176]

4.3. Melatonin and Glucose in Invertebrates and Protozoans

Regulation of glucose metabolism by melatonin does not appear to be restricted to vertebrates. Most of the studies have been accomplished in crustaceans. Thus, in the crab *Eriocheir sinensis*, melatonin injection provoked hyperglycemia by inducing crustacean hyperglycemic hormone (CHH) mRNA synthesis and a similar effect was observed in *Uca pugilator* [190] and in *Neohelice granulata* [100], thus demonstrating an ancient relationship between melatonin and glucose.

Apart from invertebrates, even some protozoans with melatonin synthetic ability, e.g., *Tetrahymena*, were seen to respond to insulin in an autocrine mode, but the relationship between the indole and glucose metabolism has not yet been approached [191].

5. Concluding Remarks

There is no doubt that melatonin is a ubiquitously molecule, present in evolutionarily different organisms, from protozoa to mammals or higher plants. Although the pineal gland is responsible for the serum night peak, it is also synthesized in a variety of tissues and organs, therefore indicating a well-conserved function(s) in all living cells. Considering that glucose is one of the preferred sources for energy and carbon and the particular relationship that seems to be between both, melatonin-related glucose homeostasis might be one of the primitive functions of the indole. Here we have reviewed most of the data that link melatonin with glucose metabolism, including glucose control of melatonin synthesis, the physiological role of pineal melatonin in controlling insulin secretion, and finally novel findings relating GLUT1/SLC2A transporter and melatonin uptake, among others.

However, there are still several questions that remain to be answered regarding melatonin and glucose: (i) is melatonin one of the major and important factors that control glucose metabolism in cells and tissues? (ii) Are membrane receptors the exclusive mediators of what seems to be a major melatonin-glucose interrelation? (iii) Could GLUT/SLC2A glucose transporters also mediate and control glucose homeostasis by interacting with the melatonin uptake? (iv) How universal throughout the different phyla is the glucose homeostasis exerted by the indole?

These questions urgently need an experimental approach, since they have multiple implications for evolutionary aspects, but also in pathologies with high incidence, such as diabetes and the metabolic syndrome.

Funding: This research was funded by Ministerio de Economía y Competitividad, Gobierno de España, co-funded by FEDER, grant number MINECO-17-BFU2016-79139-R.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lerner, A.B.; Case, J.D.; Takahashi, Y.; Lee, T.H.; Mori, W. Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J. Am. Chem. Soc.* **1958**, *80*, 2587. [[CrossRef](#)]
2. Lerner, A.B.; Case, J.D.D.; Takahashi, Y. Isolation of melatonin and 5-methoxyindole-3-acetic acid from bovine pineal glands. *J. Biol. Chem.* **1960**, *235*, 1992–1997. [[PubMed](#)]
3. McCord, C.P.; Allen, F.P. Evidences associating pineal gland function with alterations in pigmentation. *J. Exp. Zool.* **1917**, *23*, 207–224. [[CrossRef](#)]
4. Axelrod, J.; Weissbach, H. Enzymatic O-methylation of N-acetylserotonin to melatonin. *Science* **1960**, *131*, 1312. [[CrossRef](#)] [[PubMed](#)]
5. Wurtman, R.J.; Axelrod, J.; Phillips, L.S. Melatonin synthesis in the pineal gland: Control by light. *Science* (80-) **1963**, *142*, 1071–1073. [[CrossRef](#)]
6. Reiter, R.J. The pineal and its hormones in the control of reproduction in mammals. *Endocr. Rev.* **1980**, *1*, 109–131. [[CrossRef](#)] [[PubMed](#)]
7. Hoffman, R.A.; Reiter, R.J. Pineal gland influence on gonads of male Hamsters. *Science* **1965**, *148*, 1609–1611. [[CrossRef](#)] [[PubMed](#)]

8. Reiter, R.J. Comparative Physiology: Pineal Gland. *Annu. Rev. Physiol.* **1973**, *35*, 305–328. [[CrossRef](#)] [[PubMed](#)]
9. Reiter, R.J.; Hoffman, J.C.; Rubin, P.H. Pineal gland: Influence on gonads of male rats treated with androgen 3 days after birth. *Science (80-)* **1968**, *160*, 420–421. [[CrossRef](#)]
10. Arendt, J. Role of the pineal gland and melatonin in seasonal reproductive function in mammals. *Oxf. Rev. Reprod. Biol.* **1986**, *8*, 266–320. [[PubMed](#)]
11. Brainard, G.C.; Richardson, B.A.; King, T.S.; Reiter, R.J. The influence of different light spectra on the suppression of pineal melatonin content in the Syrian hamster. *Brain Res.* **1984**, *294*, 333–339. [[CrossRef](#)]
12. Reiter, R.J. Action Spectra, Dose-Response Relationships, and Temporal Aspects of Light's Effects on the Pineal Gland. *Ann. N. Y. Acad. Sci.* **1985**, *453*, 215–230. [[CrossRef](#)] [[PubMed](#)]
13. Reiter, R.J.; Craft, C.M.; Johnson, J.E.; King, T.S.; Richardson, B.A.; Vaughan, G.M.; Vaughan, M.K. Age-associated reduction in nocturnal pineal melatonin levels in female rats. *Endocrinology* **1981**, *109*, 1295–1297. [[CrossRef](#)] [[PubMed](#)]
14. Pang, S.F.; Tang, P.L. Decreased serum and pineal concentrations of melatonin and *N*-acetylserotonin in aged male hamsters. *Horm. Res.* **1983**, *17*, 228–234. [[CrossRef](#)] [[PubMed](#)]
15. Reiter, R.J. The ageing pineal gland and its physiological consequences. *BioEssays* **1992**, *14*, 169–175. [[CrossRef](#)] [[PubMed](#)]
16. Reiter, R.J. Melatonin: The chemical expression of darkness. *Mol. Cell. Endocrinol.* **1991**, *79*, C153–C158. [[CrossRef](#)]
17. Guo, X.H.; Li, Y.H.; Zhao, Y.S.; Zhai, Y.Z.; Zhang, L.C. Anti-aging effects of melatonin on the myocardial mitochondria of rats and associated mechanisms. *Mol. Med. Rep.* **2017**, *15*, 403–410. [[CrossRef](#)] [[PubMed](#)]
18. Odinkova, I.; Baburina, Y.; Kruglov, A.; Fadeeva, I.; Zvyagina, A.; Sotnikova, L.; Akatov, V.; Krestina, O. Effect of melatonin on rat heart mitochondria in acute heart failure in aged rats. *Int. J. Mol. Sci.* **2018**, *19*, 1555. [[CrossRef](#)] [[PubMed](#)]
19. Maestroni, G.J.M.M. The immunoneuroendocrine role of melatonin. *J. Pineal Res.* **1993**, *14*, 1–10. [[CrossRef](#)] [[PubMed](#)]
20. Bartsch, C.; Bartsch, H. Melatonin in cancer patients and in tumor-bearing animals. *Adv. Exp. Med. Biol.* **1999**, *467*, 247–264. [[PubMed](#)]
21. Blask, D.E.; Dauchy, R.T.; Sauer, L.A.; Krause, J.A.; Brainard, G.C. Light during darkness, melatonin suppression and cancer progression. *Neuroendocrinol. Lett.* **2002**, *23*, 52–56. [[PubMed](#)]
22. Tamarkin, L.; Cohen, M.; Reichert, C.; Reichert, C.; Lippman, M.; Chabner, B. Melatonin Inhibition and Pinealectomy Enhancement of 7,12-Dimethylbenz(a)anthracene-induced Mammary Tumors in the Rat. *Cancer Res.* **1981**, *41*, 4432–4436. [[PubMed](#)]
23. Chen, S.J.; Huang, S.H.; Chen, J.W.; Wang, K.C.; Yang, Y.R.; Liu, P.F.; Lin, G.J.; Sytwu, H.K. Melatonin enhances interleukin-10 expression and suppresses chemotaxis to inhibit inflammation in situ and reduce the severity of experimental autoimmune encephalomyelitis. *Int. Immunopharmacol.* **2016**, *31*, 169–177. [[CrossRef](#)] [[PubMed](#)]
24. Mocikova, K.; Mnichova, M.; Kubatka, P.; Bojkova, B.; Ahlers, I.; Ahlersova, E. Mammary carcinogenesis induced in Wistar:Han rats by the combination of ionizing radiation and dimethylbenz(a)anthracene: Prevention with melatonin. *Neoplasma* **2000**, *47*, 227–229.
25. Ozaki, Y.; Lynch, H.J. Presence of melatonin in plasma and urine of pinealectomized rats. *Endocrinology* **1976**, *99*, 641–644. [[CrossRef](#)] [[PubMed](#)]
26. Cardinali, D.P.; Wurtman, R.J. Hydroxyindole-*O*-Methyl Transferases in Rat Pineal, Retina and Harderian Gland. *Endocrinology* **1972**, *91*, 247–252. [[CrossRef](#)] [[PubMed](#)]
27. Bubenik, G.A.; Brown, G.M.; Uhler, I.; Grota, L.J. Immunohistological localization of *N*-acetylindolealkylamines in pineal gland, retina and cerebellum. *Brain Res.* **1974**, *81*, 233–242. [[CrossRef](#)]
28. Raikhlin, N.T.; Kvetnoy, I.M.; Tolkachev, V.N. Melatonin may be synthesised in enterochromaffin cells. *Nature* **1975**, *255*, 344–345. [[CrossRef](#)] [[PubMed](#)]
29. Bubenik, G.A.; Brown, G.M.; Grota, L.J. Immunohistochemical localization of melatonin in the rat Harderian gland. *J. Histochem. Cytochem.* **1976**, *24*, 1173–1177. [[CrossRef](#)] [[PubMed](#)]
30. Arendt, J.; Paunier, L.; Sizonenko, P.C. Melatonin radioimmunoassay. *J. Clin. Endocrinol. Metab.* **1975**, *40*, 347–350. [[CrossRef](#)] [[PubMed](#)]

31. Vakkuri, O.; Lamsa, E.; Rahkamaa, E.; Ruotsalainen, H.; Leppaluoto, J. Iodinated melatonin: Preparation and characterization of the molecular structure by mass and ¹H NMR spectroscopy. *Anal. Biochem.* **1984**, *142*, 284–289. [[CrossRef](#)]
32. Vakkuri, O.; Leppaluoto, J.; Vuolteenaho, O. Development and validation of a melatonin radioimmunoassay using radioiodinated melatonin as tracer. *Eur. J. Endocrinol.* **1984**, *106*, 152–157. [[CrossRef](#)]
33. Laudon, M.; Zisapel, N. Characterization of central melatonin receptors using ¹²⁵I-melatonin. *FEBS Lett.* **1986**, *197*, 9–12. [[CrossRef](#)]
34. Duncan, M.J.; Takahashi, J.S.; Dubocovich, M.L. Characterization of 2-[¹²⁵I]iodomelatonin binding sites in hamster brain. *Eur. J. Pharmacol.* **1986**, *132*, 333–334. [[CrossRef](#)]
35. Weaver, D.R.; Namboodiri, M.A.A.; Reppert, S.M. Iodinated melatonin mimics melatonin action and reveals discrete binding sites in fetal brain. *FEBS Lett.* **1988**, *228*, 123–127. [[CrossRef](#)]
36. Reppert, S.M.; Weaver, D.R.; Rivkees, S.A.; Stopa, E.G. Putative melatonin receptors in a human biological clock. *Science* **1988**, *242*, 78–81. [[CrossRef](#)] [[PubMed](#)]
37. Hevia, D.; Botas, C.; Sainz, R.M.; Quiros, I.; Blanco, D.; Tan, D.X.; Gomez-Cordoves, C.; Mayo, J.C. Development and validation of new methods for the determination of melatonin and its oxidative metabolites by high performance liquid chromatography and capillary electrophoresis, using multivariate optimization. *J. Chromatogr. A* **2010**, *1217*, 1368–1374. [[CrossRef](#)] [[PubMed](#)]
38. Acuña-Castroviejo, D.; Escames, G.; Venegas, C.; Díaz-Casado, M.E.; Lima-Cabello, E.; López, L.C.; Rosales-Corral, S.; Tan, D.X.; Reiter, R.J. Extrapineal melatonin: Sources, regulation, and potential functions. *Cell. Mol. Life Sci.* **2014**, *71*, 2997–3025. [[CrossRef](#)] [[PubMed](#)]
39. Chen, C.Q.; Fichna, J.; Bashashati, M.; Li, Y.Y.; Storr, M. Distribution, function and physiological role of melatonin in the lower gut. *World J. Gastroenterol.* **2011**, *17*, 3888–3898. [[CrossRef](#)] [[PubMed](#)]
40. Bähr, I.; Muhlbauer, E.; Schucht, H.; Peschke, E. Melatonin stimulates glucagon secretion in vitro and in vivo. *J. Pineal Res.* **2011**, *50*, 336–344. [[CrossRef](#)] [[PubMed](#)]
41. Slominski, A.T.; Hardeland, R.; Zmijewski, M.A.; Slominski, R.M.; Reiter, R.J.; Paus, R. Melatonin: A Cutaneous Perspective on its Production, Metabolism, and Functions. *J. Investig. Dermatol.* **2018**, *138*, 490–499. [[CrossRef](#)] [[PubMed](#)]
42. Slominski, A. The cutaneous serotonergic/melatonergic system: Securing a place under the sun. *FASEB J.* **2005**, *19*, 176–194. [[CrossRef](#)] [[PubMed](#)]
43. Yu, Y.; Lv, Y.; Shi, Y.; Li, T.; Chen, Y.; Zhao, D.; Zhao, Z. The Role of Phyto-Melatonin and Related Metabolites in Response to Stress. *Molecules* **2018**, *23*, 1887. [[CrossRef](#)] [[PubMed](#)]
44. Pöggeler, B.; Balzer, I.; Hardeland, R.; Lerchl, A. Pineal hormone melatonin oscillates also in the dinoflagellate *Gonyaulax polyedra*. *Naturwissenschaften* **1991**, *78*, 268–269. [[CrossRef](#)]
45. Manchester, L.C.; Poeggeler, B.; Alvares, F.L.; Ogden, G.B.; Reiter, R.J. Melatonin immunoreactivity in the photosynthetic prokaryote *Rhodospirillum rubrum*: Implications for an ancient antioxidant system. *Cell. Mol. Biol. Res.* **1995**, *41*, 391–395. [[PubMed](#)]
46. Balzer, I. Recent progress in understanding the temporal behavior of unicellular organisms. *Braz. J. Med. Biol. Res.* **1996**, *29*, 95–99. [[PubMed](#)]
47. Macías, M.; Rodríguez-Cabezas, M.N.; Reiter, R.J.; Osuna, A.; Acuña-Castroviejo, D. Presence and effects of melatonin in *Trypanosoma cruzi*. *J. Pineal Res.* **1999**, *27*, 86–94. [[CrossRef](#)] [[PubMed](#)]
48. Ganguly, S.; Mummaneni, P.; Steinbach, P.J.; Klein, D.C.; Coon, S.L. Characterization of the *Saccharomyces cerevisiae* Homolog of the Melatonin Rhythm Enzyme Arylalkylamine *N*-Acetyltransferase (EC 2.3.1.87). *J. Biol. Chem.* **2001**, *276*, 47239–47247. [[CrossRef](#)] [[PubMed](#)]
49. Vivien-Roels, B.; Pevet, P.; Beck, O.; Fevre-Montange, M. Identification of melatonin in the compound eyes of an insect, the locust (*Locusta migratoria*), by radioimmunoassay and gas chromatography-mass spectrometry. *Neurosci. Lett.* **1984**, *49*, 153–157. [[CrossRef](#)]
50. Arnoult, F.; Vivien-Roels, B.; Pévet, P.; Vernet, G. Melatonin in the nemertine worm *lineus lacteus*: Identification and daily variations. *Neurosignals* **1994**, *3*, 296–301. [[CrossRef](#)]
51. Blanc, A.; Vivien-Roels, B.; Pévet, P.; Attia, J.; Buisson, B. Melatonin and 5-methoxytryptophol (5-ML) in nervous and/or neurosensory structures of a gastropod mollusc (*Helix aspersa maxima*): Synthesis and diurnal rhythms. *Gen. Comp. Endocrinol.* **2003**, *131*, 168–175. [[CrossRef](#)]
52. Vivien-Roels, B.; Pevet, P. Is melatonin an evolutionary conservative molecule involved in the transduction of photoperiodic information in all living organisms? *Adv. Pineal Res.* **1986**, *1*, 61–68.

53. Balzer, I.; Kapp, H. Occurrence and comparative physiology of melatonin in evolutionary diverse organisms. In *The Redox State and Circadian Rhythms*; Vanden Driessche, T., Petiau-de Vries, G.M., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000; pp. 95–119. ISBN 0-7923-6453-8.
54. Dubbels, R.; Reiter, R.J.J.; Klenke, E.; Goebel, A.; Schnakenberg, E.; Ehlers, C.; Schiwara, H.W.W.; Schloot, W. Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. *J. Pineal Res.* **1995**, *18*, 28–31. [[CrossRef](#)] [[PubMed](#)]
55. Hattori, A.; Migita, H.; Iigo, M.; Itoh, M.; Yamamoto, K.; Ohtani-Kaneko, R.; Hara, M.; Suzuki, T.; Reiter, R.J. Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. *Biochem. Mol. Biol. Int.* **1995**, *35*, 627–634. [[PubMed](#)]
56. Tan, D.X.; Hardeland, R.; Manchester, L.C.; Korkmaz, A.; Ma, S.; Rosales-Corral, S.; Reiter, R.J. Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. *J. Exp. Bot.* **2012**, *63*, 577–597. [[CrossRef](#)] [[PubMed](#)]
57. Tan, D.X.; Hardeland, R.; Manchester, L.C.; Paredes, S.D.; Korkmaz, A.; Sainz, R.M.; Mayo, J.C.; Fuentes-Broto, L.; Reiter, R.J. The changing biological roles of melatonin during evolution: From an antioxidant to signals of darkness, sexual selection and fitness. *Biol. Rev.* **2010**, *85*, 607–623. [[CrossRef](#)] [[PubMed](#)]
58. Hardeland, R. Melatonin in plants and other phototrophs: Advances and gaps concerning the diversity of functions. *J. Exp. Bot.* **2015**, *66*, 627–646. [[CrossRef](#)] [[PubMed](#)]
59. Arnao, M.B.; Hernández-Ruiz, J. Functions of melatonin in plants: A review. *J. Pineal Res.* **2015**, *59*, 133–150. [[CrossRef](#)] [[PubMed](#)]
60. Reiter, R.J.; Tan, D.X.; Rosales-Corral, S.; Manchester, L.C. The universal nature, unequal distribution and antioxidant functions of melatonin and its derivatives. *Mini-Rev. Med. Chem.* **2013**, *13*, 373–384. [[PubMed](#)]
61. Quay, W.B. Retinal and pineal hydroxyindole-o-methyl transferase activity in vertebrates. *Life Sci.* **1965**, *4*, 983–991. [[CrossRef](#)]
62. Raikhlin, N.T.; Kvetnoy, I.M. Melatonin and enterochromaffine cells. *Acta Histochem.* **1976**, *55*, 19–24. [[CrossRef](#)]
63. Kvetnoy, I.; Yuzhakov, V. Extrapineal melatonin: Non-traditional localization and possible significance for oncology. In *Advances in Pineal Research*; Maestroni, G.J.M., Conti, A., Reiter, R.J., Eds.; John Libbey and Company: London, UK, 1994; Volume 7, pp. 199–212.
64. Kvetnoy, I.M.; Kvetnaia, T.V.; Yuzhakov, V.V. Role of Extrapineal Melatonin and Related APUD Series Peptides in Malignancy. In *The Pineal Gland and Cancer*; Springer: Berlin/Heidelberg, Germany, 2001; pp. 259–274.
65. Bubenik, G.A.; Brown, G.M.; Grota, L.J. Immunohistological investigations of *N*-Acetylserotonin in the rat cerebellum after parachlorophenylalanine treatment. *Experientia* **1976**, *32*, 579–581. [[CrossRef](#)] [[PubMed](#)]
66. Pang, S.F.; Brown, G.M.; Grota, L.J.; Chambers, J.W.; Rodman, R.L. Determination of *N*-acetylserotonin and melatonin activities in the pineal gland, retina, Harderian gland, brain and serum of rats and chickens. *Neuroendocrinology* **1977**, *23*, 1–13. [[CrossRef](#)] [[PubMed](#)]
67. Menendez-Pelaez, A.; Howes, K.A.; Gonzalez-Brito, A.; Reiter, R.J. *N*-Acetyltransferase activity, hydroxyindole-*O*-methyltransferase activity, and melatonin levels in the Harderian glands of the female Syrian hamster: Changes during the light: Dark cycle and the effect of 6-parachlorophenylalanine administration. *Biochem. Biophys. Res. Commun.* **1987**, *145*, 1231–1238. [[CrossRef](#)]
68. Bubenik, G.A. Localization of Melatonin in the Digestive Tract of the Rat. *Horm. Res.* **1980**, *12*, 313–323. [[CrossRef](#)] [[PubMed](#)]
69. Biesalski, H.K.; Welker, H.A.; Thalmann, R.; Vollrath, L. Melatonin and other serotonin derivatives in the guinea pig membranous cochlea. *Neurosci. Lett.* **1988**, *91*, 41–46. [[CrossRef](#)]
70. Lopez-Gonzalez, M.A.; Guerrero, J.M.; Delgado, F. Presence of the pineal hormone melatonin in rat cochlea: Its variations with lighting conditions. *Neurosci. Lett.* **1997**, *238*, 81–83. [[CrossRef](#)]
71. Finocchiaro, L.M.; Nahmod, V.E.; Launay, J.M. Melatonin biosynthesis and metabolism in peripheral blood mononuclear leucocytes. *Biochem. J.* **1991**, *280 Pt 3*, 727–731. [[CrossRef](#)]
72. Abe, M.; Itoh, M.T.; Miyata, M.; Ishikawa, S.; Sumi, Y. Detection of melatonin, its precursors and related enzyme activities in rabbit lens. *Exp. Eye Res.* **1999**, *68*, 255–262. [[CrossRef](#)] [[PubMed](#)]

73. Slominski, A.; Pisarchik, A.; Semak, I.; Sweatman, T.; Wortsman, J.; Szczesniewski, A.; Slugocki, G.; McNulty, J.; Kauser, S.; Tobin, D.J.; et al. Serotonergic and melatonergic systems are fully expressed in human skin. *FASEB J.* **2002**, *16*, 896–898. [[CrossRef](#)] [[PubMed](#)]
74. Slominski, A.; Baker, J.; Rosano, T.G.; Guisti, L.W.; Ermak, G.; Grande, M.; Gaudet, S.J. Metabolism of serotonin to *N*-acetylserotonin, melatonin, and 5-methoxytryptamine in hamster skin culture. *J. Biol. Chem.* **1996**, *271*, 12281–12286. [[CrossRef](#)] [[PubMed](#)]
75. Tijmes, M.; Pedraza, R.; Valladares, L. Melatonin in the rat testis: Evidence for local synthesis. *Steroids* **1996**, *61*, 65–68. [[CrossRef](#)]
76. Itoh, M.T.; Ishizuka, B.; Kuribayashi, Y.; Amemiya, A.; Sumi, Y. Melatonin, its precursors, and synthesizing enzyme activities in the human ovary. *Mol. Hum. Reprod.* **1999**, *5*, 402–408. [[CrossRef](#)] [[PubMed](#)]
77. Itoh, M.T.; Ishizuka, B.; Kudo, Y.; Fusama, S.; Amemiya, A.; Sumi, Y. Detection of melatonin and serotonin *N*-acetyltransferase and hydroxyindole-*O*-methyltransferase activities in rat ovary. *Mol. Cell. Endocrinol.* **1998**, *136*, 7–13. [[CrossRef](#)]
78. Tan, D.X.; Manchester, L.C.; Reiter, R.J.; Qi, W.B.; Zhang, M.; Weintraub, S.T.; Cabrera, J.; Sainz, R.M.; Mayo, J.C. Identification of highly elevated levels of melatonin in bone marrow: Its origin and significance. *Biochim. Biophys. Acta Gen. Subj.* **1999**, *1472*, 206–214. [[CrossRef](#)]
79. Conti, A.; Conconi, S.; Hertens, E.; Skwarlo-Sonta, K.; Markowska, M.; Maestroni, J.M. Evidence for melatonin synthesis in mouse and human bone marrow cells. *J. Pineal Res.* **2000**, *28*, 193–202. [[CrossRef](#)] [[PubMed](#)]
80. Carrillo-Vico, A.; Calvo, J.R.; Abreu, P.; Lardone, P.J.; García-Mauriño, S.; Reiter, R.J.; Guerrero, J.M. Evidence of melatonin synthesis by human lymphocytes and its physiological significance: Possible role as intracrine, autocrine, and/or paracrine substance. *FASEB J.* **2004**, *18*, 537–539. [[CrossRef](#)] [[PubMed](#)]
81. Iwasaki, S.; Nakazawa, K.; Sakai, J.; Kometani, K.; Iwashita, M.; Yoshimura, Y.; Maruyama, T. Melatonin as a local regulator of human placental function. *J. Pineal Res.* **2005**, *39*, 261–265. [[CrossRef](#)] [[PubMed](#)]
82. Maldonado, M.D.; Mora-Santos, M.; Naji, L.; Carrascosa-Salmoral, M.P.; Naranjo, M.C.; Calvo, J.R. Evidence of melatonin synthesis and release by mast cells. Possible modulatory role on inflammation. *Pharmacol. Res.* **2010**, *62*, 282–287. [[CrossRef](#)] [[PubMed](#)]
83. Tilden, A.R.; Becker, M.A.; Amma, L.L.; Arciniega, J.; McGaw, A.K. Melatonin production in an aerobic photosynthetic bacterium: An evolutionarily early association with darkness. *J. Pineal Res.* **1997**, *22*, 102–106. [[CrossRef](#)] [[PubMed](#)]
84. Jiao, J.; Ma, Y.; Chen, S.; Liu, C.; Song, Y.; Qin, Y.; Yuan, C.; Liu, Y. Melatonin-Producing Endophytic Bacteria from Grapevine Roots Promote the Abiotic Stress-Induced Production of Endogenous Melatonin in Their Hosts. *Front. Plant Sci.* **2016**, *7*, 1387. [[CrossRef](#)] [[PubMed](#)]
85. Balzer, I.; Hardeland, R. Melatonin in algae and higher plants—Possible new roles as a phytohormone and antioxidant. *Bot. Acta* **1996**, *109*, 180–183. [[CrossRef](#)]
86. Poeggeler, B.; Hardeland, R. Detection and quantification of melatonin in a dinoflagellate, *Gonyaulax polyedra*: Solutions to the problem of methoxyindole destruction in non-vertebrate material. *J. Pineal Res.* **1994**, *17*, 1–10. [[CrossRef](#)] [[PubMed](#)]
87. Sprenger, J.; Hardeland, R.; Fuhrberg, B.; Han, S.-Z. Melatonin and Other 5-Methoxylated Indoles in Yeast: Presence in High Concentrations and Dependence on Tryptophan Availability. *Cytologia (Tokyo)* **1999**, *64*, 209–213. [[CrossRef](#)]
88. Köhida, L.; Vakkuri, O.; Keresztesi, M.; Leppäluoto, J.; Csaba, G. Induction of melatonin synthesis in *Tetrahymena pyriformis* by hormonal imprinting—A unicellular “factory” of the indoleamine. *Cell. Mol. Biol. (Noisy-le-grand)* **2003**, *49*, 521–524.
89. Itoh, M.T.; Shinozawa, T.; Sumi, Y. Circadian rhythms of melatonin-synthesizing enzyme activities and melatonin levels in planarians. *Brain Res.* **1999**, *830*, 165–173. [[CrossRef](#)]
90. Migliori, M.L.; Romanowski, A.; Simonetta, S.H.; Valdez, D.; Guido, M.; Golombek, D.A. Daily variation in melatonin synthesis and arylalkylamine *N*-acetyltransferase activity in the nematode *Caenorhabditis elegans*. *J. Pineal Res.* **2012**, *53*, 38–46. [[CrossRef](#)] [[PubMed](#)]
91. Subaraja, M.; Vanisree, A.J. Neurotransmissional, structural, and conduction velocity changes in cerebral ganglions of *Lumbricus terrestris* on exposure to acrylamide. *Environ. Sci. Pollut. Res.* **2016**, *23*, 17123–17131. [[CrossRef](#)] [[PubMed](#)]
92. Finocchiaro, L.; Callebert, J.; Launay, J.M.; Jallon, J.M. Melatonin Biosynthesis in *Drosophila*: Its Nature and Its Effects. *J. Neurochem.* **1988**, *50*, 382–387. [[CrossRef](#)] [[PubMed](#)]

93. Richter, K.; Peschke, E.; Peschke, D. A neuroendocrine releasing effect of melatonin in the brain of an insect, *Periplaneta americana* (L.). *J. Pineal Res.* **2000**, *28*, 129–135. [[CrossRef](#)] [[PubMed](#)]
94. Wetterberg, L.; Hayes, D.K.; Halberg, F. Circadian rhythm of melatonin in the brain of the face fly, *Musca autumnalis* De Geer. *Chronobiologia* **1987**, *14*, 377–381. [[PubMed](#)]
95. Markowska, M.; Bentkowski, P.; Kloc, M.; Pijanowska, J. Presence of melatonin in *Daphnia magna*. *J. Pineal Res.* **2009**, *46*, 242–244. [[CrossRef](#)] [[PubMed](#)]
96. Vivien-Roels, B.; Pévet, P. Melatonin: Presence and formation in invertebrates. *Experientia* **1993**, *49*, 642–647. [[CrossRef](#)]
97. Tilden, A.R.; Rasmussen, P.; Awantang, R.M.; Furlan, S.; Goldstein, J.; Palsgrove, M.; Sauer, A. Melatonin cycle in the fiddler crab *Uca pugilator* and influence of melatonin on limb regeneration. *J. Pineal Res.* **1997**, *23*, 142–147. [[CrossRef](#)] [[PubMed](#)]
98. Aguzzi, J.; Sanchez-Pardo, J.; García, J.A.; Sardà, F. Day-night and depth differences in haemolymph melatonin of the Norway lobster, *Nephrops norvegicus* (L.). *Deep Sea Res. Part I Oceanogr. Res. Pap.* **2009**, *56*, 1894–1905. [[CrossRef](#)]
99. Farca Luna, A.J.; Heinrich, R.; Reischig, T. The circadian biology of the marbled crayfish. *Front. Biosci. (Elite Ed.)* **2010**, *2*, 1414–1431. [[PubMed](#)]
100. Maciel, F.E.; Geihs, M.A.; Cruz, B.P.; Vargas, M.A.; Allodi, S.; Marins, L.F.; Nery, L.E.M. Melatonin as a signaling molecule for metabolism regulation in response to hypoxia in the crab *Neohelice granulata*. *Int. J. Mol. Sci.* **2014**, *15*, 22405–22420. [[CrossRef](#)] [[PubMed](#)]
101. Yang, X.; Xu, M.; Huang, G.; Zhang, C.; Pang, Y.; Yang, Z.; Cheng, Y. The hyperglycemic effect of melatonin in the Chinese mitten crab, *Eriocheir sinensis*. *Front. Physiol.* **2018**, *9*, 270. [[CrossRef](#)] [[PubMed](#)]
102. Mechawar, N.; Anctil, M. Melatonin in a primitive metazoan: Seasonal changes of levels and immunohistochemical visualization in neurons. *J. Comp. Neurol.* **1997**, *387*, 243–254. [[CrossRef](#)]
103. Abran, D.; Anctil, M.; Ali, M.A. Melatonin activity rhythms in eyes and cerebral ganglia of *Aplysia californica*. *Gen. Comp. Endocrinol.* **1994**, *96*, 215–222. [[CrossRef](#)] [[PubMed](#)]
104. Tan, D.X.; Chen, L.D.; Poeggeler, B.; Manchester, L.; Reiter, R.J. Melatonin: A potent, endogenous hydroxyl radical scavenger. *Endocr. J.* **1993**, *1*, 59–60.
105. Reiter, R.J.; Mayo, J.C.; Tan, D.X.; Sainz, R.M.; Alatorre-Jimenez, M.; Qin, L. Melatonin as an antioxidant: Under promises but over delivers. *J. Pineal Res.* **2016**, *61*, 253–278. [[CrossRef](#)] [[PubMed](#)]
106. Slominski, A.T.; Semak, I.; Fischer, T.W.; Kim, T.K.; Kleszczyński, K.; Hardeland, R.; Reiter, R.J. Metabolism of melatonin in the skin: Why is it important? *Exp. Dermatol.* **2017**, *26*, 563–568. [[CrossRef](#)] [[PubMed](#)]
107. Ueck, M.; Wake, K. The pinealocyte—A paraneuron? A review. *Arch. Histol. Jpn.* **1977**, *40*, 261–278. [[CrossRef](#)] [[PubMed](#)]
108. McNulty, J.A.; Fox, L.M.; Lisco, S.J. Pinealocyte Dense-Cored Vesicles and Synaptic Ribbons: A Correlative Ultrastructural-Biochemical Investigation in Rats and Mice. *J. Pineal Res.* **1987**, *4*, 45–59. [[CrossRef](#)] [[PubMed](#)]
109. Romijn, H.J.; Mud, M.T.; Wolters, P.S. Diurnal variations in number of Golgi-dense core vesicles in light pinealocytes of the rabbit. *J. Neural Transm.* **1976**, *38*, 231–237. [[CrossRef](#)] [[PubMed](#)]
110. Benson, B.; Krasovich, M. Circadian rhythm in the number of granulated vesicles in the pinealocytes of mice. Effects of sympathectomy and melatonin treatment. *Cell Tissue Res.* **1977**, *184*, 499–506. [[CrossRef](#)] [[PubMed](#)]
111. Kappers, J.A. Localization of indoleamine and protein synthesis in the mammalian pineal gland. *J. Neural Transm. Suppl.* **1978**, *13*, 13–24.
112. Juillard, M.T.; Collin, J.P. Pools of serotonin in the pineal gland of the mouse: The mammalian pinealocyte as a component of the diffuse neuroendocrine system. *Cell Tissue Res.* **1980**, *213*, 273–291. [[CrossRef](#)] [[PubMed](#)]
113. Tricoire, H.; Malpoux, B.; Møller, M. Cellular lining of the sheep pineal recess studied by light-, transmission-, and scanning electron microscopy: Morphologic indications for a direct secretion of melatonin from the pineal gland to the cerebrospinal fluid. *J. Comp. Neurol.* **2003**, *456*, 39–47. [[CrossRef](#)] [[PubMed](#)]
114. Miguez, J.M.; Simonneaux, V.; Pévet, P. The role of the intracellular and extracellular serotonin in the regulation of melatonin production in rat pinealocytes. *J. Pineal Res.* **1997**, *23*, 63–71. [[CrossRef](#)] [[PubMed](#)]
115. Olcese, J.; Münker, M. Extracellular serotonin promotes melatonin release from cultured rat pinealocytes: Evidence for an S2-type receptor-mediated autocrine feedback. *Brain Res.* **1994**, *643*, 150–154. [[CrossRef](#)]
116. Moriyama, Y.; Hayashi, M.; Yamada, H.; Yatsushiro, S.; Ishio, S.; Yamamoto, A. Synaptic-like microvesicles, synaptic vesicle counterparts in endocrine cells, are involved in a novel regulatory mechanism for the synthesis and secretion of hormones. *J. Exp. Biol.* **2000**, *203*, 117–125. [[PubMed](#)]

117. Shida, C.; Castrucci, A.M.L.; Lamy-Freund, M.T. High melatonin solubility in aqueous medium. *J. Pineal Res.* **1994**, *16*, 198–201. [[CrossRef](#)] [[PubMed](#)]
118. Li, X.; Wang, S. Binding of glutathione and melatonin to human serum albumin: A comparative study. *Colloids Surf. B Biointerfaces* **2015**, *125*, 96–103. [[CrossRef](#)] [[PubMed](#)]
119. Oren, I.; Fleishman, S.J.; Kessel, A.; Ben-Tal, N. Free diffusion of steroid hormones across biomembranes: A simplex search with implicit solvent model calculations. *Biophys. J.* **2004**, *87*, 768–779. [[CrossRef](#)] [[PubMed](#)]
120. Costa, E.J.X.; Lopes, R.H.; Lamy-Freund, M.T. Permeability of pure lipid bilayers to melatonin. *J. Pineal Res.* **1995**, *19*, 123–126. [[CrossRef](#)] [[PubMed](#)]
121. Costa, E.J.X.; Shida, C.S.; Biaggi, M.H.; Ito, A.S.; Lamy-Freund, M.T. How melatonin interacts with lipid bilayers: A study by fluorescence and ESR spectroscopies. *FEBS Lett.* **1997**, *416*, 103–106. [[CrossRef](#)]
122. Saija, A.; Tomaino, A.; Trombetta, D.; Pellegrino, M.L.; Tita, B.; Caruso, S.; Castelli, F. Interaction of melatonin with model membranes and possible implications in its photoprotective activity. *Eur. J. Pharm. Biopharm.* **2002**, *53*, 209–215. [[CrossRef](#)]
123. Severcan, F.; Sahin, I.; Kazanci, N. Melatonin strongly interacts with zwitterionic model membranes-evidence from Fourier transform infrared spectroscopy and differential scanning calorimetry. *Biochim. Biophys. Acta Biomembr.* **2005**, *1668*, 215–222. [[CrossRef](#)] [[PubMed](#)]
124. Dies, H.; Cheung, B.; Tang, J.; Rheinstädter, M.C. The organization of melatonin in lipid membranes. *Biochim. Biophys. Acta Biomembr.* **2015**, *1848*, 1032–1040. [[CrossRef](#)] [[PubMed](#)]
125. Venegas, C.; García, J.A.; Escames, G.; Ortiz, F.; López, A.; Doerrier, C.; García-Corzo, L.; López, L.C.; Reiter, R.J.; Acuña-Castroviejo, D. Extrapineal melatonin: Analysis of its subcellular distribution and daily fluctuations. *J. Pineal Res.* **2012**, *52*, 217–227. [[CrossRef](#)] [[PubMed](#)]
126. Melchiorri, D.; Reiter, R.J.; Sewerynek, E.; Chen, L.D.; Nisticó, G. Melatonin reduces kainate-induced lipid peroxidation in homogenates of different brain regions. *FASEB J.* **1995**, *9*, 1205–1210. [[CrossRef](#)] [[PubMed](#)]
127. García, J.J.; Reiter, R.J.; Guerrero, J.M.; Escames, G.; Yu, B.P.; Oh, C.S.; Muñoz-Hoyos, A. Melatonin prevents changes in microsomal membrane fluidity during induced lipid peroxidation. *FEBS Lett.* **1997**, *408*, 297–300. [[CrossRef](#)]
128. García, J.J.; López-Pingarrón, L.; Almeida-Souza, P.; Tres, A.; Escudero, P.; García-Gil, F.A.; Tan, D.X.; Reiter, R.J.; Ramírez, J.M.; Bernal-Pérez, M. Protective effects of melatonin in reducing oxidative stress and in preserving the fluidity of biological membranes: A review. *J. Pineal Res.* **2014**, *56*, 225–237. [[CrossRef](#)] [[PubMed](#)]
129. Yu, H.; Dickson, E.J.; Jung, S.-R.; Koh, D.-S.; Hille, B. High membrane permeability for melatonin. *J. Gen. Physiol.* **2016**, *147*, 63–76. [[CrossRef](#)] [[PubMed](#)]
130. Bubenik, G.A.; Purtill, R.A.; Brown, G.M.; Grotta, L.J. Melatonin in the retina and the harderian gland. Ontogeny, diurnal variations and melatonin treatment. *Exp. Eye Res.* **1978**, *27*, 323–333. [[CrossRef](#)]
131. Escames, G.; López, L.C.; Tapias, V.; Utrilla, P.; Reiter, R.J.; Hitos, A.B.; León, J.; Rodríguez, M.I.; Acuña-Castroviejo, D. Melatonin counteracts inducible mitochondrial nitric oxide synthase-dependent mitochondrial dysfunction in skeletal muscle of septic mice. *J. Pineal Res.* **2006**, *40*, 71–78. [[CrossRef](#)] [[PubMed](#)]
132. Mayo, J.C.; Sainz, R.M.; González-Menéndez, P.; Hevia, D.; Cernuda-Cernuda, R. Melatonin transport into mitochondria. *Cell. Mol. Life Sci.* **2017**, *74*, 3927–3940. [[CrossRef](#)] [[PubMed](#)]
133. Reiter, R.J.; Rosales-Corral, S.; Tan, D.X.; Jou, M.J.; Galano, A.; Xu, B. Melatonin as a mitochondria-targeted antioxidant: One of evolution's best ideas. *Cell. Mol. Life Sci.* **2017**, *74*, 3863–3881. [[CrossRef](#)] [[PubMed](#)]
134. Hevia, D.; Sainz, R.M.; Blanco, D.; Quirós, I.; Tan, D.X.; Rodríguez, C.; Mayo, J.C. Melatonin uptake in prostate cancer cells: Intracellular transport versus simple passive diffusion. *J. Pineal Res.* **2008**, *45*, 247–257. [[CrossRef](#)] [[PubMed](#)]
135. Hevia, D.; Rodríguez-García, A.; Alonso-Gervós, M.; Quirós-González, I.; Cimadevilla, H.M.; Gómez-Cordovés, C.; Sainz, R.M.; Mayo, J.C.; Hevia, D.; Rodríguez-García, A.; et al. Cell Volume and Geometric Parameters Determination in Living Cells Using Confocal Microscopy and 3D Reconstruction. Available online: http://www.nature.com/protocolexchange/protocols/2264%0Ahttp://www.nature.com/protocolexchange/protocols/2264#/anticipated_results (accessed on 13 June 2017).
136. Hevia, D.; González-Menéndez, P.; Quiros-González, I.; Miar, A.; Rodríguez-García, A.; Tan, D.X.; Reiter, R.J.; Mayo, J.C.; Sainz, R.M. Melatonin uptake through glucose transporters: A new target for melatonin inhibition of cancer. *J. Pineal Res.* **2015**, *58*, 234–250. [[CrossRef](#)] [[PubMed](#)]

137. Deng, D.; Xu, C.; Sun, P.; Wu, J.; Yan, C.; Hu, M.; Yan, N. Crystal structure of the human glucose transporter GLUT1. *Nature* **2014**, *510*, 121–125. [[CrossRef](#)] [[PubMed](#)]
138. Rumsey, S.C.; Kwon, O.; Xu, G.W.; Burant, C.F.; Simpson, I.; Levine, M. Glucose transporter isoforms GLUT1 and GLUT3 transport dehydroascorbic acid. *J. Biol. Chem.* **1997**, *272*, 18982–18989. [[CrossRef](#)] [[PubMed](#)]
139. Montel-Hagen, A.; Kinet, S.; Manel, N.; Mongellaz, C.; Prohaska, R.; Battini, J.L.; Delaunay, J.; Sitbon, M.; Taylor, N. Erythrocyte Glut1 triggers dehydroascorbic acid uptake in mammals unable to synthesize vitamin C. *Cell* **2008**, *132*, 1039–1048. [[CrossRef](#)] [[PubMed](#)]
140. Huo, X.; Wang, C.; Yu, Z.; Peng, Y.; Wang, S.; Feng, S.; Zhang, S.; Tian, X.; Sun, C.; Liu, K.; et al. Human transporters, PEPT1/2, facilitate melatonin transportation into mitochondria of cancer cells: An implication of the therapeutic potential. *J. Pineal Res.* **2017**, *62*, e12390. [[CrossRef](#)] [[PubMed](#)]
141. Miyamoto, K.I.; Shiraga, T.; Morita, K.; Yamamoto, H.; Haga, H.; Taketani, Y.; Tamai, I.; Sai, Y.; Tsuji, A.; Takeda, E. Sequence, tissue distribution and developmental changes in rat intestinal oligopeptide transporter. *Biochim. Biophys. Acta Gene Struct. Exp.* **1996**, *1305*, 34–38. [[CrossRef](#)]
142. Bailey, M.J.; Coon, S.L.; Carter, D.A.; Humphries, A.; Kim, J.S.; Shi, Q.; Gaildrat, P.; Morin, F.; Ganguly, S.; Hogenesch, J.B.; et al. Night/day changes in pineal expression of >600 genes: Central role of adrenergic/cAMP signaling. *J. Biol. Chem.* **2009**, *284*, 7606–7622. [[CrossRef](#)] [[PubMed](#)]
143. Milcou, I.; Nanu, L.; Marcean, R. Existence of a hypoglycemic pineal hormone synergistic with insulin. *Ann. Endocrinol. (Paris)* **1957**, *18*, 612–620. [[PubMed](#)]
144. Röjdmarm, S.; Wetterberg, L. Short-term fasting inhibits the nocturnal melatonin secretion in healthy man. *Clin. Endocrinol. (Oxf.)* **1989**, *30*, 451–457. [[CrossRef](#)] [[PubMed](#)]
145. Röjdmarm, S. Influence of short-term fasting on the pituitary-testicular axis in normal men. *Horm. Res.* **1987**, *25*, 140–146. [[PubMed](#)]
146. Röjdmarm, S.; Rössner, S.; Wetterberg, L. Effect of short-term fasting on nocturnal melatonin secretion in obesity. *Metabolism* **1992**, *41*, 1106–1109. [[CrossRef](#)]
147. Welker, H.A.; Vollrath, L. The effects of a number of short-term exogenous stimuli on pineal serotonin-*N*-acetyltransferase activity in rats. *J. Neural Transm.* **1984**, *59*, 69–80. [[CrossRef](#)] [[PubMed](#)]
148. Peschke, E.; Wolgast, S.; Bazwinsky, I.; Pönicke, K.; Mühlbauer, E. Increased melatonin synthesis in pineal glands of rats in streptozotocin induced type 1 diabetes. *J. Pineal Res.* **2008**, *45*, 439–448. [[CrossRef](#)] [[PubMed](#)]
149. Do Carmo Buonfiglio, C.; Peliciari-Garcia, R.A.; do Amaral, F.G.; Peres, R.; Araujo Nogueira, T.C.; Afeche, S.C.; Cipolla-Neto, J. Early-stage retinal melatonin synthesis impairment in streptozotocin-induced diabetic wistar rats. *Investig. Ophthalmol. Vis. Sci.* **2011**, *52*, 7416–7422. [[CrossRef](#)] [[PubMed](#)]
150. Peschke, E.; Frese, T.; Chankiewicz, E.; Peschke, D.; Preiss, U.; Schneyer, U.; Spessert, R.; Mühlbauer, E. Diabetic Goto Kakizaki rats as well as type 2 diabetic patients show a decreased diurnal serum melatonin level and an increased pancreatic melatonin-receptor status. *J. Pineal Res.* **2006**, *40*, 135–143. [[CrossRef](#)] [[PubMed](#)]
151. Frese, T.; Bach, A.G.; Mühlbauer, E.; Pönicke, K.; Brömme, H.J.; Welp, A.; Peschke, E. Pineal melatonin synthesis is decreased in type 2 diabetic Goto-Kakizaki rats. *Life Sci.* **2009**, *85*, 526–533. [[CrossRef](#)] [[PubMed](#)]
152. Corbalán-Tutau, D.; Madrid, J.A.; Nicolás, F.; Garaulet, M. Daily profile in two circadian markers “melatonin and cortisol” and associations with metabolic syndrome components. *Physiol. Behav.* **2014**, *123*, 231–235. [[CrossRef](#)] [[PubMed](#)]
153. Cano, P.; Jiménez-Ortega, V.; Larrad, Á.; Toso, C.F.R.; Cardinali, D.P.; Esquifino, A.I. Effect of a high-fat diet on 24-h pattern of circulating levels of prolactin, luteinizing hormone, testosterone, corticosterone, thyroid-stimulating hormone and glucose, and pineal melatonin content, in rats. *Endocrine* **2008**, *33*, 118–125. [[CrossRef](#)] [[PubMed](#)]
154. Bizot-Espiard, J.G.; Double, A.; Guardiola-Lemaitre, B.; Delagrangé, P.; Ktorza, A.; Penicaud, L. Diurnal rhythms in plasma glucose, insulin, growth hormone and melatonin levels in fasted and hyperglycaemic rats. *Diabetes Metab.* **1998**, *24*, 235–240. [[PubMed](#)]
155. Peschke, E.; Bähr, I.; Mühlbauer, E. Melatonin and pancreatic islets: Interrelationships between melatonin, insulin and glucagon. *Int. J. Mol. Sci.* **2013**, *14*, 6981–7015. [[CrossRef](#)] [[PubMed](#)]
156. Peschke, E. Melatonin, endocrine pancreas and diabetes. *J. Pineal Res.* **2008**, *44*, 26–40. [[CrossRef](#)] [[PubMed](#)]
157. Lardone, P.J.; Sanchez, N.; Guerrero, J.M. Melatonin and Glucose Metabolism: Clinical Relevance. *Curr. Pharm. Des.* **2014**, *20*, 4841–4853. [[PubMed](#)]

158. Qian, J.; Scheer, F.A.J.L. Circadian System and Glucose Metabolism: Implications for Physiology and Disease. *Trends Endocrinol. Metab.* **2016**, *27*, 282–293. [[CrossRef](#)] [[PubMed](#)]
159. Bailey, C.J.; Atkins, T.W.; Matty, A.J. Melatonin inhibition of insulin secretion in the rat and mouse. *Horm. Res. Paediatr.* **1974**, *5*, 21–28. [[CrossRef](#)] [[PubMed](#)]
160. Gorray, K.; Quay, W.; Ewart, R.B. Effects of Pinealectomy and Pineal Incubation Medium and Sonicates on Insulin Release by Isolated Pancreatic Islets In Vitro. *Horm. Metab. Res.* **1979**, *11*, 432–436. [[CrossRef](#)] [[PubMed](#)]
161. Diaz, B.; Blázquez, E. Effect of pinealectomy on plasma glucose, insulin and glucagon levels in the rat. *Horm. Metab. Res.* **1986**, *18*, 225–229. [[CrossRef](#)] [[PubMed](#)]
162. Lima, F.B.; Machado, U.F.; Bartol, I.; Seraphim, P.M.; Sumida, D.H.; Moraes, S.M.; Hell, N.S.; Okamoto, M.M.; Saad, M.J.; Carvalho, C.R.; et al. Pinealectomy causes glucose intolerance and decreases adipose cell responsiveness to insulin in rats. *Am. J. Physiol.* **1998**, *275*, E934–E941. [[CrossRef](#)] [[PubMed](#)]
163. Champney, T.H.; Brainard, G.C.; Richardson, B.A.; Reiter, R.J. Experimentally-induced diabetes reduces nocturnal pineal melatonin content in the Syrian hamster. *Comp. Biochem. Physiol. Part A Physiol.* **1983**, *76*, 199–201. [[CrossRef](#)]
164. Nagorny, C.L.F.; Sathanoori, R.; Voss, U.; Mulder, H.; Wierup, N. Distribution of melatonin receptors in murine pancreatic islets. *J. Pineal Res.* **2011**, *50*, 412–417. [[CrossRef](#)] [[PubMed](#)]
165. Mühlbauer, E.; Albrecht, E.; Hofmann, K.; Bazwinsky-Wutschke, I.; Peschke, E. Melatonin inhibits insulin secretion in rat insulinoma β -cells (INS-1) heterologously expressing the human melatonin receptor isoform MT2. *J. Pineal Res.* **2011**, *51*, 361–372. [[CrossRef](#)] [[PubMed](#)]
166. Bazwinsky-Wutschke, I.; Mühlbauer, E.; Albrecht, E.; Peschke, E. Calcium-signaling components in rat insulinoma β -cells (INS-1) and pancreatic islets are differentially influenced by melatonin. *J. Pineal Res.* **2014**, *56*, 439–449. [[CrossRef](#)] [[PubMed](#)]
167. She, M.; Laudon, M.; Yin, W. Melatonin receptors in diabetes: A potential new therapeutical target? *Eur. J. Pharmacol.* **2015**, *744*, 220–223. [[CrossRef](#)] [[PubMed](#)]
168. Zibolka, J.; Mühlbauer, E.; Peschke, E. Melatonin influences somatostatin secretion from human pancreatic δ -cells via MT1 and MT2 receptors. *J. Pineal Res.* **2015**, *58*, 198–209. [[CrossRef](#)] [[PubMed](#)]
169. Stumpf, I.; Mühlbauer, E.; Peschke, E. Involvement of the cGMP pathway in mediating the insulin-inhibitory effect of melatonin in pancreatic β -cells. *J. Pineal Res.* **2008**, *45*, 318–327. [[CrossRef](#)] [[PubMed](#)]
170. Peschke, E.; Bach, A.G.; Mühlbauer, E. Parallel signaling pathways of melatonin in the pancreatic β -cell. *J. Pineal Res.* **2006**, *40*, 184–191. [[CrossRef](#)] [[PubMed](#)]
171. Li, Y.; Wu, H.; Liu, N.; Cao, X.; Yang, Z.; Lu, B.; Hu, R.; Wang, X.; Wang, J.; Li, Y.; et al. Melatonin exerts an inhibitory effect on insulin gene transcription via MTNR1B and the downstream Raf-1/ERK signaling pathway. *Int. J. Mol. Med.* **2018**, *41*, 955–961. [[CrossRef](#)] [[PubMed](#)]
172. Mssig, K.; Staiger, H.; MacHicao, F.; Hring, H.U.; Fritsche, A. Genetic variants in MTNR1B affecting insulin secretion. *Ann. Med.* **2010**, *42*, 387–393. [[CrossRef](#)] [[PubMed](#)]
173. Tam, C.H.T.; Ho, J.S.K.; Wang, Y.; Lee, H.M.; Lam, V.K.L.; Germer, S.; Martin, M.; So, W.Y.; Ma, R.C.W.; Chan, J.C.N.; et al. Common polymorphisms in MTNR1B, G6PC2 and GCK are associated with increased fasting plasma glucose and impaired beta-cell function in Chinese subjects. *PLoS ONE* **2010**, *5*, e11428. [[CrossRef](#)] [[PubMed](#)]
174. Sparsø, T.; Bonnefond, A.; Andersson, E.; Bouatia-Naji, N.; Holmkvist, J.; Wegner, L.; Grarup, N.; Gjesing, A.P.; Banasik, K.; Cavalcanti-Proença, C.; et al. G-allele of intronic rs10830963 in MTNR1B confers increased risk of impaired fasting glycemia and type 2 diabetes through an impaired glucose-stimulated insulin release: Studies involving 19,605 Europeans. *Diabetes* **2009**, *58*, 1450–1456. [[CrossRef](#)] [[PubMed](#)]
175. Tuomi, T.; Nagorny, C.L.F.F.; Singh, P.; Bennet, H.; Yu, Q.; Alenkvist, I.; Isomaa, B.; Östman, B.; Söderström, J.; Pesonen, A.K.; et al. Increased Melatonin Signaling Is a Risk Factor for Type 2 Diabetes. *Cell Metab.* **2016**, *23*, 1067–1077. [[CrossRef](#)] [[PubMed](#)]
176. Mulder, H. Melatonin signalling and type 2 diabetes risk: Too little, too much or just right? *Diabetologia* **2017**, *60*, 826–829. [[CrossRef](#)] [[PubMed](#)]
177. Simões, D.; Riva, P.; Peliciari-Garcia, R.A.; Cruzat, V.F.; Graciano, M.F.; Munhoz, A.C.; Taneda, M.; Cipolla-Neto, J.; Carpinelli, A.R. Melatonin modifies basal and stimulated insulin secretion via NADPH oxidase. *J. Endocrinol.* **2016**, *231*, 235–244. [[CrossRef](#)] [[PubMed](#)]

178. Park, J.H.; Shim, H.M.; Na, A.Y.; Bae, K.C.; Bae, J.H.; Im, S.S.; Cho, H.C.; Song, D.K. Melatonin prevents pancreatic β -cell loss due to glucotoxicity: The relationship between oxidative stress and endoplasmic reticulum stress. *J. Pineal Res.* **2014**, *56*, 143–153. [[CrossRef](#)] [[PubMed](#)]
179. Costes, S.; Boss, M.; Thomas, A.P.; Matveyenko, A.V. Activation of Melatonin Signaling Promotes β -Cell Survival and Function. *Mol. Endocrinol.* **2015**, *29*, 682–692. [[CrossRef](#)] [[PubMed](#)]
180. Li, T.; Ni, L.; Zhao, Z.; Liu, X.; Lai, Z.; Di, X.; Xie, Z.; Song, X.; Wang, X.; Zhang, R.; et al. Melatonin attenuates smoking-induced hyperglycemia via preserving insulin secretion and hepatic glycogen synthesis in rats. *J. Pineal Res.* **2018**, *64*, e12475. [[CrossRef](#)] [[PubMed](#)]
181. Mayo, J.C.; Hevia, D.; Quiros-Gonzalez, I.; Rodriguez-Garcia, A.; Gonzalez-Menendez, P.; Cepas, V.; Gonzalez-Pola, I.; Sainz, R.M. IGFBP3 and MAPK/ERK signaling mediates melatonin-induced antitumor activity in prostate cancer. *J. Pineal Res.* **2017**, *62*, e12373. [[CrossRef](#)] [[PubMed](#)]
182. Sharma, S.; Singh, H.; Ahmad, N.; Mishra, P.; Tiwari, A. The role of melatonin in diabetes: Therapeutic implications. *Arch. Endocrinol. Metab.* **2015**, *59*, 391–399. [[CrossRef](#)] [[PubMed](#)]
183. Lo, C.C.; Lin, S.H.; Chang, J.S.; Chien, Y.W. Effects of melatonin on glucose homeostasis, antioxidant ability, and adipokine secretion in ICR mice with NA/STZ-induced hyperglycemia. *Nutrients* **2017**, *9*, 1187. [[CrossRef](#)] [[PubMed](#)]
184. Rubio-Sastre, P.; Scheer, F.A.J.L.; Gómez-Abellán, P.; Madrid, J.A.; Garaulet, M. Acute Melatonin Administration in Humans Impairs Glucose Tolerance in Both the Morning and Evening. *Sleep* **2014**, *37*, 1715–1719. [[CrossRef](#)] [[PubMed](#)]
185. Rigas, A.N.; Bittles, A.H.; Hadden, D.R.; Montgomery, D.A. Circadian variation of glucose, insulin, and free fatty acids during long-term use of oral hypoglycaemic agents in diabetes mellitus. *Br. Med. J.* **1968**, *4*, 25–28. [[CrossRef](#)] [[PubMed](#)]
186. Gagliardino, J.J.; Hernández, R.E. Circadian variation of the serum glucose and immunoreactive insulin levels. *Endocrinology* **1971**, *88*, 1532–1534. [[CrossRef](#)] [[PubMed](#)]
187. Boden, G.; Ruiz, J.; Urbain, J.L.; Chen, X. Evidence for a circadian rhythm of insulin secretion. *Am. J. Physiol. Metab.* **1996**, *271*, E246–E252. [[CrossRef](#)] [[PubMed](#)]
188. Zibolka, J.; Bazwinsky-Wutschke, I.; Mühlbauer, E.; Peschke, E. Distribution and density of melatonin receptors in human main pancreatic islet cell types. *J. Pineal Res.* **2018**, *65*, e12480. [[CrossRef](#)] [[PubMed](#)]
189. Bouatia-Naji, N.; Bonnefond, A.; Cavalcanti-Proença, C.; Sparsø, T.; Holmkvist, J.; Marchand, M.; Delplanque, J.; Lobbens, S.; Rocheleau, G.; Durand, E.; et al. A variant near MTNR1B is associated with increased fasting plasma glucose levels and type 2 diabetes risk. *Nat. Genet.* **2009**, *41*, 89–94. [[CrossRef](#)] [[PubMed](#)]
190. Tilden, A.; McGann, L.; Schwartz, J.; Bowe, A.; Salazar, C. Effect of melatonin on hemolymph glucose and lactate levels in the fiddler crab *Uca pugilator*. *J. Exp. Zool.* **2001**, *290*, 379–383. [[CrossRef](#)] [[PubMed](#)]
191. Köhidai, L.; Vakkuri, O.; Keresztesi, M.; Leppäluoto, J.; Csaba, G. Melatonin in the unicellular *Tetrahymena pyriformis*: Effects of different lighting conditions. *Cell Biochem. Funct.* **2002**, *20*, 269–272. [[CrossRef](#)] [[PubMed](#)]

