Cell. Mol. Life Sci.
DOI 10.1007/s00018-014-1579-2

Cellular and Molecular Life Sciences

REVIEW

Extrapineal melatonin: sources, regulation, and potential functions

Darío Acuña-Castroviejo · Germaine Escames · Carmen Venegas · María E. Díaz-Casado · Elena Lima-Cabello · Luis C. López · Sergio Rosales-Corral · Dun-Xian Tan · Russel J. Reiter

Received: 11 November 2013 / Revised: 26 January 2014 / Accepted: 27 January 2014

Abstract Endogenous melatonin is synthesized from tryptophan via 5-hydroxytryptamine. It is considered an indoleamine from a biochemical point of view because the melatonin molecule contains a substituted indolic ring with an amino group. The circadian production of melatonin by the pineal gland explains its chronobiotic influence on organismal activity, including the endocrine and non-endocrine rhythms. Other functions of melatonin, including its antioxidant and anti-inflammatory properties, its genomic effects, and its capacity to modulate mitochondrial homeostasis, are linked to the redox status of cells and tissues. With the aid of specific melatonin antibodies, the presence of melatonin has been detected in multiple extrapineal tissues including the brain, retina, lens, cochlea, Harderian gland, airway epithelium, skin, gastrointestinal tract, liver, kidney, thyroid, pancreas, thymus, spleen, immune system cells, carotid body, reproductive tract, and endothelial cells. In most of these tissues, the melatonin-synthesizing enzymes have been identified. Melatonin is present in essentially all biological fluids including cerebrospinal fluid, saliva, bile, synovial fluid, amniotic fluid, and breast milk. In several of these fluids, melatonin concentrations exceed those in the blood. The importance of the continual availability of melatonin at the cellular level is important for its physiological regulation of cell homeostasis, and may be relevant to its therapeutic applications. Because of this, it is essential to compile information related to its peripheral production and regulation of this ubiquitously acting indoleamine. Thus, this review emphasizes the presence of melatonin in extrapineal organs, tissues, and fluids of mammals including humans.

Keywords Melatonin receptors · Oxidative stress · Free radicals · Mitochondria · Cytoprotection · Homeostasis

D. Acuña-Castroviejo (✉) · G. Escames · C. Venegas · M. E. Díaz-Casado · E. Lima-Cabello · L. C. López
Instituto de Biotecnología, Centro de Investigación Biomédica, Parque Tecnológico de Ciencias de la Salud, Universidad de Granada, Avda. del Conocimiento s/n, Armilla, 18100 Granada, Spain
e-mail: dacuna@ugr.es

D. Acuña-Castroviejo · G. Escames · C. Venegas · M. E. Díaz-Casado · E. Lima-Cabello · L. C. López
Departamento de Fisiología, Facultad de Medicina, Universidad de Granada, Granada, Spain

D. Acuña-Castroviejo
Unidad de Gestión Clínica de Laboratorios, Hospital Universitario San Cecilio, Granada, Spain

S. Rosales-Corral · D.-X. Tan · R. J. Reiter
Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, USA

Introduction

Brief historic overview

For a number of years after the discovery of melatonin (*N*-acetyl-5-methoxytryptamine) by Lerner in 1958 [1], melatonin was considered to be exclusively produced in the pineal gland related to the control of the circadian and circannual rhythms including seasonal reproduction. Following the identification of melatonin synthesis in the pineal gland, the presence of melatonin-related enzymes was subsequently uncovered in the retina and cerebellum [2, 3]. This was followed by its identification in many other peripheral tissues and organs. The existence of the key enzymes of melatonin synthesis in many tissues [4], the presence of melatonin receptors in numerous tissues,

Published online: 20 February 2014

and the discovery of the widespread antioxidant and anti-inflammatory properties of melatonin, caused scientists to reclassify melatonin from being exclusively a hormone to that of a multi-tasking molecule [5–7].

The initial studies pointing to the existence of non-pineal sources of melatonin were related to the retina [2], with the identification of hydroxindole-*O*-methyltransferase (HIOMT), now called *N*-acetylserotonin-*O*-methyltransferase (ASMT), in this tissue. Soon thereafter, Vlahakes and Wurtman [8] showed the presence of ASMT in the rat Harderian gland. The presence of melatonin in the retina, Harder's gland, and cerebellum of pinealectomized rats was also found [3, 9]. These data, including the detection of aralkylamine-*N*-acetyltransferase (AANAT) and ASMT, suggested the ability of these tissues to synthesize melatonin independently of the pineal. Also, in 1975 Raikhlin et al. [10] immunocytochemically identified the presence of melatonin in enterochromaffin cells of the intestinal mucosa. They estimated a melatonin content in the gastrointestinal tract roughly 400 times greater than in the pineal gland, and 10–100 times higher than in the plasma. Gastrointestinal melatonin amounts were initially found not to be controlled by the photoperiod, but by the rhythms in food intake [10, 11]. At about the same time, melatonin was identified in the plasma and urine of pinealectomized rats [12]. In this study, the daily urinary excretion of melatonin decreased by 80 % after pinealectomy, and it did not exhibit a circadian variation. These scientific reports initiated the age of "extrapineal melatonin". More recently, however, other authors showed a nocturnal melatonin peak in chicken duodenum, which could not be explained by food intake in this diurnal animal [13]. With the use of more specific molecular biology techniques including PCR, the expression of the mRNAs for AANAT and ASMT have now been detected in a variety of tissues and organs including thymus, spleen, heart, skeletal muscle, liver, stomach, gut, placenta, testes, ovaries, cerebral cortex, and striatum [4, 14]. Because these tissues express both AANAT and ASMT, they likely synthesize melatonin from serotonin.

Except for some tissues such as the retina, the common absence of day:night variations in extrapineal melatonin synthesis suggests signaling pathway(s) other than the photoperiod regulating the production of this indoleamine [15]. Moreover, many extrapineal tissues have higher concentrations of melatonin than plasma throughout the 24 h, and the intracellular melatonin from these tissues is not generally released into the circulation [15]. These observations suggest that extrapineal melatonin acts locally possibly protecting cells from oxidative and inflammatory damage. Since melatonin is a highly effective antioxidant [16–19], the lack of a circadian fluctuation in extrapineal tissues also helps to explain one melatonin inconsistency. Thus, the nocturnal melatonin peak provides important photoperiodic information to every cell to ensure proper chronobiotic synchronization [20]. However, the production of reactive oxygen (ROS) and reactive nitrogen (RNS) species takes place mainly during the phases of metabolic, motor, and neural activity, when oxygen consumption is maximal; these activities do not necessarily occur at night, e.g., in diurnal animals. Phylogenetically, the potential of a cell to increase melatonin output as required probably constitutes an adaptive mechanism involved in the survival of cells and also tissues. Thus, melatonin may be produced in non-pineal cells as a protective mechanism against the metabolites of aerobic metabolism.

Melatonin receptors

The effects of melatonin in the body are mediated by two main pathways that include receptor-mediated and non-receptor mediated effects. Melatonin receptors include membrane and nuclear binding sites. Among the former, two types of melatonin receptors, MT1 and MT2, which belong to the family of guanine nucleotide-binding regulatory protein (G protein)-coupled receptors, have been fully characterized in cell membranes [21–23]. Activation of MT1 receptors by melatonin leads to different responses, most of them depending on the inhibition of cAMP, through G proteins, and via increases in cytosolic calcium through Gq_{11} [24]. Binding of melatonin to MT2 receptors leads to cAMP and cGMP inhibition [25]. Both MT1 and MT2 receptors can couple also to PLC-dependent pathways [26]. MT1 melatonin receptors are related to reproductive and metabolic functions, and vasoconstriction, whereas MT2 receptors are involved in the control of circadian rhythms and dopamine release in the retina, and vasodilatation, among other actions [23]. A third melatonin binding site, formerly MT3, has been identified as a quinone reductase 2, and it is related to the xenobiotic metabolism of the cell [27]; this enzyme, however, does not fullfit the criteria for a melatonin receptor. Melatonin binding sites in the nucleus have been also reported [28]; they were later identified as ROR orphan receptors that include three subtypes (α, β, γ) and four splicing variants of the α-subtype [29]. Only the splice variant c of the RORα subtype and the RORγ subtype are related to melatonin actions in the nucleus. Some of the genomic effects of melatonin are related to its interaction with these type of receptors [30]. Melatonin also interacts with cytosolic proteins including calmodulin and calreticulin, which are involved in the cytoskeleton regulation and control of nuclear receptors, respectively [31, 32].

Melatonin synthesis pathways

Melatonin synthesis occurs in the pineal gland and extrapineal tissues. In all tissues, melatonin is produced from

tryptophan via serotonin. In the pineal, its production is controlled by a circadian signal from the suprachiasmatic nucleus (SCN), which in turn is associated to the photoperiod [33]. Signals from the SCN reach the pinealocyte through a complex pathway involving the paraventricular nucleus and the upper thoracic intermediolateral cell column. From here, fibers project to the superior cervical ganglia that send postganglionic sympathetic noradrenergic fibers to the pineal gland. During the night, this pathway is active and releases norepinephrine from the sympathetic fibers which, in turn, activate adenylate cyclase via β_1-adrenergic receptors in the pinealocyte [34]. Other modulators of the pineal melatonin synthesis included vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating polypeptide (PACAP) [35], and α1-adrenoceptors. Regarding the latter, a recent report showed that α1-adrenoceptors stimulate melatonin synthesis, but after their heteromerization with dopamine D4 receptors, melatonin production is blocked [36]. Thus, the dopamine D4 receptor can inhibit melatonin release by forming circadian-controlled complexes with adrenergic receptors, preventing the indoleamine modulation of circadian cycles of the body. The rapid increase in cAMP-dependent AANAT rapidly converts serotonin to *N*-acetylserotonin [37], which is metabolized to melatonin by ASMT. Although preliminary studies showed that AANAT was the rate-limiting enzyme in the melatonin synthesis, recent data suggest that this role at least on some occasions corresponds to ASMT [38]. Once formed, melatonin is not stored in the pineal gland but is rapidly secreted into the spinal fluid and into the blood, where it appears in free and albumin-bound fractions [39]. Except for some tissues as the retina, extrapineal melatonin synthesis occurs through the same metabolic pathway as that described in the pineal gland, although it is not regulated by the photoperiod.

Significant differences in the regulation of melatonin synthesis exist among animal species [40]. In contrast to that in lower mammals, the human pineal constitutively expresses AANAT and ASMT mRNA and protein, suggesting a major role of their post-transcriptional regulation in the rhythmic melatonin synthesis [41]. These findings led to suggest that the activity of these enzymes is regulated by protein–protein interaction [42]. In this model, the protein complex, named "melatoninosome" by the authors, contains the phosphorylated active form of AANAT (p31T-AANAT), ASMT and 14-3-3 protein. The presence of S-antigen (arrestin) closely to this complex suggest its role in melatoninosome assembly. Thus, the presence of these enzymes by themselves does not mean that melatonin is being synthesized. Although the occurrence of mRNAs of AANAT and ASMT has been proven in most of the tissues tested, including humans, it is not known whether these enzymes form complexes to synthesize melatonin. Also, the existence of unspecific/less specific *N*-acetyltransferases and/or *O*-methyltransferases isoforms with different activities [43] may also account for differences in the regulation of extrapineal melatonin synthesis. When known, features of the extrapineal regulation of melatonin production are included in each section of this review.

Melatonin actions: low vs. high concentrations

Among other actions, this review emphasizes that circulating melatonin coming from the pineal plus melatonin produced by extrapineal tissues, collaborate in the maintenance of homeostasis, protecting the cell against oxidative stress. Circulating melatonin reaches concentrations up to 0.5 nM in mammalian blood, while extrapineal concentrations vary depending on the tissue, but they can reach micromolar levels. The importance of pineal vs. extrapineal melatonin is that both counteract free radical damage, although through different mechanism's of action, i.e., antioxidant and free radical scavenger activities, respectively. Circulating melatonin binds and activates MT1/MT2 membrane receptors that inhibit adenylate cyclase via G_i protein, reducing cAMP content. Consequently, there is an inhibition of protein kinase A and cAMP response element binding protein/activation transcription factor. Through this pathway, melatonin can modulate antioxidant gene transcription [44]. Melatonin activation of MT1/MT2 receptors also triggers other signaling pathways including phospholipase C/protein kinase (PLC/PKC), mitogen-activated protein kinases (MAPK), and extracellular-signal-regulated kinase (ERK1/2) pathways. These pathways are involved in multiple regulatory processes, from chronobiological regulation to cell responses to a variety of injuries including apoptosis [45–48]. The effects of melatonin depend on its dose and the presence of either MT1, MT2, or both receptors in the cell. Studies with variable native MT1 and MT2 receptors showed that nanomolar, but not micromolar melatonin concentrations decreased ERK activity in the presence of MT1/MT2 receptors. Deficiencies in MT1 and MT2 yield melatonin activation of ERK. Thus, the relative MT1/MT2 distribution in different brain areas and in different tissues and the melatonin levels may drive its signaling pathways [49, 50].

Melatonin inhibits the calcium-calmodulin complex in the cytosol [51], which may influence the RORα melatonin receptor, influencing gene transcription of antioxidant enzymes [52]. High concentrations of melatonin have been used in multiple experimental conditions in vivo, and consistently exhibit antioxidant activity [53, 54]. Two main mechanisms are involved in these actions of melatonin: (1) a direct free radical scavenger activity [55] that generates a series of intermediates during its interaction with ROS and nitrogen species, all of them which are also free radical

scavengers. This was defined as the free radical scavenging cascade reaction of the melatonin family; (2) an indirect antioxidant activity that results of an increased expression and activity of endogenous antioxidant enzymes. This mechanism, which was initially related to an effect of melatonin through the nuclear RORα receptors [56], may also depend on the activation of melatonin membrane receptors, because it was shown recently that high doses of melatonin increased the mRNA expression and protein content of MT1/MT2 receptors in rat liver [57]. This wide spectrum of melatonin actions, and the extra-and intracellular production of melatonin, support the endocrine, paracrine and autocrine actions of this indoleamine to protect the cells from free radical damage. Moreover, the existence of some mechanisms controlling the intracellular distribution of melatonin allowing it to reach its targets including the nucleus and mitochondria, may explain the requirements of large doses of melatonin to counteract hyperoxidative status in multiple models of diseases elsewhere reported. Some of these situations are considered in this review.

Melatonin duality: antioxidant/pro-oxidant effects

Although most of the studies report antioxidant actions of melatonin, in some conditions the indoleamine may induce pro-oxidant activity. The latter mainly occur in severely injured cells and in cancer cells. The dual effects of melatonin on intracellular redox status include an antioxidant/antiproliferative response or a pro-oxidant/cytotoxic effect [58]. Among other mechanism, the intracellular pro-oxidant activity of melatonin in cancer cells depends on the NF-κB activation, which in turns drives the synthesis of melatonin, transforming melatonin in a pro-apoptotic molecule [59, 60]. The transient ROS production induced by melatonin in different cancer types may involve changes in the glutathione/glutathione disulfide ratio. When the ratio decreases, melatonin drives the cell fate to apoptosis, whereas melatonin has the opposite effect when the glutathione/glutathione disulfide ratio increases. These and other pro-apoptotic pathways are activated by melatonin, and they have been recently revised [61]. Thus, whereas the antioxidant/pro-oxidant balance of melatonin effects may depend on the redox environment of the cell, its pro-oxidant activity may explain its oncostatic actions [62]. The latter may serve to control the fate of the cell, promoting in last instance a proapoptotic response to eliminate cells that, otherwise, are potentially dangerous for the organism.

The melatonin-dependent redox status of the cell may have additional consequences. It is known that peroxiredoxins, a type of thiol peroxidases that degrade hydroperoxides to water, are involved in the redox sensing of the cell [63]. In eukaryotic cells, circadian timekeeping involves both transcriptional and non-transcriptional mechanisms, the latter involving oxidation–reduction cycles of peroxiredoxin [64, 65]. Melatonin affects the expression of peroxiredoxin and may influence its oxidation–reduction cycles modifying the redox status of the cell [66]. Thus, melatonin does not only may modify the chronobiology of the normal cell through its interaction with the peroxiredoxin pathway, but it may have importance in the cytotoxic effect of the indoleamine in cancer cells.

Sources of extrapineal melatonin

With the use of highly sensitive antibodies against melatonin and molecular biology techniques, melatonin was also identified in extrapineal tissues including the retina, Harderian gland, gut mucosa, cerebellum, airway epithelium, liver, kidney, adrenals, thymus, thyroid, pancreas, ovary, carotid body, placenta, endometrium, mast cells, natural killer cells, eosinophilic leukocytes, platelets, and endothelial cells [14, 67]; indeed, melatonin has been detected in all organs which have been examined, where often high concentrations of this indoleamine have been measured. The physico-chemical properties of melatonin support its amphiphilic nature, which allows its passage across all morphophysiological barriers including cell membranes and the blood–brain barrier [15, 68, 69]. Although extrapineal melatonin generally does not gain access to the systemic circulation, there are several exceptions to this assumption, mainly in lower vertebrates such as chicks but also in rats. Here, oral overload of tryptophan to rats and chicks induced a dose-dependent increase of serum melatonin [70]. The tryptophan-induced melatonin production was related to the enterochromaffin cells of the gastrointestinal tract, which produce melatonin for regulatory purposes at duodenal level [71]. In other conditions, however, peripherally generated melatonin seems to do not enter the circulation. An answer to this question could be the existence of a molecule (protein?) that binds melatonin, acting as a "buffering system" to maintain melatonin concentrations in the cell and prevent its discharge into capillaries. Table 1 shows the levels of melatonin in biological fluids.

While it is known that some mammalian hormones are produced by multiple cell types [72, 73], none of these molecules appear to have the broad spectrum of sources as does melatonin. Thus, melatonin has a unique position among the identified signaling molecules. This reflects its essential role in the homeostatic control and probably also in cell survival. Herein, we review information related to the regulation and actions of melatonin in organs and tissues where strong evidence of its synthesis exists. While other organs including kidney and adrenal gland seem to

Table 1 Evidences of extrapineal melatonin synthesis in mammals

Tissue	Species	Experimental condition	Enzyme expression	Melatonin detection	Melatonin levels	Enzyme activity	Precursors used	Metabolites measured	References
Brain	Rat	Tissue homogenate, sham-PX, Px	mRNA, DNA: AANAT, ASMT	HPLC	2,000–5,000 pg/mg prot (a)	AANAT, ASMT	[^{14}C]AC [^{14}C]SAM	[^{14}C]Ac-Trp [^{14}C]melatonin	[77]
Brain, liver	Rat	Subcellular SPx, Px	n.a.	HPLC-FD	5–300 pg/mg prot	n.a.	n.a.	n.a.	[15]
ARPE-19 cells	Human	Cell culture	mRNA: TPH1, TPH2, AANAT, ASMT protein: TH1, AANAT	HPLC-FD	(b)	n.a.	n.a.	n.a.	[134]
Retina	Rat	Tissue homogenate	n.a.	n.a.	n.a.	AANAT	[^{14}C]AC	Ac-Trp	[129]
Retina	Monkey	Tissue homogenate	mRNA, protein: AANAT, ASMT	RIA	n.a.	AANAT, ASMT	[^{3}H]AC [^{3}H]SAM	[^{3}H]Ac-Trp [^{3}H]melatonin	[41]
Retina	Golden hamster	Culture	n.a.	RIA	20–55 pg/ml (c)	n.a.	n.a.	n.a.	[130]
Y79 retinoblastoma cells	Human	Culture	mRNA, DNA: AANAT, ASMT	n.a.	n.a.	n.a.	n.a.	n.a.	[133]
Lens	Rabbit	Tissue homogenate	n.a.	RIA	35–58 pg/lens	AANAT	AC SAM	Ac-Trp Melatonin	[145]
Lens	Rat	Tissue homogenate	mRNA, protein: AANAT, ASMT	n.a.	n.a.	n.a.	n.a.	n.a.	[146]
Bone marrow	Rat	Tissue homogenate, Px	n.a.	RIA, ICC	750 pg/mg prot	AANAT, ASMT	[^{14}C]AC [^{14}C]SAM	[^{14}C]Ac-Trp [^{14}C]-melatonin	[158]
PBMN	Human	Cell culture, tissue	mRNA, DNA: AANAT, ASMT	HPLC-FD	400–600 pg/ml (d)	AANAT, ASMT	AC, Trp SAM	Ac-Trp Melatonin	[150]
Thymus	Rat	Tissue homogenate, SPx, Px	mRNA, DNA: AANAT, ASMT	HPLC-FD	0–9,500 pg/mg prot (e)	AANAT, ASMT	AC, Trp SAM	Ac-Trp melatonin	[152]
Thymus spleen	Rat	Tissue homogenate	mRNA, DNA: AANAT, ASMT	ELISA	1–40,000 pg/mg prot	AANAT, ASMT	[^{14}C]AC [^{14}C]SAM	[^{14}C]Ac-Trp [^{14}C]melatonin	[14]
RBL-2H3 mast cells	Rat	In vitro culture	n.a.	ELISA	6–15 pg/ml (f)	AANAT, ASMT	[^{14}C]AC [^{14}C]SAM	[^{14}C]Ac-Trp [^{14}C]melatonin	[161]
SKMEL188 human melanoma cells	Human, roe	Cell culture	mRNA: TPH, ASMT	LC/MS	(g)	TPH, AANAT, ASMT	[^{3}H]TP AC, Trp, SAM	[^{3}H]HTP NAS Ac-Trp, melatonin	[164]
Skin	Hamster	Cell culture	n.a.	HPLC, GC/MS	n.a.	n.a.	[^{3}H]HT	[3H]NAS [3H]melatonin [3H]5MT NAS	[163]
Ovary	Rat, human	Tissue homogenate	n.a.	HPLC-FD, RIA	139–348 pg/mg prot	AANAT, ASMT	AC, Trp SAM, NAS	Ac-Trp Melatonin	[250, 252]

Table 1 continued

Tissue	Species	Experimental condition	Enzyme expression	Melatonin detection	Melatonin levels	Enzyme activity	Precursors used	Metabolites measured	References
Placenta	Human	Cell culture, villous trophoblast	mRNA: AANAT, ASMT	n.a.	n.a.	AANAT, ASMT	[^{14}C]AC [^{14}C]SAM	[^{14}C]Ac-Trp [^{14}C]melatonin	[266]
Liver, kidney, heart	Rat	Tissue homogenate	mRNA, DNA:AANAT, ASMT	ELISA	900–2,500 pg/mg prot	AANAT, ASMT	[^{14}C]AC [^{14}C] SAM	[^{14}C]Ac-Trp [^{14}C]melatonin	[14]
Thymus, spleen, platelets, lung, heart, kidney, muscle, liver, stomach fundus, gut, testis, spinal cord, brain	Rat	Tissue homogenate	mRNA: AANAT, ASMT	n.a.	n.a.	n.a.	n.a.	n.a.	[4]

References include only those related to the original report of melatonin synthesis in a given tissue/organ; more information in the text

(a), day/night difference at 60 days after birth; (b), undetectable in untreated cells, evidenced after incubation with 100 μM tryptophan; (c), melatonin released from the cultured retinas; (d), melatonin content in peripheral blood mononuclear cells after 24–72 h of incubation; (e), maximum day/night levels at 7 days after birth; (f), melatonin released to the incubation medium; (g) melatonin levels not indicated; n.a., not assayed; 5MT, 5-methoxytryptamine; AANAT, aralkylamine *N*-acetyltransferase; AC, acetyl coenzyme A; Ac-Trp, *N*-acetyltryptamine; ASMT, acetylserotonin *O*-methyltransferase; [^{14}C]AC, [^{14}C]-acetyl-coenzyme A; [^{14}C]Ac-Trp, [^{14}C]*N*-acetyl tryptamine; [^{14}C]SAM, *S*-[methyl-14C]adenosyl-L-methionine; ELISA, enzyme-linked immunosorbent assay; GC/MS, gas chromatographic/mass spectroscopy; [^{3}H]HTP, [^{3}H]5-hydroxy tryptophan; [^{3}H]TP, [^{3}H]tryptophan; HPLC, high performance liquid chromatography; HPLC-FD, HPLC with fluorometric detection; HT, serotonin; ICC, immunocytochemistry; LC/MS, liquid chromatography-mass spectrometry; NAS, *N*-acetyl serotonin; PBMN, human peripheral blood mononuclear cells; Px, pinealectomy; RIA, radioimmunoassay; SAM, *S*-adenosyl-L-methionine; SPx, sham pinealectomy; TPH1 and TPH2, tryptophan hydroxylases 1 and 2, respectively; Trp, tryptamine.sss

have the machinery to synthesize melatonin and express its receptors [14], there is not sufficient experimental evidence supporting the local production of melatonin and they are not included in this review. Table 1 summarizes the information related to the extrapineal synthesis of melatonin.

Melatonin in the brain

The presence of melatonin in the brain was suspected because it is released into the cerebral ventricles both from the systemic circulation [74, 75] and from the pineal gland [76]. The means by which pineal melatonin is transferred to the cerebrospinal fluid (CSF) has been summarized in a recent review [77]. In addition to brain melatonin being derived from other sites, the possibility of the brain production of melatonin was addressed by Jiménez-Jorge et al. [78]. These authors measured the melatonin content and the expression and activity of AANAT and ASMT in the rat developing brain during fetal and postnatal stages. Significant daily changes in brain AANAT and ASMT were found together with fluctuations in melatonin content. Since rat pineal gland does not produce melatonin during fetal development or until 2 weeks after birth [79], it was concluded that rat brain synthesizes melatonin rhythmically during these early periods. Support for this idea also comes from the observation that pinealectomy does not influence whole-brain melatonin levels during this time [78].

The brain area(s) capable of melatonin synthesis is not yet clear. Elevated AANAT activity has been reported in hippocampus, striatum, cerebellum, olfactory bulb, and prefrontal cortex [80, 81]. Also, the hypothalamus contains high melatonin amounts at night, and melatonin may be released from this brain area [82]. In some brain regions, including nucleus gracilis, pons, medulla, cerebellum, and cerebral cortex, high amounts of melatonin were measured [15, 67, 83, 84]. High activity of AANAT and melatonin concentrations in cerebral cortex and cerebellum suggest that at least these brain areas are capable of melatonin production.

To date, there is no agreement as to the amounts of melatonin synthesized in the brain through the day; findings from different species have yielded inconsistent data [43]. This issue was recently addressed by measuring the content of brain melatonin during a 24-h cycle under different experimental conditions, including pinealectomy and continuous light exposure [15]. In this study, melatonin was estimated using HPLC with fluorescent detection. The authors examined the melatonin content in four subcellular fractions, i.e., membrane, cytosol, nucleus, and mitochondria from rat cerebral cortex. Melatonin content in these fractions was higher than in plasma, reaching values of 300 pg/mg prot in the mitochondria. Although fluctuations in the melatonin content in some subcellular fractions were found, they did not coincide with the normal melatonin cycle. Interestingly, when rats were pinealectomized, intracellular contents of melatonin significantly increased up to four times in the cell membrane compared to the amounts in this compartment in sham-pinealectomized rats (Fig. 1). The effects of pinealectomy were reproduced when the animals were maintained under continuous light, which blunts endogenous melatonin production by the pineal gland [85]. Also, luzindole, an antagonist of MT1/MT2 receptors, treatment did not influence the brain content of melatonin, suggesting that MT1/MT2 melatonin receptors are unrelated to brain melatonin content. This study also provided no evidence for the release of melatonin from brain cells and, in contrast to the pineal, brain production of melatonin is unrelated to the photoperiodic environment. It was suggested that the changes in the subcellular brain melatonin distribution are related to changes in their metabolic activity and to generation of toxic oxygen derivatives [15].

It remains unclear what cell type is responsible for the production of melatonin in the brain. Cultured astrocytes and C6 rat glioma cells both produce melatonin [86, 87]. Recently, high amounts of melatonin were detected in cultured neural stem cells from the subventricular area of adult mice (Escames et al., unpublished). The production of melatonin was further increased when these cells had differentiated into mature neurons. The virtual absence of glial cells in these cultures suggests that neurons themselves synthesize melatonin.

The synthesis and the presence of melatonin in neurons and/or glia indicate that locally generated melatonin may exert intra-, para-, and autocrine functions in brain, possibly regulating neuronal homeostasis through the control of neurotransmitter release and neurogenesis. Many brain neurotransmitters and their receptors exhibit a circadian rhythm influenced by the day:night fluctuations in pineal-derived melatonin [88–96]. Melatonin also regulates the circadian rhythm of some neuronal membrane properties including the ATPase pump activity [97]. Additionally, melatonin exerts anti-excitotoxic and protective effects in brain by reducing glutamate activity and increasing GABA [53, 92, 98–100]. Together, these effects reflect the ability of melatonin to protect neuronal cell membranes from hyperexcitability [101–105]. Melatonin modulates the electrical activity of the SCN neurons, acting primarily via MT1/MT2 receptors [106]. The chronobiotic actions of melatonin not only serves to influence the normal control of the daily brain activity, but also may be important in terms of its therapeutic application to restore abnormal circadian rhythms in individuals with neurodegenerative diseases [107, 108]. Moreover, the anti-inflammatory and antioxidant properties of melatonin are important in protecting neurons from damage during hypoxia, toxin exposure, etc. [92, 109–113], as well as reducing deterioration

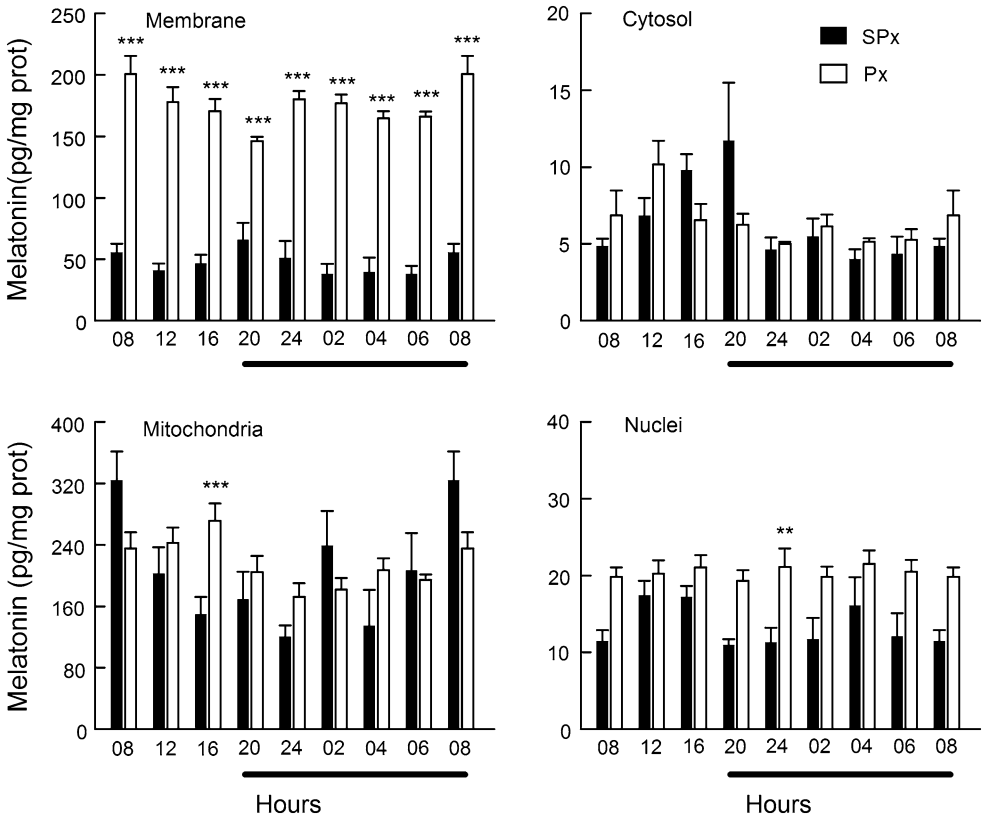

Fig. 1 Daily changes in the melatonin levels of membrane, cytosol, mitochondria, and nuclei from rat cerebral cortex of sham-operated (SPx) and pinealectomized (Px) rats. Animals were maintained in a 12:12 h cycle and killed at the indicated hours. *Black bars* indicate the night period. ** $p < 0.01$ and *** $p < 0.001$ vs. SPx

of the central nervous system during aging and age-related pathologies, e.g., Alzheimer's disease (AD), etc. [107, 114].

Melatonin stimulates dendritogenesis [115], and modulates cytoskeletal organization in the cells of rat hippocampus [116]. Thus, therapeutic doses of melatonin may help not only in reducing the brain damage but also in the treatment of neurodegenerative and neuropsychiatric diseases by repairing damaged tissue and promoting synaptic connections [117]. Melatonin promotes endogenous brain neurogenesis through MT2 receptors after brain injury in mice [118], and maintains hippocampal neurogenesis and cognitive functions after irradiation [119]. Melatonin-induced neurogenesis has also been detected in mouse hippocampus [120, 121], and the dentate gyrus [122, 123]. Melatonin and its role in neurogenesis is a new field of research with promising results [124].

Melatonin in the retina

The retina is another major site of melatonin formation in many vertebrates including all mammals, where it exerts multiple regulatory functions [125, 126]. Using specific melatonin antibodies, Bubenik et al. [127] first reported the presence and daily rhythm of melatonin in histological preparations from retinas of 2-day-old rat pups; these concentrations increased with age up to day 20. The rhythm of immunoreactive melatonin in the rat retina is not affected by pinealectomy, supporting the existence of an independent retinal oscillator controlling the indoleamine rhythm [9]. However, exogenous melatonin administration, or pinealectomy, increased the content of melatonin in the rat retina [9, 128]. Whereas the former may depend on the uptake of circulating melatonin by the retina, the latter agrees with similar effects of pinealectomy in the rat liver and brain recently reported [15], and suggests some type of control derived from the pineal gland over extrapineal melatonin production.

Several experiments have led to the identification and the rhythmic production and regulation of retinal AANAT and melatonin [129–131]. Retinas from the golden hamster (*Mesocricetus auratus*) cultured in vitro for 5 days rhythmically produce melatonin. Several experimental manipulations, including different light cycles, continuous darkness, and circadian short-period mutation 'tau', affected the rhythm of melatonin in these retinas [130]. At least in the CH3 mouse, the rhythm, but not the synthesis, of melatonin is related to the integrity of rod photoreceptors [130]. With in situ hybridization and laser capture microdissection, AANAT was detected in the inner nuclear layer and ganglion cell layer of the rat retinas, but only rhythmic AANAT transcription was found in the outer nuclear layer [132]. The expression of both AANAT and ASMT has been studied in Y79 human retinoblastoma cells in monolayer culture [125, 133].

These experiments revealed that cAMP, which increases AANAT activity and melatonin synthesis, does not elevate AANAT but rather reduce ASMT expression. The authors suggest that in Y79 cells, the AANAT activity does not depend on its expression but results from protein activation.

A complete evaluation of melatonin synthesis was performed in the retina of *Macaca mulatta* [41]. With PCR and Northern-blot analysis, the authors detected the expression of AANAT and ASMT in the rhesus monkey retinas. ASMT expression was very low and detected only at night. In turn, AANAT mRNA levels were similar over the 24 h, and AANAT activity increased up to fourfold during the night. These results agree with previous studies in Y79 human retinoblastoma cells [133], supporting that AANAT protein activation at posttranscriptional level is of primary importance in the regulation of melatonin synthesis in the retina [133]. Moreover, in contrast to the rodent, the rhesus monkey and human only needs to activate AANAT, without requiring changes in gene expression, to rapidly synthesize melatonin [41].

The retinal pigment epithelium seems to be another source of extrapineal melatonin synthesis in the eye. Expression of tryptophan hydroxylases (TPH1 and TPH2), AANAT, and ASMT have been documented in cultured ARPE-19 cells, a human retinal pigment cell line [134]. These cells also express melatonin receptors MT2, RORα1, and RORα4, and quinone oxidoreductase (NQO2). Moreover, 5-hydroxytryptophan, *N*-acetylserotonin, and melatonin, were detected by HPLC with a fluorometric detector. Thus, ARPE-19 cells express fully functional machinery for melatonin synthesis and effects for local homeostasis in an intra-, auto- and/or paracrine fashion [134].

Thus, local synthesis of melatonin in the retina may have clinical implications in the pathogenesis of a number of disorders of the eye, including keratopathies, corneal healing, age-related macular degeneration, and UVB/light-induced pathologies. This suggestion is based on the different actions that melatonin may have in the retina, i.e., receptor-mediated and non-receptor-mediated effects. With regard to the former, melatonin receptors have been identified in many ocular tissues, including the neural retina, retinal pigment epithelium, ciliary body, cornea, sclera, and lens. One of them is that it entrains the biological clock located in this tissue modulating dopamine release through MT1/MT2 receptors [125]. The melatonin/dopamine interaction in the retina mediates darkness-adaptive changes, i.e., dopamine mediates light-adaptive changes while melatonin inhibits nocturnal dopamine release through MT1/MT2 receptors [135]. In turn, stimulation of dopamine receptors suppresses the nocturnal increase of AANAT enzyme activity in the retina and thus melatonin synthesis [136].

The second action of melatonin in the retina is related to its antioxidant capacity. In this regard, melatonin may be physiologically relevant for the protection of the photoreceptor outer segment from photo-oxidative stress [134, 137–139]. It has been suggested that melatonin protects the photoreceptor outer segment of membranes from photo-oxidative stress [138, 139] and counteracts ischemic injury to chick embryo RPE cells [140]. The mechanisms involved in the protection of RPE by melatonin have been reported [137]. When human ARP-19 cells were cultured in the presence of H_2O_2 (0.5 mM), H_2O_2 induced a reduction of 71 % in cell viability. When the cells were pretreated with low (10^{-10} M) or high (10^{-6} M) melatonin concentrations, the cells were protected against H_2O_2 toxicity for at least 48 h. The protective effect of melatonin significantly increased with its concentration. When the ARP-19 cells were co-cultured with H_2O_2 plus luzindole, the authors showed that at low concentrations (10^{-10}–10^{-8} M) melatonin elevated cell viability through the activation of its MT1/MT2 membrane receptors, while high concentrations of melatonin (10^{-6}–10^{-4} M) protected the cells by a direct antioxidant activity not involving an interaction with MT1/MT2 receptors [137].

Melatonin in the lens

The ciliary body [141], the lacrimal glands [142], and the lens [143] are other loci of melatonin production in the orbital cavity. The first indication of a relationship between melatonin and lens was provided by Quay [144] in 1984 when he reported an increase in lenticular weight after the systemic administration of melatonin to hamsters, an effect unrelated to the pineal gland. Subsequently, Abe et al. [145] reported the presence of tryptophan, 5-hydroxytryptophan, and melatonin in rabbit lens extracts using HPLC with fluorometric detection. These authors also detected the activities of the two key enzymes mediating melatonin synthesis, i.e., AANAT and ASMT. Kinetic studies and photoperiod entrainment experiments further documented the capacity of the rabbit lens to synthesize melatonin. Using PCR and Western-blot techniques, the expression of AANAT and ASMT mRNAs were identified in adult male rat lens [146]. Histochemical analysis revealed the presence of AANAT protein in the cortical fiber cells of the rat lens, which also contain serotonin and melatonin. Moreover, the AANAT activity in rat lens displays a circadian rhythm [143] including under continuous darkness; this supports the existence of a circadian clock in rat lens, which controls AANAT activity and thus melatonin production.

Melatonin in the cochlea

Melatonin synthesis has been reported in the auditory tract of guinea pigs, including in the membranous cochlea [147], and small amounts of this indoleamine were also measured

in the cochlear nerve. Melatonin was also found in cochlea of the rat where it exhibits a significant daily variation [148]. There are no additional data, however, documenting the expression and/or activities of the enzymes that convert serotonin to melatonin in these tissues.

Melatonin in the immune system

Several cells and tissues related to the immune system contain and/or produce melatonin. Among them, thymus, mast cells, natural killer cells, eosinophilic leukocytes, platelets, and endothelial cells contain melatonin in different concentrations [14, 149]. Human lymphocytes also produce large amounts of melatonin, with values up to five times greater than plasma concentrations at night [150, 151]. The authors also reported the mRNA expression and activity of AANAT and ASMT in human lymphocytes. An increased AANAT and reduced ASMT activities after phytohemagglutinin stimulation was shown, but the meaning of these changes in terms of melatonin production was not determined [151]. That lymphocytes synthesize melatonin was further demonstrated after blocking AANAT and ASMT mRNAs; this was followed by the reduction of melatonin levels. Multiple actions of lymphocyte melatonin including intra-, auto-, and paracrine regulation of IL-2 and/or IL-2R, are supported by experimental data [151].

Human and rat thymus synthesize melatonin [152, 153]. The thymus from 18-day fetal rats contain melatonin and express AANAT and ASMT mRNAs and activity. These enzymes exhibit significant day/night differences with highest levels during the day; the thymic maximal activities are reached during adulthood. In contrast, the melatonin content of the thymus also expresses a daily rhythm with peak levels at night; so the melatonin cycle is out of phase with those of AANAT and ASMT. Due to the transfer of melatonin from the mother to the fetus, this apparent discrepancy was presumably related to thymic uptake of circulating maternal melatonin from the fetal blood [152]. The thymus from adult rats, however, had lower melatonin content at night, and it was hypothesized that high circulating nighttime melatonin concentrations of pineal origin inhibited thymic melatonin synthesis. In support of this possibility, several reports showed that pinealectomy influences extrapineal melatonin synthesis and/or content, including in the brain [154, 155], Harderian gland [9], and retina [9, 128]. The effects of circulating melatonin and pinealectomy on extrapineal melatonin production was recently reported in rat liver and brain [15], further suggesting that the intact pineal modulates the synthesis of melatonin at peripheral sites.

Melatonin and serotonin production has been identified in rat peritoneal cavity-isolated macrophages [156]. In the presence of tryptophan, macrophages exhibited higher AANAT activity and melatonin production. High levels of plasma melatonin and elevated production of iNOS by macrophages were also reported in hypertensive patients, and they were reduced after treatment with lacidipine, a Ca^{2+}-channel blocker [157]. Whether macrophages contribute to the rise of plasma melatonin levels before treatment, or it depends only of its pineal production, remains unclear. These data may reflect a role for melatonin in activated macrophages, probably protecting them from the damage induced by the high levels of $NO^{\bullet}$ and/or the product, peroxynitrite, which they produce under some conditions.

Bone marrow constitutes a tissue with significantly elevated amounts of melatonin. Tan et al. [158] first identified the presence of melatonin in rat bone marrow using immunocytochemistry, radioimmunoassay, HPLC with electrochemical detection, and mass spectrometry analysis. Nocturnal content of melatonin in rat bone marrow were almost two orders of magnitude higher than plasma concentrations. The production of melatonin was supported by the high amounts of melatonin that remained in this tissue after pinealectomy [158]. The concentrations of intracellular melatonin detected in bone marrow increased after exogenous melatonin administration, leading to the suggestion that, at least in part, melatonin could be sequestered from blood by bone marrow cells. High amounts of melatonin were subsequently confirmed in both mouse and human bone marrow cells [159]. This study also reported AANAT activity in the bone marrow obtained from different sources, including mouse strains (C57/Bl/6, C3H/He, and SJl/J) and Nalm-6 cells from human bone marrow; the latter cells also express ASMT mRNA. Collectively, these data point to the synthesis of melatonin by cells normally present in bone marrow.

Mast cells express high-affinity IgE Fc receptors, and are one of the main effector cells of the immune system against parasitic and some bacterial infections [160]. The fact that they derive from bone marrow progenitors makes it possible that they may also synthesize melatonin. These cells contain elevated amounts of melatonin that further increase after their stimulation with phorbol-12-myristate 13-acetate plus calcium ionophore A23187 [161]. Both unstimulated and stimulated mast cells exhibit AANAT and ASMT activities, suggesting they synthesize melatonin. The presence of MT1/MT2 receptors in mast cells further supports a regulatory mechanism related to the uptake of extracellular melatonin by mast cells or, alternatively, directly for the melatonin produced by them via autocrine or paracrine mechanisms.

Melatonin in the skin

In 1993, the research group of Slominski [162] identified two NAT isoforms in the Syrian hamster skin. One

of these isoforms, NAT-2, yields *N*-acetylserotonin, the melatonin precursor, suggesting the capacity of the skin cells to generate melatonin. Soon thereafter, also in hamster skin cultures, the biotransformation of [^{3}H]serotonin to its metabolites [^{3}H]*N*-acetylserotonin, [^{3}H]*N*-acetyl-5-methoxytryptamine, and [^{3}H]5-methoxytryptamine was reported [163]. The synthesis of melatonin by the skin was stimulated by forskolin, supporting the ability of hamster skin not only to synthesize melatonin but also its inactivation through its catabolism to 5-methoxytryptamine. Human melanoma cells reportedly convert L-tryptophan to serotonin and melatonin [164], and they express tryptophan hydroxylase and ASMT. These data prove that melanoma cells produce melatonin that could be related to the inhibition of their proliferation [165, 166]. Other functions of melatonin may involve melanogenesis and/or redox status regulation in these cells. The expression of genes involved in melatonin synthesis were reported in a variety of skin samples including normal basal epidermal cells, basal cell carcinoma and melanoma, melanoma cell lines, squamous cell carcinoma cells, normal epidermal and follicular melanocytes, normal neonatal and adult epidermal and follicular keratinocytes, and fibroblasts from dermis and follicular papilla [167]. The expression of AANAT and ASMT, as well as other enzymes in the melatonin pathway, and the presence of serotonin and melatonin, have been identified in human skin, providing strong evidence of the in situ synthesis of the latter.

MT1/MT2 melatonin receptors and 5HT2B and 5HT7 serotonin receptors are present in rodent and human skin [168, 169], suggesting that the human skin not only synthesizes melatonin but it is also a target for this indoleamine. Some of these actions involve the presence of MT1/MT2 and RORα receptors, and include regulation of hair growth, pigmentation, and inhibition of melanoma cell proliferation as well as the mitotic activity of other skin cells. Other functions are related to the antioxidative activity of melatonin and include protection against UV and X-ray radiation, thermal injury, burns, and any other agent capable of inducing oxidative damage in the skin [170–172]. Of special importance is the ability of melatonin to protect against UVB radiation, which is highly harmful to the skin. UVB exposure induces melatonin catabolism to 2-hydroxymelatonin and AFMK, both of which also possess significant antioxidant capacity [173]. Thus, melatonin's antioxidative cascade reported elsewhere [55] is also operative in skin. The skin represents, therefore, a tissue able to produce melatonin but also a target for the multiple protective melatonin actions [174], by acting through intra-, auto-, or paracrine mechanisms [170, 175].

Melatonin may have additional benefits in terms of skin annexa. In mammals, hair density protects against extremes of air temperature; the seasonal changes in fur thickness are under the control of the photoperiod. Consequently, it is reasonable to assume that seasonal changes in melatonin, which are also under the control of the photoperiod, serve as the basis for annual fluctuations in hair growth throughout the year. In this regard, the relationship between photoperiodic changes and the moulting stages in sheep and ram are modulated by melatonin [176, 177].

Melatonin in the gastrointestinal tract

It was proposed as early as 1975 that enterochromaffin cells from the gastrointestinal tract (GIT) might synthesize melatonin [10, 11]. The existence of a local production of melatonin in the GIT comes from the studies in postnatal rats. While pineal does not produce melatonin until the second week of life, melatonin was detected in the GIT tissue of 2-day-old rats [178], increasing to the adult content at 21 days postnatal [179]. Additional studies using several methodological approaches including radioimmunoassay and HPLC confirmed the presence of melatonin in the GIT of several species [180]. These data, and the presence of AANAT and ASMT activities in the GIT, further supported the GIT synthesis of melatonin independent of the pineal [4, 181].

Melatonin levels in the GIT are 10–100 times higher than in serum [182, 183], and the total amount of melatonin in the GIT was calculated to be up 400 times more than in the pineal [184–186]. Melatonin was also found in high concentrations in the GIT of several non-mammalian vertebrates, with the highest content (>1,000 pg/g) reported in the stomach of the garter snake [187].

Exogenous melatonin administration increases its content in the GIT [70, 179, 188]. Under some conditions, GIT melatonin concentrations may increase at night [180, 189]. These observations led to the assumption that at least some gut melatonin might come from the pineal gland, which after its release, is taken up from the circulation [179, 186, 190]. However, several decades ago it was found that the synthesis of melatonin in the GIT remain unchanged after pinealectomy [10], an observation that has been confirmed [191]; thus, melatonin in the gut is believed to be gut derived. However, daytime levels of melatonin in the blood of pinealectomized rats disappear with fasting, supporting a possible food source of blood melatonin [192, 193]. Most foods contain melatonin [194, 195] and when they are consumed, this indoleamine is absorbed by the gut into the circulation [195, 196]. Finally, taking into account that liver diseases reduce the metabolism of melatonin, such conditions would be expected to lead to its elevation in the circulation. The possibility that pinealectomy may influence the hepatic breakdown of melatonin also cannot be ruled out.

The exclusive source of melatonin in the GIT is still a matter of discussion. GIT melatonin levels are detectable

soon after birth in postnatal mice, with regional differences in terms of its concentration in jejunum, ileum, and colon, with each of the levels being greater than those in the stomach [197]. Luminal fluids of gut also contain melatonin [198], which probably comes from the mucosa and from the bile [199]. Because melatonin is also produced by microorganisms in large amounts [200, 201], the intestinal flora could be an alternative source of melatonin. Clearly, GIT melatonin may derive from several sources including other tissues, bile, intestinal flora, or food. Since melatonin amounts in the digestive tract generally do not show daily fluctuations, the photoperiod seems to have little regulatory effect in GIT melatonin content. Additionally, experiments with pinealectomized animals, showing that this procedure does not influence the content of melatonin in the digestive tract, indicated these values are pineal-independent [191].

Because many foods contain melatonin, at least a portion of the melatonin content in the GIT likely comes from that eaten [182, 185, 195, 196, 202]. The melatonin content varies widely in foods including edible plants [203–207], which may explain the species-dependent differences in the GIT melatonin concentrations based on the food normally consumed [182, 185, 202]. This may also explain in part the high melatonin amounts in the GIT during the day, when diurnal animals feed.

With regard to the functions and mechanisms of action of melatonin in the GIT and adnexa including the liver, the presence of melatonin binding was identified in the GIT tract, mainly in the mucosa. For the mucosa, which contains epithelial lining cells and underlying connective tissue including the muscularis mucosa, the nuclear fraction had the highest binding levels, followed by the microsomal, mitochondrial, and cytosol [28, 32, 208–210]. Of importance, it was reported that the effects of melatonin on GIT smooth muscle depend mainly on MT2 membrane receptors [211, 212], indicating the lack of involvement of nuclear receptors. Thus, the effects of melatonin mediated by ROR nuclear receptors in the GIT require evaluation.

As in other tissues, melatonin actions in the GIT include intra-, auto-, and paracrine effects. In this regard, enterochromaffin cells release serotonin into the lumen that stimulates local enteric neural reflexes to initiate secretion and propulsive motility [213]. Melatonin produced by enterochromaffin cells of the GIT mucosa may be released to the blood vessels or diffuse to reach the outer smooth muscle layers, acting as an antagonist of the contractile effects of serotonin [212, 214, 215] thereby causing relaxation [180, 189]. Melatonin may also regulate the myenteric nervous system [212, 216], transmembrane transport of electrolytes and ions [217], water content in the gut [197], and mitotic activity [218]. In response to neuronal stimuli, proximal duodenum enterochromaffin cells discharge melatonin, which binds to MT2 receptors causing calcium release and bicarbonate ions secretion from adjacent lining cells; this serves to neutralize the acid content of the stomach as it is released into the duodenum [71, 219–221]. Duodenal melatonin also plays an important role in the neurohumoral control of duodenal mucosa barrier via nicotinic receptor [222, 223]. Updated reviews have recently been published on these matters [180, 189, 212, 224–226]. Thus, enterochromaffin cells exert multiple functions in the GIT, which are modulated by the melatonin/serotonin balance.

Melatonin seems to have a variety of functions in other GIT annexa including liver. The hepatocytes express AANAT and ASMT mRNAs and activities. One portion of liver melatonin comes from the GIT via the hepatic portal vein [70, 202, 227]. Menéndez-Peláez et al. [209] reported the presence of melatonin in the liver, especially in the hepatocyte nuclei. Soon thereafter, the presence of nuclear receptors for melatonin were reported in rat liver [28, 210] and confirmed [29, 32]. The concentration of hepatic melatonin was calculated as 15-fold higher than in serum [190]. The liver is the main catabolic tissue for circulating melatonin, via cytochrome P450 [228, 229]. However, below a certain concentration in blood, corresponding to daytime levels of melatonin, this indoleamine may not catabolized by the liver [186, 190]. Hepatocytes also release melatonin into the bile, where it reaches remarkably high concentrations [199, 230] (also see below). Clearly, the presence of melatonin and melatonin receptors in liver portend important functions for this indoleamine in this organ. Among them, inhibitory effects of melatonin on hepatic cancer have been documented [231, 232].

Melatonin is present in human GIT [183]. The colon of the fetus has high concentrations of melatonin, and it was suggested that this indoleamine participates in meconium production [180]. Melatonin content in the GIT early after birth could also come from the mother, since melatonin crosses the placenta and remains in the circulation for at least 72 h in newborn infants [233–235]. A transient postprandial elevation of circulating melatonin in blood, which was related to the release of melatonin from the GIT, was related to the sleepiness after the midday meal [202]. This effect of melatonin is also associated with the reduction of the core body temperature [236, 237], although it is yet unclear whether the slight increase of melatonin levels in blood after a meal is sufficient to cause this effect. Studies in pigs showed that the melatonin produced in the lower GIT may be responsible for the rise in blood melatonin after feeding [182].

Melatonin in the reproductive tract

A potential association of the pineal gland with the reproductive system was identified as early as 1898, when the relationship between a human pineal tumor and an

alteration in puberty was reported [228]. In a recent review, however, it was noted that the pineal tumors described were not pinealomas but choriocarcinomas, which produce human chorionic gonadotrophin, thus causing premature pseudopuberty [238]. Six years later after the identification of melatonin [1], Hoffman and Reiter [239] found that exposure of male hamsters to short daily photoperiods caused gonadal atrophy. Surgical removal of the pineal gland prevented the gonadal inhibition. These data pointed to a relationship between photoperiod, pineal activity, and reproduction. In subsequent years, multiple aspects of the main pineal product, melatonin, and reproduction have been uncovered documenting the existence of a complex regulation by which melatonin controls seasonal reproduction [240, 241]. The role of melatonin on reproductive physiology in determining the sexual status in photoperiodic mammals is now clear [242–244].

Preovulatory follicular fluid of the human ovary, obtained by laparoscopy, revealed the presence of significantly higher amounts of melatonin than in serum, with a positive correlation between them [245]. Follicular fluid melatonin displays circadian and circannual rhythms, which may be related to human fertility [246]. Human follicular cells contain melatonin and MT1 and, to a lesser extent, MT2 mRNA is present in human granulosa cells [247, 248]. Mature follicles contain high amounts of melatonin compared to those that are immature; the melatonin content is positively correlated with progesterone production [249], suggesting a regulatory role for melatonin on human reproduction and/or local steroidogenesis. Melatonin may be synthesized in the granulosa cells and released into the follicular fluid, where it is concentrated [250], although the initial lack of detection of AANAT mRNA in the ovary [251] suggested that ovarian melatonin comes from the blood. However, subsequently, the presence of AANAT and ASMT activities in the rat ovary were reported; and these ovaries also were found to contain melatonin. The kinetic analysis of AANAT and ASMT provided apparent Kms close to those in the pineal gland, suggesting that the ovary does in fact synthesize melatonin [252]. The activities of AANAT and ASMT were also detected in rat testis, with the highest activity in the interstitial cells and the lowest in the seminiferous tubules [253]. The high levels of serotonin produced by the testis interstitial cells [254], and the presence of melatonin binding sites in these cells [255], are consistent with the production of melatonin in the testis and with specific actions in this organ. The synthesis and presence of melatonin in multiple sites of the ovary and testes reflect its potential intra-, auto-, and paracrine regulation of reproductive physiology, which guarantees the quality of the egg and sperm.

It now seems established that melatonin is produced by and acts on the ovary, thereby controlling its function [242, 256–258], and it may aid in the improvement of oocyte quality in these circumstances [259]. High intrafollicular levels of 8-OHdG have been reported in oocytes obtained from women during in vitro fertilization and embryo transfer, who presented infertility with elevated degenerated oocytes. Following the administration of 600 mg/day vitamin E plus 3 mg/day melatonin, which induced an fourfold rise in intrafollicular melatonin, a reduction in the levels of a damaged DNA product, 8-OHdG, with a significant improvement in oocyte quality and fertilization were recorded. Even doses of melatonin of 200 mg/kg/day from gestational day 6 had no effect on prenatal survival, fetal body weight, or the incidence of fetal malformations in rats [260]. Due to the fact that many chromosomal alterations in the fetus are a result of oxidative stress, further studies should be done to assess whether melatonin may be also helpful in the prevention of chromosomal abnormalities [257].

The existence of a maternal-fetal transfer of melatonin in pregnant women was suggested after the identification of melatonin in the umbilical artery and vein cord of human neonates with a circadian rhythm resembling that of plasma melatonin in the mother [233, 235, 261]. The rhythm of plasma melatonin disappeared 72 h after delivery in the neonates, supporting its maternal origin [235]. The maternal-fetal transfer of melatonin was further shown in pregnant women who underwent a cesarean section after receiving a single oral dose of 3 mg of melatonin [261], with a significant correlation between maternal and umbilical cord blood melatonin levels. Thus, the fetus receives a photoperiodic signal during gestation, which may be necessary for synchronizing fetal physiology. Throughout gestation, maternal serum melatonin gradually increases, reaching maximal values at the time of delivery; after parturition the values return to normal levels [234, 262–264].

Based on the available evidence, it is likely that melatonin plays a role in the feto–maternal interface, helping to maintain a successful pregnancy. In this connection, the mRNA expression of AANAT and ASMT has been detected in human placenta during the first trimester of pregnancy [265]. With the use of PCR, Western blot, and analysis of its activity, the presence and physiological activity of AANAT and ASMT in villous trophoblastic cells has been documented [266]. Thus, increased melatonin levels in maternal blood during pregnancy may be, at least in part, of placental origin. Immunohistochemical analysis identified the presence of MT1 and MT2 in villous cytotrophoblast and syncytiotrophoblast of normal-term placenta, as well as in endothelial cells surrounding the fetal capillaries and in the villous mesenchymal core [266]. The mRNAs and proteins of three receptors were also expressed in primary cultures of villous cytotrophoblast and syncytiotrophoblast. The mRNA expression of MT1 and MT2 melatonin

receptors was also found in the placenta, further supporting a role of melatonin in regulating placental function through intra-, auto-, and/or paracrine actions. Although melatonin reduces cAMP production through the activation of its membrane receptors, it was shown that higher levels of melatonin increase myometrial cAMP [267]. Thus, the possibility exists that the elevated amounts of melatonin in the placenta may induce the production of hGC, a cAMP-dependent process [265]. However, other authors have published contrary results [268], although it involved a different experimental model (normal first-trimester trophoblasts cells vs. choriocarcinoma cells). Taking into account the role of hCG in villous trophoblast differentiation [269], and the role of melatonin in promoting hCG, a role for melatonin in the regulation of villous trophoblast differentiation and endocrine function was proposed [266].

During the prepubertal period until females become fertile, melatonin is high relative to values in adult women. These levels reportedly drop throughout puberty and then remain constant until 35–40 years, when they decrease with age. During all stages, melatonin displays its typical circadian rhythm, with the possibly some seasonal variations. Although humans are not seasonal reproducers, they reportedly display some changes in terms of birth and conception [270] and embryos quality [271] throughout the year. These changes have been associated with the seasonal variations in melatonin levels [272]. Melatonin may influence the reproductive axis in other ways; high levels of melatonin have been related to low reproductive rates and hypothalamic amenorrhea [273]. Seasonality varies with the geographic area, and it involves changes in the sperm quality, embryo quality, sperm chromatin concentration, multiple fetuses, etc. Circulating melatonin levels increase with pregnancy and they may modulate the timing of labor. In pregnant rats, but not documented in humans, the elevation of blood melatonin during pregnancy seems to depend on increased pineal production and not on its secretion from the placenta. This production of melatonin may be stimulated by placental hormones [258]. Blind people have higher melatonin levels, coinciding with lower sexual hormone levels [274]. An association between pineal tumors with advanced or delayed puberty has been reported [275], although these data require confirmation and may not be related directly to changes in melatonin.

Melatonin in biological fluids other than blood

Melatonin is present at high concentrations, sometimes higher than in serum, in different biological fluids (Table 1). Fluids in which melatonin is detectable include CSF, saliva, bile, synovial fluid, breast milk, and amniotic fluid. The sources of melatonin in these fluids and its functions remain largely unknown. Table 2 summarizes the presence of melatonin in biological fluids.

Melatonin in cerebrospinal fluid

While melatonin in the CSF may be primarily pineal-derived, extrapineal melatonin may be released into the CSF from the brain and/or via the choroid plexus from the blood. The presence of a circadian rhythm of melatonin in the CSF parallel to that of the blood supports the view that melatonin is released directly into the third ventricle with ready access to the brain, affecting all neural structures [76]. The highest melatonin content has been detected in fluids of the third ventricle, with lower concentrations in the lateral ventricles in sheep and humans [276, 277]. At least some of the melatonin in the CSF is discharged from the pineal recess directly into the third ventricle of the sheep. Human studies also revealed that melatonin can directly enter the CSF possibly through the

Table 2 Melatonin levels in human biological fluids (when available)

Fluid	Melatonin (pg/ml)	Species	References
Cerebrospinal fluid	1.47–542	Human	[74]
Bile	2,000–11,000	Pig, rat, guinea pig, rabbit, human, monkey	[199]
Follicular fluid	36.5 ± 4.8	Human	[245]
	12.4–23.8	Human	[246]
Amniotic fluid (labor)	25–158	Human	[301]
Saliva	11–63	Human	[296, 297]
Synovial fluid (RA)	79.8 ± 38.9	Human	[299]
Breast milk (night)	36–99	Human	[305, 307, 308]
Anterior chamber of lens	28–70*	Rabbit	[143]

References include only those related to the original report of melatonin in a given fluid; more information in the text

RA rheumatoid arthritis

* Expressed in pg/lens

pineal recess [278]. Melatonin levels in CSF fluctuate as in the blood, with a circadian rhythm peaking at night and low levels during the day [76, 276]. In sheep, nighttime melatonin levels were reported to be several hundred-fold higher than in plasma, where it maintained at higher levels since it is not metabolized in this fluid [279]. In humans, however, there is less unequivocal data because of the difficulties in obtaining CSF samples throughout the day and night. It was calculated, however, that the nocturnal levels of melatonin in human CSF are much higher than in blood [280]. The high levels of N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), a unique melatonin metabolite, in rat brain and CSF fluid [281], and the presence of the melatonin-synthesizing machinery in the brain [77], suggest the CSF melatonin could, in part, be derived from the brain. Thus, besides its intracrine actions on brain cells, melatonin in the CSF may have paracrine effects throughout the nervous system.

Melatonin levels increase in the CSF of patients after severe traumatic brain injury where they correlate with the level of oxidative stress [282]. CSF of patients with meningitis also has elevated levels of AFMK [281]; AFMK is known to be an important immunomodulatory agent [283]. In another pathology, i.e., AD, CSF levels of melatonin are significantly reduced [284], presumably related to its rapid utilization as a free radical scavenger [114].

Melatonin in the bile

It is clear now that one part of the circulating melatonin comes from the GIT through hepatic portal vein [70, 182, 202, 285], where it protects the liver and biliary tract from various irritants [225]. The amount of melatonin transported to the biliary system depends on the melatonin content of the liver and its hepatic catabolism. Although the exact percentage of melatonin passing through the liver that is metabolized it is not yet clear, some studies indicate that it depends on the blood melatonin concentration. So, below daytime concentrations of melatonin, the liver does not metabolize melatonin, whereas the catabolism increases with higher concentrations [186]. Beyond the known catabolic activity of the liver, the meaning of hepatic melatonin catabolism is a matter of discussion. It was reported that portocaval anastomosis disrupted the pineal melatonin rhythm in rats [286], and liver cirrhosis disrupts the sleep rhythm in patients, although in this case plasma melatonin alterations were not related to sleep disturbances [287]. Thus, the rapid decay in the melatonin peak at night may depend mainly on the degree of its liver metabolism and less on melatonin uptake into the cells. In the liver, the presence of melatonin was recently revised, when its subcellular distribution with higher concentrations than in the serum was documented [15]. Much of this melatonin escapes from liver metabolism and it is excreted in the bile. It seems that the gallbladder from many species including rat, guinea pig, rabbit, pig, monkey, and humans concentrate melatonin up to 11,000 pg/ml in the bile [199]. These values are as much as 40-times higher than those in the GIT, and 2–3 orders of magnitude higher than in serum during the day. Messner et al. [230] also detected considerable amounts of melatonin excreted through the human bile (51 ng/24 h). The meaning of these amounts is unclear; however, due to the antioxidant and anti-inflammatory properties of melatonin, a protective effect in the own gallbladder and intestinal epithelium induced by bile acids, oxidized cholesterol derivatives, and the metabolic activity of intestinal epithelium during digestive processes, is suggested. These actions reflect a regulation of the gut-liver axis by melatonin.

The question of the source of high amounts of melatonin in the hepatobiliary tract was addressed suggesting that GIT releases melatonin to the portal circulation, which reaches the liver and is then excreted into the bile. While this is, in fact, a source of hepatic melatonin, there are new data supporting the synthesis of melatonin by the liver. Hepatocytes contain AANAT and ASMT [4, 14], and they have access to tryptophan and/or any other melatonin metabolic precursors. Moreover, a recent study showed that liver of pinealectomized rats or rats subjected to continuous light have higher melatonin concentrations than sham-operated animals [15] (Fig. 2). The intracellular content of hepatic melatonin changes throughout the day and they were not related to serum melatonin variations or food intake, because animals were fasted before the study. Hepatocyte content of melatonin was not affected by luzindole, and the level increased after subcutaneous melatonin administration. Thus, the liver probably synthesizes melatonin, and the changes reported in its concentrations probably depend on the metabolic requirements of the liver.

Melatonin in saliva

Many hormones, peptides, and other molecules are transferred from the circulation into the saliva [288]. Salivary glands may also inactivate and/or activate inactive hormones, such as the conversion of salivary androstenedione to testosterone and cortisol to cortisone [289, 290]. Biological actions of salivary hormones range from the control to the oral immune response to cell proliferation and wound repair, and social behavior [291]. Saliva is also a good pathway by which melatonin enters the oral cavity from the blood via plasma ultrafiltration by the salivary glands; alternatively, these glands may produce melatonin. Immunohistochemical analysis revealed the presence of melatonin in the salivary gland of rats [292], and the accumulation of this indoleamine in the saliva [293].

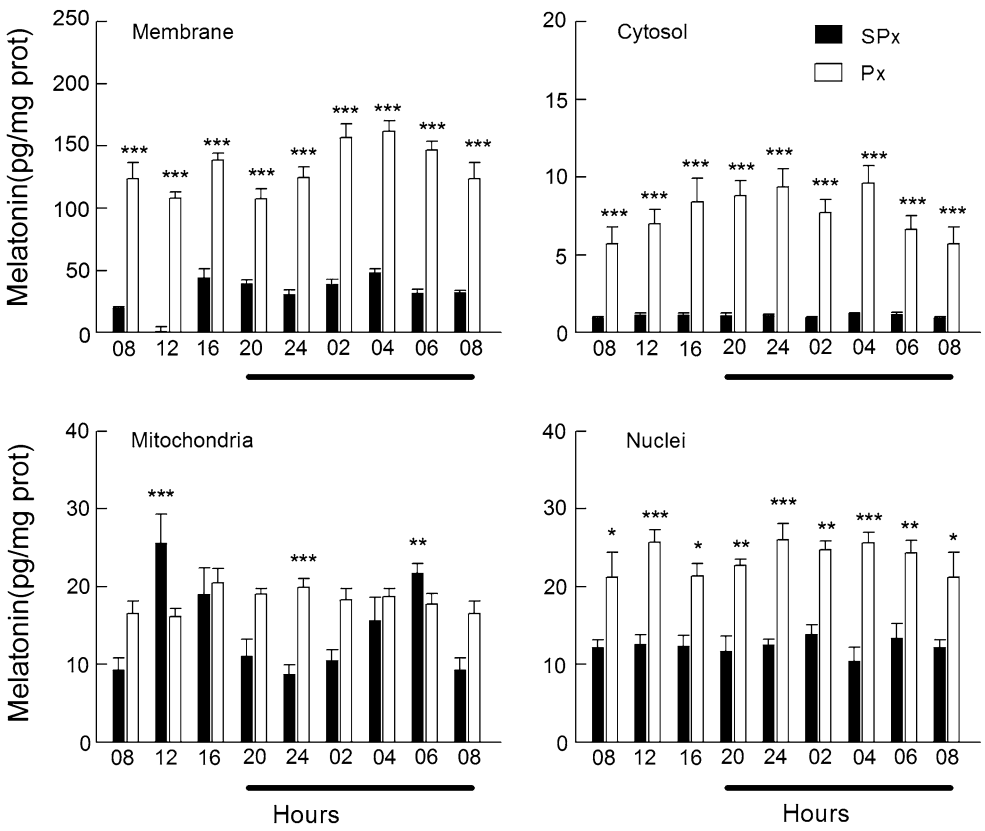

Fig. 2 Daily changes in the melatonin content of membranes, cytosol, mitochondria, and nuclei from rat liver of sham-operated (SPx) and pinealectomized (Px) rats. Animals were maintained in a 12:12 h cycle and killed at the indicated hours. *Black bar* indicates the night period. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ vs. SPx

The presence of AANAT was reported in epithelial cells of striated ducts of the rat parotid, submandibular, and sublingual glands, and in human submandibular gland [294]. Also, rat oral mucosa and parotid glands express MT1 and MT2 receptors [294, 295]. Melatonin synthesis may occur in salivary glands where it may have paracrine or autocrine actions.

Melatonin is excreted in the saliva, and its levels are about 30 % of the serum concentrations; this corresponds to the free melatonin in blood [39]. Thus, salivary melatonin depends on its serum levels [296], and it displays a circadian rhythm that closely resembles that in serum [297]. Regarding the functions of salivary melatonin, it was proposed that it may control protein synthesis of the rat parotid gland by a mechanism involving an nNOS-dependent mechanism [298]. While the specific nature of these proteins is not yet identified, the protective effects of melatonin in the oral cavity suggest that it may induce the synthesis of proteins involved in oral defense.

Together, the results summarized here support the idea of the beneficial effects of melatonin in maintaining the normal redox status in the oral cavity. These findings also suggest the therapeutic use of melatonin alone or in combination with GH to improve bone formation and reduce the time required for osteointegration of dental implants.

Melatonin in synovial fluid

Melatonin is present in the synovial fluid, although its source is unclear [299]. As mentioned above, melatonin exerts important immunomodulatory properties, acting on specific receptors in immune cells. Among other effects, melatonin stimulates the production of IL-2 by lymphocytes and macrophages [151]. The fact that plasma melatonin increases at night, in advance of the cyclic increase in the manifestations of rheumatoid arthritis [300], led investigators to examine the effects of melatonin in this disease. Higher concentrations of melatonin were measured in the synovial fluid from these patients, relative to levels in controls. Moreover, synovial macrophages in patients suffering from rheumatoid arthritis have specific melatonin binding sites compatible with its membrane receptors. The elevated amount of melatonin in the synovial fluid of these patients suggests that it may be locally produced [159] and may act through intra-, auto-, and paracrine mechanisms. This report also suggested that the high content of melatonin induces iNOS and/or nNOS to produce the elevated levels of $NO^{\bullet}$ found in these patients.

Melatonin in amniotic fluid

The amounts of melatonin in the amniotic fluid during pregnancy and labor are low [301]. During human labor, serum

melatonin was positively correlated with amniotic fluid melatonin, which also displays a diurnal rhythm although with lower levels than in serum [302]. The concentrations of melatonin were not related to the physical stress of labor or with any other endocrine change associated with it [262, 263]. The elevate concentrations of melatonin in amniotic fluid (and low ACTH levels) was proposed as an endocrine marker of a high risk of fetal abnormalities [303]. Moreover, in pregnant mice, LPS-induced intra-uterine fetal death and growth retardation were significantly attenuated by melatonin administration without changing the levels of TNFα and IL-1β in amniotic fluid [304]. Moreover, the elevated levels of melatonin found in the neonatal cord blood [233], together with the fact that melatonin administration to pregnant women had no side-effects and ameliorated and protected the fetus [261], supports the absence of any pathologies related to high levels of melatonin. Nevertheless, further studies are necessary to evaluate the exact role of melatonin in amniotic fluid.

Melatonin in breast milk

Melatonin has been identified in human milk of mothers 3–4 days after delivery. While no detectable melatonin was found during the day, they were measurable at night with the values being threefold lower than in the serum [305]. Daily variations in milk melatonin remained stable until 3 months after delivery. Because the newborn pineal does not produce melatonin rhythmically [234], the circadian rhythm of milk melatonin may serve the newborn for chronobiotic regulation. This hypothesis was tested by Cubero et al. [306]. They measured the content of tryptophan in breast milk in 16 infants of 12 weeks of age divided into two groups of natural or artificial feeding. Tryptophan amounts in milk showed a circadian rhythm peaking at 03:00 h, which coincided with the urinary excretion of 6-sulfatoxymelatonin in these infants. The variations of tryptophan in milk were associated with sleep efficiency of the neonates, whereas neonates under artificial feeding showed significantly poorer sleep. Thus, not only melatonin in milk but also the variations of tryptophan in milk, which resemble those of melatonin, may serve as a chronobiotic signal to communicate time of day information to breast-fed infants leading to consolidate the sleep-wake rhythm. Once the newborn circadian system matures, endogenous melatonin production occurs and provides chronobiologic information.

Melatonin in milk may have other important consequences for the newborn's health. In a study examining the etiologies of infantile colic and sleep disturbances, it was found that infants exclusively fed with breast milk had a significantly lower incidence of colic attack, lower severity of irritable attacks, and a trend for longer nocturnal sleep than infants fed with artificial milk [307]. Daily changes in melatonin were also assessed in diurnal and nocturnal samples of colostrum from healthy puerperal mothers and from mothers with mastitis [308]. Melatonin exhibits a circadian rhythm in the colostrum from normal mothers, but it is not apparent in mothers with mastitis. Among other conclusions, the data suggest the reduction in the transfer of melatonin or the metabolism (as a radical scavenger) of melatonin in breast milk during the inflammatory process. This could significantly impact the chronobiotic properties of natural milk feeding. The reader is reminded that during the intrauterine period, the fetus receives a circadian melatonin signal from the mother, and this signal disappears soon after delivery [233, 235]. Newborns have the capacity to produce melatonin [234], but not rhythmically until 4 months after delivery [309]. Consequently, newborns lack the endogenous signal to synchronize their functions rhythmically. Clearly, the melatonin in human milk functions as a potential chronobiotic [310] and thus breast milk feeding provides newborns an external synchronizer that they cannot themselves generate.

Several conclusions should be drawn from these data [311]: (a) the importance of nocturnal breast feeding in darkness or, alternatively, under red light, to prevent the light-induced melatonin suppression; (b) artificial milk formulas should be differentiate into diurnal and nocturnal milks, the latter containing melatonin and/or tryptophan as precursor of melatonin synthesis by the newborn [312], and (c) milk banks also should classify milk obtained from donors during day vs. those collected at night in darkness, if this ever occurs.

Studies on melatonin safety and toxicity

Melatonin is an uncommonly safe molecule. A reduction in endogenous physiological melatonin levels throughout life seems to cause greater organismal detriments [313] than does its excess or long-term usage. Low toxicity of high doses of melatonin was revealed in a thorough study by Jahnke et al. [260] conducted under the auspices of the National Institute of Environmental Health Sciences in the United States. In the study in question, several doses of melatonin, including at least three doses that by any standard would be considered massive, were administered to different groups of rats on days 6–19 of pregnancy (roughly equivalent to administering melatonin daily to pregnant women from week 15 to week 39 of pregnancy). The doses used by Jahnke et al. [260] were 0, 1, 10, 100, 150, or 200 mg/kg daily. These exceptionally large amounts of melatonin did not influence pregnancy in any noticeable way. These doses of melatonin exceed 70,000 times the doses recommended for sleep induction. For the three

highest doses, in some animals there was a slight aversion to treatment, some mild sedation (subjectively estimated) and a slight reduction in food intake and weight gain by the mothers. No untoward effects were observed, however, in either the mothers (serum 17 β-estradiol, progesterone, prolactin, or luteinizing hormone levels or glandular area of mammary tissue) or in the fetuses. There was no maternal morbidity/mortality. The fetuses (roughly 370 in each of the treatment groups) were surgically removed just prior to normal delivery and were examined in detail morphologically (external malformations, skeletal and visceral abnormalities); nothing unusual was uncovered. Interestingly, the pups of mothers treated with only diluent had a statistically insignificantly higher incidence of embryological malformations than did any of the melatonin-exposed offspring.

While the study of Jahnke et al. [260] has limitations since no biochemical/molecular parameters in the fetuses were evaluated and potential long-term behavioral consequences were not a consideration, the otherwise thoroughness of the investigation is of interest since no abnormalities of any type were noted in either the mothers or the fetuses, even at the melatonin dose of 200 mg/kg daily. According to the human equivalent dose calculation [314], it corresponds to a 70-kg human taking roughly 2.13 g of melatonin daily. An earlier investigation using mice reported no behavioral modifications after melatonin (100 mg/kg) in the short term [315].

Several clinical attempts to identify potential toxicities of melatonin have been performed using pharmacological doses of this indoleamine over relatively short intervals [316–318]. In the studies reviewed, no serious negative findings were reported. The doses of melatonin in these trials varied but surely caused blood levels of melatonin to rise above normal endogenous values.

Melatonin has been given to humans for a 1-month period at a relatively high dose (1 g daily taken per os) [319, 320]. They measured a number of blood and endocrine parameters at the conclusion of the study and reported nothing exceptional. Some drowsiness was noted by the subjects, but a thorough examination of the functions of the eyes, liver, kidney, and bone marrow revealed no evidence of toxicity. One gram of melatonin taken acutely each day surely caused extremely high pharmacological concentrations of this indoleamine in the blood.

Melatonin is widely used as an over-the-counter product in some countries and as a prescription medication in others. It has been available for more than two decades; an estimate of the total number of individuals who use melatonin daily has not been tabulated according to the knowledge of the present authors. On the other hand, no reports of illness have been linked to chronic melatonin use. The number of controlled studies that have been systematically performed in humans is limited. Considering the estimated large number of humans who routinely use melatonin, these individuals should be explored by epidemiologists for the purpose of comparing health parameters in humans who have regularly used melatonin for years vs. those who have never used this indoleamine.

There have been some unusual, extremely rare reactions to short-term melatonin use, e.g., one case of gynecomastia in a male [321] and one incident where it aggravated the signs of Crohn's disease [322]. Such unusual reactions should not be unexpected since every molecule, under some circumstances, occasionally initiates a reaction that is contrary to the norm. Interestingly, others have proposed the use of melatonin as a treatment for ulcerative colitis [323]. In the controlled studies that have been reported on melatonin usage, minor symptoms, e.g., headache, nausea, dizziness, etc., have been claimed but at essentially the same frequency as seen in the placebo-treated individuals.

Conclusions

The existence of multiple sites in the body where melatonin is synthesized may reflect an adaptive mechanism throughout the evolution. The normal physiological function of the body requires a homeostatic equilibrium among all organs and tissues. The chronobiotic system works to maintain all of these functions and uses melatonin as the main endogenous synchronizer. Melatonin is produced from tryptophan through serotonin, and it is synthesized in phylogenetically very ancient one-cell organisms including cyanobacteria, algae, and trypanosomes [324, 325]. The presence of melatonin very early in evolution suggests its importance for cell physiology. Primitive cells probably developed a defense mechanism against the UV-induced free radical generation, decomposing tryptophan, a primitive amino acid, into a series of metabolites with antioxidant properties. Among them, melatonin was probably the most efficient antioxidant because its metabolism to other antioxidative metabolites, AFMK and AMK, so that primitive cells used the melatonin antioxidant's cascade [326]. If these cells produced melatonin at a constant rate, its intracellular concentrations probably decreased during the day, when most of the UV irradiation occurred, because of its scavenger activity. This could be the origin of the circadian rhythms associated with melatonin changes throughout the day. These two functions, chronobiotic and free radical scavenging, were then associated with melatonin during the evolution. Their efficacy in adaptations of the organism to the environment is likely because melatonin is currently performing the same functions, and its chemical structure has remained unchanged over billions of years of evolution.

From these considerations, and the data herein reviewed, we can assume that melatonin has a double role in the body: synchronizing the organism's functions and protecting the

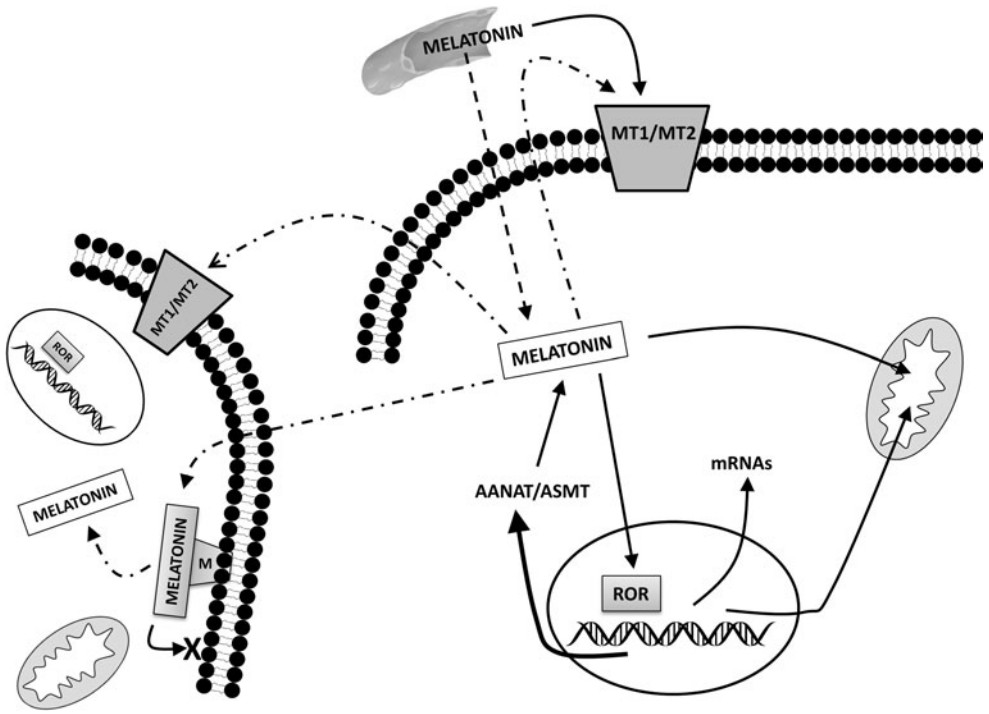

Fig. 3 Most of the melatonin present in extrapineal tissues is produced locally for regulatory processes. This melatonin may act via intracrine, autocrine, or paracrine mechanisms to maintain cell homeostasis

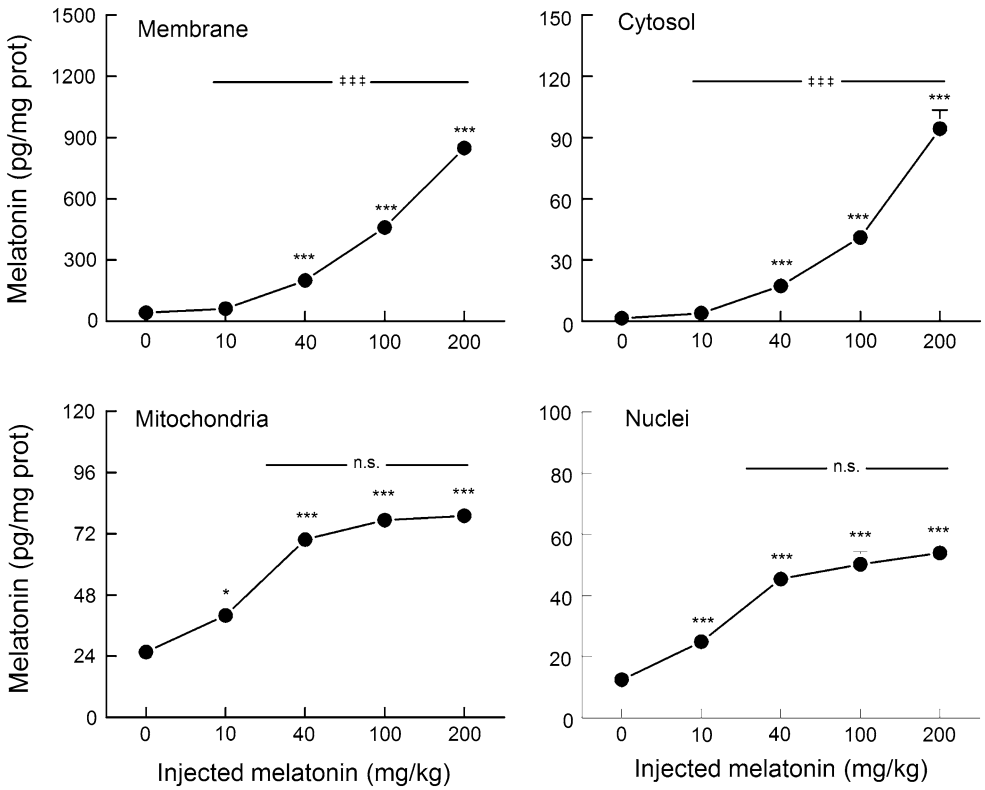

Fig. 4 Dose-dependent changes in the subcellular distribution of melatonin in rat liver. Control rats were intraperitoneally injected with either vehicle (0) or 10, 40, 100, or 200 mg/kg melatonin at 10:00 h, and killed 2 h later. *$p < 0.05$, and ***$p < 0.001$ vs. 0

cells from oxidative/inflammatory damage. This explains why extrapineal melatonin acts in a different manner than that of the pineal, and it has different regulatory mechanisms. While pineal-produced melatonin may to some extent regulate extrapineal melatonin synthesis, the latter is likely controlled by additional mechanisms including the redox status of the cell. Moreover, antioxidant and anti-inflammatory properties of melatonin requires higher concentrations of this indoleamine than those produced and released by the pineal gland; this may also explain the higher concentrations of melatonin in tissues and organs compared with its serum levels. Finally, the synthesis of melatonin by the same cells expressing its membrane and nuclear receptors support the intra-, auto-, and paracrine actions of this indoleamine, thereby refining its homeostatic potential (Fig. 3).

Another important consideration is the clinical relevance of melatonin. Due to the requirements of high melatonin content in the cell for its protective roles, for therapeutic purposes melatonin should possibly be used at high doses, which yield pharmacological levels in blood, but physiologically relevant levels in subcellular fractions. Following pharmacological studies in animals, the therapeutic doses of melatonin in humans range from 50 to 500 mg/day [15] (Fig. 4). These doses are required for the saturation of the intracellular therapeutic targets of melatonin. Nevertheless, studies on the regulatory mechanisms of the synthesis, metabolism, and action of melatonin at different subcellular levels would be necessary for the optimal understanding of its clinical applications. Taking into account that some disruption of the circadian rhythm could be produced when giving high doses of melatonin, the choice of the right timing of its administration is mandatory. Thus, the circadian rhythm of melatonin should be analyzed, which can be easily done with a non-invasive method such as in the saliva. Moreover, when high therapeutic doses of melatonin are used, the first consideration to have in mind is to counteract an oxidative and/or inflammatory stress; once the normal condition is reached, we can reduce the melatonin dose to restore any circadian disruption.

Acknowledgments This study was partially supported by grants from the Instituto de Salud Carlos III (RD12/0043/0005, PI08-1664), and from the Consejería de Innovación, Ciencia y Empresa, Junta de Andalucía (P07-CTS-03135 and CTS-101), Spain. ED is a fellow from the Consejería de Innovación, Ciencia y Empresa, Junta de Andalucía, Spain.

References

1. Lerner AB, Case JD, Takahasi Y, Lee TH, Mori W (1958) Isolation of melatonin, the pineal gland factor that lightens melanocytes. J Am Chem Soc 80:2587
2. Cardinali DP, Rosner JM (1971) Retinal localization of the hydroxyindole-*O*-methyl transferase (HIOMT) in the rat. Endocrinology 89:301–303
3. Bubenik GA, Brown GM, Uhlir I, Grota LJ (1974) Immunohistological localization of *N*-acetylindolealkylamines in pineal gland, retina and cerebellum. Brain Res 81:233–242
4. Stefulj J, Hortner M, Ghosh M, Schauenstein K, Rinner I, Wolfler A, Semmler J, Liebmann PM (2001) Gene expression of the key enzymes of melatonin synthesis in extrapineal tissues of the rat. J Pineal Res 30:243–247
5. Reiter RJ (1991) Melatonin: that ubiquitously-acting pineal hormone. News Physiol Sci 6:223–227
6. Tan DX, Manchester LC, Hardeland R, Lopez-Burillo S, Mayo JC, Sainz RM, Reiter RJ (2003) Melatonin: a hormone, a tissue factor, an autocoid, a paracoid, and an antioxidant vitamin. J Pineal Res 34:75–78
7. Reiter RJ, Fuentes-Broto L, Tan DX (2010) Melatonin: a multitasking molecule. Prog Brain Res 181:127–151
8. Vlahakes GJ, Wurtman RJ (1972) A Mg 2+ dependent hydroxyindole *O*-methyltransferase in rat Harderian gland. Biochim Biophys Acta 261:194–197
9. Reiter RJ, Richardson BA, Matthews SA, Lane SJ, Ferguson BN (1983) Rhythms in immunoreactive melatonin in the retina and Harderian gland of rats: persistence after pinealectomy. Life Sci 32:1229–1236
10. Raikhlin NT, Kvetnoy IM, Tolkachev VN (1975) Melatonin may be synthesised in enterochromaffin cells. Nature 255:344–345
11. Raikhlin NT, Kvetnoy IM (1976) Melatonin and enterochromaffine cells. Acta Histochem 55:19–24
12. Ozaki Y, Lynch HJ (1976) Presence of melatonin in plasma and urine or pinealectomized rats. Endocrinology 99:641–644
13. Poeggeler B, Cornelissen G, Huether G, Hardeland R, Jozsa R, Zeman M, Stebelova K, Olah A, Bubenik G, Pan W, Otsuka K, Schwartzkopff O, Bakken EE, Halberg F (2005) Chronomics affirm extending scope of lead in phase of duodenal vs. pineal circadian melatonin rhythms. Biomed Pharmacother 59(Suppl 1):S220–S224
14. Sanchez-Hidalgo M, de la Lastra CA, Carrascosa-Salmoral MP, Naranjo MC, Gomez-Corvera A, Caballero B, Guerrero JM (2009) Age-related changes in melatonin synthesis in rat extrapineal tissues. Exp Gerontol 44:328–334
15. Venegas C, García JA, Escames G, Ortiz F, López A, Doerrier C, García Corzo L, López LC, Reiter RJ, Acuña-Castroviejo D (2012) Extrapineal melatonin: analysis of its subcellular distribution and daily fluctuations. J Pineal Res 52:217–227
16. Tan DX, Chen LD, Poeggeler B, Manchester LC, Reiter RJ (1993) Melatonin: a potent, endogenous hydroxyl radical scavenger. Endocr J 1:52–60
17. Reiter RJ, Paredes SD, Manchester LC, Tan DX (2009) Reducing oxidative/nitrosative stress: a newly-discovered genre for melatonin. Crit Rev Biochem Mol Biol 44:175–200
18. Korkmaz A, Reiter RJ, Topal T, Manchester LC, Oter S, Tan DX (2009) Melatonin: an established antioxidant worthy of use in clinical trials. Mol Med 15:43–50
19. Bonnefont-Rousselot D, Collin F, Jore D, Gardes-Albert M (2011) Reaction mechanism of melatonin oxidation by reactive oxygen species in vitro. J Pineal Res 50:328–335
20. Hardeland R, Madrid JA, Tan DX, Reiter RJ (2012) Melatonin, the circadian multioscillator system and health: the need for detailed analyses of peripheral melatonin signaling. J Pineal Res 52:139–166
21. Reppert SM, Weaver DR, Ebisawa T (1994) Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. Neuron 13:1177–1185
22. Reppert SM, Godson C, Mahle CD, Weaver DR, Slaugenhaupt SA, Gusella JF (1995) Molecular characterization of a second melatonin receptor expressed in human retina and brain: the Mel1b melatonin receptor. Proc Natl Acad Sci USA 92:8734–8738

23. Dubocovich ML, Markowska M (2005) Functional MT1 and MT2 melatonin receptors in mammals. Endocrine 27:101–110
24. Brydon L, Roka F, Petit L, de CP, Tissot M, Barrett P, Morgan PJ, Nanoff C, Strosberg AD, Jockers R (1999) Dual signaling of human Mel1a melatonin receptors via G(i2), G(i3), and G(q/11) proteins. Mol Endocrinol 13:2025–2038
25. Petit L, Lacroix I, de CP, Strosberg AD, Jockers R (1999) Differential signaling of human Mel1a and Mel1b melatonin receptors through the cyclic guanosine 3′-5′-monophosphate pathway. Biochem Pharmacol 58:633–639
26. Garcia-Perganeda A, Pozo D, Guerrero JM, Calvo JR (1997) Signal transduction for melatonin in human lymphocytes: involvement of a pertussis toxin-sensitive G protein. J Immunol 159:3774–3781
27. Nosjean O, Ferro M, Coge F, Beauverger P, Henlin JM, Lefoulon F, Fauchere JL, Delagrange P, Canet E, Boutin JA (2000) Identification of the melatonin-binding site MT3 as the quinone reductase 2. J Biol Chem 275:31311–31317
28. Acuña-Castroviejo D, Reiter RJ, Menéndez Peláez A, Pablos MI, Burgos A (1994) Characterization of high-affinity melatonin binding sites in purified cell nuclei of rat liver. J Pineal Res 16:100–112
29. Becker-Andre M, Wiesenberg I, Schaeren-Wiemers N, Andre E, Missbach M, Saurat JH, Carlberg C (1994) Pineal gland hormone melatonin binds and activates an orphan of the nuclear receptor superfamily. J Biol Chem 269:28531–28534
30. Wiesenberg I, Missbach M, Carlberg C (1998) The potential role of the transcription factor RZR/ROR as a mediator of nuclear melatonin signaling. Restor Neurol Neurosci 12:143–150
31. Benitez-King G, Huerto-Delgadillo L, Anton-Tay F (1993) Binding of 3H-melatonin to calmodulin. Life Sci 53:201–207
32. Macias M, Escames G, León J, Coto-Montes A, Sbihi Y, Osuna A, Acuña-Castroviejo D (2003) Calreticulin-melatonin. An unexpected relationship. Eur J Biochem 270:832–840
33. Moore RY (2007) Suprachiasmatic nucleus in sleep-wake regulation. Sleep Med 8(Suppl 3):27–33
34. Sugden D, Klein DC (1983) Beta-adrenergic receptor control of rat pineal hydroxyindole-*O*-methyltransferase. Endocrinology 113:348–353
35. Schomerus C, Maronde E, Laedtke E, Korf HW (1996) Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) induce phosphorylation of the transcription factor CREB in subpopulations of rat pinealocytes: immunocytochemical and immunochemical evidence. Cell Tissue Res 286:305–313
36. Gonzalez S, Moreno-Delgado D, Moreno E, Perez-Capote K, Franco R, Mallol J, Cortes A, Casado V, Lluis C, Ortiz J, Ferre S, Canela E, McCormick PJ (2012) Circadian-related heteromerization of adrenergic and dopamine D(4) receptors modulates melatonin synthesis and release in the pineal gland. PLoS Biol 10:e1001347
37. Klein DC (2007) Arylalkylamine *N*-acetyltransferase: "the Timezyme". J Biol Chem 282:4233–4237
38. Ribelayga C, Pevet P, Simonneaux V (2000) HIOMT drives the photoperiodic changes in the amplitude of the melatonin peak of the Siberian hamster. Am J Physiol Regul Integr Comp Physiol 278:R1339–R1345
39. Cardinali DP, Lynch HJ, Wurtman RJ (1972) Binding of melatonin to human and rat plasma proteins. Endocrinology 91:1213–1218
40. Stehle JH, Saade A, Rawashdeh O, Ackermann K, Jilg A, Sebesteny T, Maronde E (2011) A survey of molecular details in the human pineal gland in the light of phylogeny, structure, function and chronobiological diseases. J Pineal Res 51:17–43
41. Coon SL, Del OE, Young WS III, Klein DC (2002) Melatonin synthesis enzymes in *Macaca mulatta*: focus on arylalkylamine *N*-acetyltransferase (EC 2.3.1.87). J Clin Endocrinol Metab 87:4699–4706
42. Maronde E, Saade A, Ackermann K, Goubran-Botros H, Pagan C, Bux R, Bourgeron T, Dehghani F, Stehle JH (2011) Dynamics in enzymatic protein complexes offer a novel principle for the regulation of melatonin synthesis in the human pineal gland. J Pineal Res 51:145–155
43. Hardeland R, Poeggeler B (2012) Melatonin and synthetic melatonergic agonists: actions and metabolism in the central nervous system. Cent Nerv Syst Agents Med Chem 12:189–216
44. Rodriguez C, Mayo JC, Sainz RM, Antolin I, Herrera F, Martin V, Reiter RJ (2004) Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res 36:1–9
45. Goldsmith CS, Bell-Pedersen D (2013) Diverse roles for MAPK signaling in circadian clocks. Adv Genet 84:1–39
46. Luchetti F, Canonico B, Betti M, Arcangeletti M, Pilolli F, Piroddi M, Canesi L, Papa S, Galli F (2010) Melatonin signaling and cell protection function. FASEB J 24:3603–3624
47. Jarzynka MJ, Passey DK, Johnson DA, Konduru NV, Fitz NF, Radio NM, Rasenick M, Benloucif S, Melan MA, Witt-Enderby PA (2009) Microtubules modulate melatonin receptors involved in phase-shifting circadian activity rhythms: in vitro and in vivo evidence. J Pineal Res 46:161–171
48. Luchetti F, Betti M, Canonico B, Arcangeletti M, Ferri P, Galli F, Papa S (2009) ERK MAPK activation mediates the antiapoptotic signaling of melatonin in UVB-stressed U937 cells. Free Radic Biol Med 46:339–351
49. Imbesi M, Uz T, Dzitoyeva S, Giusti P, Manev H (2008) Melatonin signaling in mouse cerebellar granule cells with variable native MT1 and MT2 melatonin receptors. Brain Res 1227:19–25
50. Baba K, Benleulmi-Chaachoua A, Journe AS, Kamal M, Guillaume JL, Dussaud S, Gbahou F, Yettou K, Liu C, Contreras-Alcantara S, Jockers R, Tosini G (2013) Heteromeric MT1/MT2 melatonin receptors modulate photoreceptor function. Sci Signal 6:ra89
51. León J, Macias M, Escames G, Camacho E, Khaldy H, Martin M, Espinosa A, Gallo MA, Acuña-Castroviejo D (2000) Structure-related inhibition of calmodulin-dependent nNOS activity by melatonin and synthetic knurenines. Mol Pharmacol 58: 967–975
52. Tomás-Zapico T, Álvarez-García O, Sierra V, Vega-Naredo I, Caballero B, García JJ, Acuña-Castroviejo D, Rodríguez MI, Tolivia D, Rodríguez-Colunga MJ, Coto-Montes A (2006) Oxidative damage in liver of senescence accelerated mice: gender-related response 4574. Can J Physiol Pharmacol 84:213–220
53. Acuña-Castroviejo D, López LC, Escames G, López A, García JA, Reiter RJ (2011) Melatonin-mitochondria interplay in health and disease. Curr Top Med Chem 11:221–240
54. Acuña-Castroviejo D, Martín M, Macías M, Escames G, León J, Khaldy H, Reiter RJ (2001) Melatonin, mitochondria and cellular bioenergetics. J Pineal Res 30:65–74
55. Tan DX, Reiter RJ, Manchester LC, Yan MT, El-Sawi M, Sainz RM, Mayo JC, Kohen R, Allegra M, Hardeland R (2002) Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. Curr Top Med Chem 2:181–197
56. Tomas-Zapico C, Coto-Montes A (2005) A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. J Pineal Res 39:99–104
57. Venegas C, García JA, Doerrier C, Volt H, Escames G, López LC, Reiter RJ, Acuña-Castroviejo D (2012) Analysis of the daily changes of melatonin receptors in the rat liver. J Pineal Res. doi:10.1111/jpi.12019
58. Sánchez-Sánchez AM, Martin V, Garcia-Santos G, Rodriguez-Blanco J, Casado-Zapico S, Suarez-Garnacho S, Antolin I,

Rodriguez C (2011) Intracellular redox state as determinant for melatonin antiproliferative vs cytotoxic effects in cancer cells. Free Radic Res 45:1333–1341
59. Muxel SM, Pires-Lapa MA, Monteiro AW, Cecon E, Tamura EK, Floeter-Winter LM, Markus RP (2012) NF-kappaB drives the synthesis of melatonin in RAW 264.7 macrophages by inducing the transcription of the arylalkylamine-*N*-acetyltransferase (AA-NAT) gene. PLoS One 7:e52010
60. Cristofanon S, Uguccioni F, Cerella C, Radogna F, Dicato M, Ghibelli L, Diederich M (2009) Intracellular prooxidant activity of melatonin induces a survival pathway involving NF-kappaB activation. Ann N Y Acad Sci 1171:472–478
61. Bizzarri M, Proietti S, Cucina A, Reiter RJ (2013) Molecular mechanisms of the pro-apoptotic actions of melatonin in cancer: a review. Expert Opin Ther Targets 17:1483–1496
62. Kadoma Y, Fujisawa S (2011) Radical-scavenging activity of melatonin, either alone or in combination with vitamin E, ascorbate or 2-mercaptoethanol as co-antioxidants, using the induction period method. In Vivo 25:49–53
63. Poynton RA, Hampton MB (2014) Peroxiredoxins as biomarkers of oxidative stress. Biochim Biophys Acta 1840:906–912
64. O'Neill JS, van OG, Dixon LE, Troein C, Corellou F, Bouget FY, Reddy AB, Millar AJ (2011) Circadian rhythms persist without transcription in a eukaryote. Nature 469:554–558
65. Edgar RS, Green EW, Zhao Y, van OG, Olmedo M, Qin X, Xu Y, Pan M, Valekunja UK, Feeney KA, Maywood ES, Hastings MH, Baliga NS, Merrow M, Millar AJ, Johnson CH, Kyriacou CP, O'Neill JS, Reddy AB (2012) Peroxiredoxins are conserved markers of circadian rhythms. Nature 485:459–464
66. Sung JH, Cho EH, Kim MO, Koh PO (2009) Identification of proteins differentially expressed by melatonin treatment in cerebral ischemic injury–a proteomics approach. J Pineal Res 46:300–306
67. Kvetnoy IM (1999) Extrapineal melatonin: location and role within diffuse neuroendocrine system. Histochem J 31:1–12
68. Menendez-Pelaez A, Reiter RJ (1993) Distribution of melatonin in mammalian tissues: the relative importance of nuclear versus cytosolic localization. J Pineal Res 15:59–69
69. Lapi D, Vagnani S, Cardaci E, Paterni M, Colantuoni A (2011) Rat pial microvascular responses to melatonin during bilateral common carotid artery occlusion and reperfusion. J Pineal Res 51:136–144
70. Huether G, Poeggeler B, Reimer A, George A (1992) Effect of tryptophan administration on circulating melatonin levels in chicks and rats: evidence for stimulation of melatonin synthesis and release in the gastrointestinal tract. Life Sci 51:945–953
71. Sjoblom M, Flemstrom G (2003) Melatonin in the duodenal lumen is a potent stimulant of mucosal bicarbonate secretion. J Pineal Res 34:288–293
72. LeRoith D, McGuinness M, Shemer J, Stannard B, Lanau F, Faria TN, Kato H, Werner H, Adamo M, Roberts CT Jr (1992) Insulin-like growth factors. Biol Signals 1:173–181
73. Mackenzie SM, Connell JM, Davies E (2012) Non-adrenal synthesis of aldosterone: a reality check. Mol Cell Endocrinol 350:163–167
74. Young SN, Gauthier S, Kiely ME, Lal S, Brown GM (1984) Effect of oral melatonin administration on melatonin, 5-hydroxyindoleacetic acid, indoleacetic acid, and cyclic nucleotides in human cerebrospinal fluid. Neuroendocrinology 39:87–92
75. Reppert SM, Perlow MJ, Tamarkin L, Klein DC (1979) A diurnal melatonin rhythm in primate cerebrospinal fluid. Endocrinology 104:295–301
76. Skinner DC, Malpaux B (1999) High melatonin concentrations in third ventricular cerebrospinal fluid are not due to Galen vein blood recirculating through the choroid plexus. Endocrinology 140:4399–4405
77. Tan DX, Manchester LC, Sanchez-Barcelo E, Mediavilla MD, Reiter RJ (2010) Significance of high levels of endogenous melatonin in Mammalian cerebrospinal fluid and in the central nervous system. Curr Neuropharmacol 8:162–167
78. Jimenez-Jorge S, Guerrero JM, Jimenez-Caliani AJ, Naranjo MC, Lardone PJ, Carrillo-Vico A, Osuna C, Molinero P (2007) Evidence for melatonin synthesis in the rat brain during development. J Pineal Res 42:240–246
79. Deguchi T (1978) Ontogenesis of circadian rhythm of melatonin synthesis in pineal gland of rat. J Neural Transm Suppl 13: 115–128
80. Uz T, Qu T, Sugaya K, Manev H (2002) Neuronal expression of arylalkylamine *N*-acetyltransferase (AANAT) mRNA in the rat brain. Neurosci Res 42:309–316
81. Uz T, Ahmed R, Akhisaroglu M, Kurtuncu M, Imbesi M, Dirim AA, Manev H (2005) Effect of fluoxetine and cocaine on the expression of clock genes in the mouse hippocampus and striatum. Neuroscience 134:1309–1316
82. Pang SF, Brown GM (1983) Regional concentrations of melatonin in the rat brain in the light and dark period. Life Sci 33:1199–1204
83. Hardeland R (2010) Melatonin metabolism in the central nervous system. Curr Neuropharmacol 8:168–171
84. Sallanon M, Claustrat B, Touret M (1982) Presence of melatonin in various cat brainstem nuclei determined by radioimmunoassay. Acta Endocrinol (Copenh) 101:161–165
85. Reiter RJ (1986) Normal patterns of melatonin levels in the pineal gland and body fluids of humans and experimental animals. J Neural Transm Suppl 21:35–54
86. Liu YJ, Zhuang J, Zhu HY, Shen YX, Tan ZL, Zhou JN (2007) Cultured rat cortical astrocytes synthesize melatonin: absence of a diurnal rhythm. J Pineal Res 43:232–238
87. Liu YJ, Meng FT, Wang LL, Zhang LF, Cheng XP, Zhou JN (2012) Apolipoprotein E influences melatonin biosynthesis by regulating NAT and MAOA expression in C6 cells. J Pineal Res 52:397–402
88. Acuña-Castroviejo D, Lowenstein PR, Rosenstein RE, Cardinali DP (1986) Diurnal variations of benzodiapine binding in rat cerebral cortex: disruption by pinealectomy. J Pineal Res 3:101–109
89. Acuña-Castroviejo D, Romero HE, Cardinali DP (1986) Changes in gamma-aminobutiryc acid hight affinity binding to cerebral cortex membranes after pinealectomy or melatonin administrations to rats. Neuroendocrinology 43:24–31
90. Acuña-Castroviejo D, Fernández B, Gomar MD, Del Aguila CM, Castillo JL (1990) Influence of the pituitary-adrenal axis on benzodiazepine receptor binding to rat cerebral cortex. Neuroendocrinology 51:97–103
91. Gomar MD, Fernández B, Castillo JL, Del Aguila CM, Acuña-Castroviejo D (1993) Melatonin counteracts pinealectomy-dependent decreases in rat brain [3H] flunitrazepam binding through an opioid mechanism. Neurosci Lett 164:149–153
92. Acuña-Castroviejo D, Escames G, Macías M, Muñoz-Hoyos A, Molina-Carballo A, Arauzo M, Montes R, Vives F (1995) Cell protective role of melatonin in the brain. J Pineal Res 19:57–63
93. Khaldy H, Leon J, Escames G, Bikjdaouene L, Garcia JJ, Acuña-Castroviejo D (2002) Circadian rhythms of dopamine and dihydroxyphenyl acetic acid in the mouse striatum: effects of pinealectomy and of melatonin treatment. Neuroendocrinology 75:201–208
94. Marquez de Prado B, Castaneda TR, Galindo A, del AA, Segovia G, Reiter RJ, Mora F (2000) Melatonin disrupts circadian rhythms of glutamate and GABA in the neostriatum of the aware rat: a microdialysis study. J Pineal Res 29:209–216
95. de Prado BM, Reiter RJ, Mora F (2003) Perfusion of melatonin into the prefrontal cortex disrupts the circadian rhythm

of acetylcholine but not of locomotor activity. J Pineal Res 35:283–287
96. Castaneda TR, de Prado BM, Prieto D, Mora F (2004) Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: modulation by light. J Pineal Res 36:177–185
97. Acuña-Castroviejo D, Castillo JL, Fernández B, Gomar MD, Del Aguila CM (1992) Modulation by pineal gland of ouabain high affinity binding sites in rat cerebral cortex. Am J Physiol 262:R698–R706
98. Castillo JL, Vives F, Reiter RJ, Acuña-Castroviejo D (1993) Pineal modulation of the rat caudate-putamen spontaneous neuronal activity: roles of melatonin and vasotocin. J Pineal Res 15:147–152
99. Escames G, Macías M, León J, García JJ, Khaldy H, Martín M, Vives F, Acuña-Castroviejo D (2001) Calcium-dependent effects of melatonin inhibition of glutamatergic response in rat striatum. J Neuroendocrinol 13:459–466
100. Khaldy H, León J, Escames G, Bikjdaouene L, Acuña-Castroviejo D (2003) Synergistic effects of melatonin and deprenyl protect against MPTP-induced mitochondrial damage and DA depletion. Neurobiol Aging 24:491–500
101. Molina-Carballo A, Muñoz-Hoyos A, Reiter RJ, Sánchez-Forte M, Moreno-Madrid F, Rufo-Campos M, Molina-Font JA, Acuña-Castroviejo D (1997) Utility of high doses of melatonin as adjunctive anticonvulsant therapy in a child with severe myoclonic experience: two years' experience. J Pineal Res 23:97–105
102. Bikjdaouene L, Escames G, León J, Ferrer JMR, Khaldy H, Vives F, Acuña-Castroviejo D (2003) Changes in brain amino acids and nitric oxide after melatonin administration to pentylenetetrazole-induced seizures in rats. J Pineal Res 35:54–60
103. Molina-Carballo A, Muñoz-Hoyos A, Sánchez-Forte M, Uberos-Fernández J, Moreno-Madrid F, Acuña-Castroviejo D (2007) Melatonin increases following convulsive seizures may be related to its anticonvulsant properties at physiological concentrations. Neuropediatrics 38:122–125
104. Philo R, Reiter RJ (1978) Characterization of pinealectomy induced convulsions in the Mongolian gerbil (*Meriones unguiculatus*). Epilepsia 19:485–492
105. Anton-Tay F, Diaz JL, Fernandez-Guardiola A (1971) On the effect of melatonin upon human brain. Its possible therapeutic implications. Life Sci I 10:841–850
106. Mason R, Brooks A (1988) The electrophysiological effects of melatonin and a putative melatonin antagonist (*N*-acetyltryptamine) on rat suprachiasmatic neurones in vitro. Neurosci Lett 95:296–301
107. Cardinali DP, Furio AM, Brusco LI (2011) The use of chronobiotics in the resynchronization of the sleep/wake cycle. Therapeutical application in the early phases of Alzheimer's disease. Recent Pat Endocr Metab Immune Drug Discov 5:80–90
108. Cardinali DP, Srinivasan V, Brzezinski A, Brown GM (2012) Melatonin and its analogs in insomnia and depression. J Pineal Res 52:365–375
109. Esposito E, Cuzzocrea S (2010) Antiinflamatory activity of melaotnin in central nervous system. Curr Neuropharmacol 8:228–242
110. Rosales-Corral S, Reiter RJ, Tan DX, Ortiz GG, Lopez-Armas G (2010) Functional aspects of redox control during neuroinflammation. Antioxid Redox Signal 13:193–247
111. Xiong YF, Chen Q, Chen J, Zhou J, Wang HX (2011) Melatonin reduces the impairment of axonal transport and axonopathy induced by calyculin A. J Pineal Res 50:319–327
112. Fu J, Zhao SD, Liu HJ, Yuan QH, Liu SM, Zhang YM, Ling EA, Hao AJ (2011) Melatonin promotes proliferation and differentiation of neural stem cells subjected to hypoxia in vitro. J Pineal Res 51:104–112
113. Kilic U, Yilmaz B, Ugur M, Yuksel A, Reiter RJ, Hermann DM, Kilic E (2012) Evidence that membrane-bound G protein-coupled melatonin receptors MT1 and MT2 are not involved in the neuroprotective effects of melatonin in focal cerebral ischemia. J Pineal Res 52:228–235
114. Rosales-Corral SA, Acuna-Castroviejo D, Coto-Montes A, Boga JA, Manchester LC, Fuentes-Broto L, Korkmaz A, Ma S, Tan DX, Reiter RJ (2012) Alzheimer's disease: pathological mechanisms and the beneficial role of melatonin. J Pineal Res 52:167–202
115. Dominguez-Alonso A, Ramirez-Rodriguez G, Benitez-King G (2012) Melatonin increases dendritogenesis in the hilus of hippocampal organotypic cultures. J Pineal Res 52:427–436
116. Jimenez-Rubio G, Ortiz-Lopez L, Benitez-King G (2012) Melatonin modulates cytoskeletal organization in the rat brain hippocampus. Neurosci Lett 511:47–51
117. Reiter RJ, Benitez-King G (2009) Melatonn reduces neuronal loss and cytoskeletal deterioration: implications for psychiatry. Salud Mental 32:3–11
118. Chern CM, Liao JF, Wang YH, Shen YC (2012) Melatonin ameliorates neural function by promoting endogenous neurogenesis through the MT2 melatonin receptor in ischemic-stroke mice. Free Radic Biol Med 52:1634–1647
119. Manda K, Reiter RJ (2010) Melatonin maintains adult hippocampal neurogenesis and cognitive functions after irradiation. Prog Neurobiol 90:60–68
120. Crupi R, Mazzon E, Marino A, La SG, Bramanti P, Spina E, Cuzzocrea S (2011) Melatonin's stimulatory effect on adult hippocampal neurogenesis in mice persists after ovariectomy. J Pineal Res 51:353–360
121. Ramirez-Rodriguez G, Ortiz-Lopez L, Dominguez-Alonso A, Benitez-King GA, Kempermann G (2011) Chronic treatment with melatonin stimulates dendrite maturation and complexity in adult hippocampal neurogenesis of mice. J Pineal Res 50:29–37
122. Rennie K, De BM, Pappas BA (2009) Melatonin promotes neurogenesis in dentate gyrus in the pinealectomized rat. J Pineal Res 47:313–317
123. Yoo DY, Kim W, Lee CH, Shin BN, Nam SM, Choi JH, Won MH, Yoon YS, Hwang IK (2012) Melatonin improves D-galactose-induced aging effects on behavior, neurogenesis, and lipid peroxidation in the mouse dentate gyrus via increasing pCREB expression. J Pineal Res 52:21–28
124. López LC, Escames G, López A, García JA, Doerrier C, Acuña-Castroviejo D (2010) Melatonin, neurogenesis, and aging brain. Open Neuroendocrinol J 3:121–133
125. Wiechmann AF, Summers JA (2008) Circadian rhythms in the eye: the physiological significance of melatonin receptors in ocular tissues. Prog Retin Eye Res 27:137–160
126. Tosini G, Baba K, Hwang CK, Iuvone PM (2012) Melatonin: an underappreciated player in retinal physiology and pathophysiology. Exp Eye Res 103:82–89
127. Bubenik GA, Purtill RA, Brown GM, Grota LJ (1978) Melatonin in the retina and the Harderian gland. Ontogeny, diurnal variations and melatonin treatment. Exp Eye Res 27:323–333
128. Yu HS, Pang SF, Tang PL (1981) Increase in the level of retinal melatonin and persistence of its diurnal rhythm in rats after pinealectomy. J Endocrinol 91:477–481
129. Miller L, Stier M, Lovenberg W (1980) Evidence for the presence of N-acetyl transferase in rat retina. Comp Biochem Physiol C 66:213–216
130. Tosini G, Menaker M (1996) Circadian rhythms in cultured mammalian retina. Science 272:419–421

131. Tosini G, Menaker M (1998) The clock in the mouse retina: melatonin synthesis and photoreceptor degeneration. Brain Res 789:221–228
132. Liu C, Fukuhara C, Wessel JH, Iuvone PM, Tosini G (2004) Localization of Aa-nat mRNA in the rat retina by fluorescence in situ hybridization and laser capture microdissection. Cell Tissue Res 315:197–201
133. Wiechmann AF, Burden MA (1999) Regulation of AA-NAT and HIOMT gene expression by butyrate and cyclic AMP in Y79 human retinoblastoma cells. J Pineal Res 27:116–121
134. Zmijewski MA, Sweatman TW, Slominski AT (2009) The melatonin-producing system is fully functional in retinal pigment epithelium (ARPE-19). Mol Cell Endocrinol 307:211–216
135. Dubocovich ML (1988) Pharmacology and function of melatonin receptors. FASEB J 2:2765–2773
136. Zawilska J, Iuvone PM (1989) Catecholamine receptors regulating serotonin *N*-acetyltransferase activity and melatonin content of chicken retina and pineal gland: D2-dopamine receptors in retina and alpha-2 adrenergic receptors in pineal gland. J Pharmacol Exp Ther 250:86–92
137. Rosen RB, Hu DN, Chen M, McCormick SA, Walsh J, Roberts JE (2012) Effects of melatonin and its receptor antagonist on retinal pigment epithelial cells against hydrogen peroxide damage. Mol Vis 18:1640–1648
138. Marchiafava PL, Longoni B (1999) Melatonin as an antioxidant in retinal photoreceptors. J Pineal Res 26:184–189
139. Siu AW, Reiter RJ, To CH (1999) Pineal indoleamines and vitamin E reduce nitric oxide-induced lipid peroxidation in rat retinal homogenates. J Pineal Res 27:122–128
140. Ogino N, Matsumura M, Shirakawa H, Tsukahara I (1983) Phagocytic activity of cultured retinal pigment epithelial cells from chick embryo: inhibition by melatonin and cyclic AMP, and its reversal by taurine and cyclic GMP. Ophthalmic Res 15:72–89
141. Rohde BH, McLaughlin MA, Chiou LY (1985) Existence and role of endogenous ocular melatonin. J Ocul Pharmacol 1:235–243
142. Mhatre MC, van Jaarsveld AS, Reiter RJ (1988) Melatonin in the lacrimal gland: first demonstration and experimental manipulation. Biochem Biophys Res Commun 153:1186–1192
143. Abe M, Itoh MT, Miyata M, Shimizu K, Sumi Y (2000) Circadian rhythm of serotonin *N*-acetyltransferase activity in rat lens. Exp Eye Res 70:805–808
144. Quay WB (1984) Increases in volume, fluid content, and lens weight of eyes following systemic administration of melatonin. J Pineal Res 1:3–13
145. Abe M, Itoh MT, Miyata M, Ishikawa S, Sumi Y (1999) Detection of melatonin, its precursors and related enzyme activities in rabbit lens. Exp Eye Res 68:255–262
146. Itoh MT, Takahashi N, Abe M, Shimizu K (2007) Expression and cellular localization of melatonin-synthesizing enzymes in the rat lens. J Pineal Res 42:92–96
147. Biesalski HK, Welker HA, Thalmann R, Vollrath L (1988) Melatonin and other serotonin derivatives in the guinea pig membranous cochlea. Neurosci Lett 91:41–46
148. Lopez-Gonzalez MA, Guerrero JM, Delgado F (1997) Presence of the pineal hormone melatonin in rat cochlea: its variations with lighting conditions. J Pineal Res 238:81–83
149. Carrillo-Vico A, Guerrero JM, Lardone PJ, Reiter RJ (2005) A review of the multiple actions of melatonin on the immune system. Endocrine 27:189–200
150. Carrillo-Vico A, Calvo JR, Abreu P, Lardone PJ, Garcia-Maurino S, Reiter RJ, Guerrero JM (2004) Evidence of melatonin synthesis by human lymphocytes and its physiological significance: possible role as intracrine, autocrine, and/or paracrine substance. FASEB J 18:537–539
151. Carrillo-Vico A, Lardone PJ, Fernandez-Santos JM, Martin-Lacave I, Calvo JR, Karasek M, Guerrero JM (2005) Human lymphocyte-synthesized melatonin is involved in the regulation of the interleukin-2/interleukin-2 receptor system. J Clin Endocrinol Metab 90:992–1000
152. Jimenez-Jorge S, Jimenez-Caliani AJ, Guerrero JM, Naranjo MC, Lardone PJ, Carrillo-Vico A, Osuna C, Molinero P (2005) Melatonin synthesis and melatonin-membrane receptor (MT1) expression during rat thymus development: role of the pineal gland. J Pineal Res 39:77–83
153. Naranjo MC, Guerrero JM, Rubio A, Lardone PJ, Carrillo-Vico A, Carrascosa-Salmoral MP, Jimenez-Jorge S, Arellano MV, Leal-Noval SR, Leal M, Lissen E, Molinero P (2007) Melatonin biosynthesis in the thymus of humans and rats. Cell Mol Life Sci 64:781–790
154. Pang SF, Tang PL, Yu HS, Yip MK (1982) The level of *N*-acetylserotonin and melatonin in the brain of male rats: diurnal variations and effects of pinealectomy. J Exp Zool 219:271–276
155. Tamarkin L, Reppert SM, Klein DC, Pratt B, Goldman BD (1980) Studies on the daily pattern of pineal melatonin in the Syrian hamster. Endocrinology 107:1525–1529
156. Martins E Jr, Ferreira AC, Skorupa AL, Afeche SC, Cipolla-Neto J, Costa Rosa LF (2004) Tryptophan consumption and indoleamines production by peritoneal cavity macrophages. J Leukoc Biol 75:1116–1121
157. Escames G, Khaldy H, León López J, González L, Acuña-Castroviejo D (2004) Changes in iNOS activity, oxidative stress and melatonin levels in hypertensive patients treated with lacidipine 4489. J Hypertens 22:629–635
158. Tan DX, Manchester LC, Reiter RJ, Qi WB, Zhang M, Weintraub ST, Cabrera J, Sainz RM, Mayo JC (1999) Identification of highly elevated levels of melatonin in bone marrow: its origin and significance. Biochim Biophys Acta 1472:206–214
159. Conti A, Conconi S, Hertens E, Skwarlo-Sonta K, Markowska M, Maestroni JM (2000) Evidence for melatonin synthesis in mouse and human bone marrow cells. J Pineal Res 28:193–202
160. Kuehn HS, Gilfillan AM (2007) G protein-coupled receptors and the modification of FcepsilonRI-mediated mast cell activation. Immunol Lett 113:59–69
161. Maldonado MD, Mora-Santos M, Naji L, Carrascosa-Salmoral MP, Naranjo MC, Calvo JR (2010) Evidence of melatonin synthesis and release by mast cells. Possible modulatory role on inflammation. Pharmacol Res 62:282–287
162. Gaudet SJ, Slominski A, Etminan M, Pruski D, Paus R, Namboodiri MA (1993) Identification and characterization of two isozymic forms of arylamine *N*-acetyltransferase in Syrian hamster skin. J Invest Dermatol 101:660–665
163. Slominski A, Baker J, Rosano TG, Guisti LW, Ermak G, Grande M, Gaudet SJ (1996) Metabolism of serotonin to *N*-acetylserotonin, melatonin, and 5-methoxytryptamine in hamster skin culture. J Biol Chem 271:12281–12286
164. Slominski A, Semak I, Pisarchik A, Sweatman T, Szczesniewski A, Wortsman J (2002) Conversion of L-tryptophan to serotonin and melatonin in human melanoma cells. FEBS Lett 511:102–106
165. Slominski A, Pruski D (1993) Melatonin inhibits proliferation and melanogenesis in rodent melanoma cells. Exp Cell Res 206:189–194
166. Cabrera J, Negrin G, Estevez F, Loro J, Reiter RJ, Quintana J (2010) Melatonin decreases cell proliferation and induces melanogenesis in human melanoma SK-MEL-1 cells. J Pineal Res 49:45–54
167. Slominski A, Pisarchik A, Semak I, Sweatman T, Wortsman J, Szczesniewski A, Slugocki G, McNulty J, Kauser S, Tobin DJ, Jing C, Johansson O (2002) Serotoninergic and melatoninergic systems are fully expressed in human skin. FASEB J 16:896–898

168. Slominski A, Pisarchik A, Zbytek B, Tobin DJ, Kauser S, Wortsman J (2003) Functional activity of serotoninergic and melatoninergic systems expressed in the skin. J Cell Physiol 196:144–153
169. Slominski A, Pisarchik A, Wortsman J (2004) Expression of genes coding melatonin and serotonin receptors in rodent skin. Biochim Biophys Acta 1680:67–70
170. Slominski A, Fischer TW, Zmijewski MA, Wortsman J, Semak I, Zbytek B, Slominski RM, Tobin DJ (2005) On the role of melatonin in skin physiology and pathology. Endocrine 27:137–148
171. Tunali T, Sener G, Yarat A, Emekli N (2005) Melatonin reduces oxidative damage to skin and normalizes blood coagulation in a rat model of thermal injury. Life Sci 76:1259–1265
172. Izykowska I, Cegielski M, Gebarowska E, Podhorska-Okolow M, Piotrowska A, Zabel M, Dziegiel P (2009) Effect of melatonin on human keratinocytes and fibroblasts subjected to UVA and UVB radiation in vitro. In Vivo 23:739–745
173. Fischer TW, Sweatman TW, Semak I, Sayre RM, Wortsman J, Slominski A (2006) Constitutive and UV-induced metabolism of melatonin in keratinocytes and cell-free systems. FASEB J 20:1564–1566
174. Fischer TW, Slominski A, Zmijewski MA, Reiter RJ, Paus R (2008) Melatonin as a major skin protectant: from free radical scavenging to DNA damage repair. Exp Dermatol 17:713–730
175. Slominski A, Tobin DJ, Zmijewski MA, Wortsman J, Paus R (2008) Melatonin in the skin: synthesis, metabolism and functions. Trends Endocrinol Metab 19:17–24
176. Lincoln GA, Ebling FJ (1985) Effect of constant-release implants of melatonin on seasonal cycles in reproduction, prolactin secretion and moulting in rams. J Reprod Fertil 73:241–253
177. Lincoln GA, Klandorf H, Anderson N (1980) Photoperiodic control of thyroid function and wool and horn growth in rams and the effect of cranial sympathectomy. Endocrinology 107:1543–1548
178. Bubenik GA, Brown GM, Grota LJ (1977) Immunohistological localization of melatonin in the rat digestive system. Experientia 33:662–663
179. Bubenik GA (1980) Localization of melatonin in the digestive tract of the rat. Effect of maturation, diurnal variation, melatonin treatment and pinealectomy. Horm Res 12:313–323
180. Bubenik GA (2002) Gastrointestinal melatonin: localization, function, and clinical relevance. Dig Dis Sci 47:2336–2348
181. Hong GX, Pang SF (1995) *N*-acetyltransferase activity in the quail (Coturnix coturnix jap) duodenum. Comp Biochem Physiol B Biochem Mol Biol 112:251–255
182. Bubenik GA, Pang SF, Hacker RR, Smith PS (1996) Melatonin concentrations in serum and tissues of porcine gastrointestinal tract and their relationship to the intake and passage of food. J Pineal Res 21:251–256
183. Vician M, Zeman M, Herichova I, Jurani M, Blazicek P, Matis P (1999) Melatonin content in plasma and large intestine of patients with colorectal carcinoma before and after surgery. J Pineal Res 27:164–169
184. Huether G (1993) The contribution of extrapineal sites of melatonin synthesis to circulating melatonin levels in higher vertebrates. Experientia 49:665–670
185. Huether G (1994) Melatonin synthesis in the gastrointestinal tract and the impact of nutritional factors on circulating melatonin. Ann N Y Acad Sci 719:146–158
186. Huether G, Messner M, Rodenbeck A, Hardeland R (1998) Effect of continuous melatonin infusions on steady-state plasma melatonin levels in rats under near physiological conditions. J Pineal Res 24:146–151
187. Bubenik GA, Pang SF (1997) Melatonin levels in the gastrointestinal tissues of fish, amphibians, and a reptile. Gen Comp Endocrinol 106:415–419
188. Bubenik GA, Ayles HL, Friendship RM, Brown GM, Ball RO (1998) Relationship between melatonin levels in plasma and gastrointestinal tissues and the incidence and severity of gastric ulcers in pigs. J Pineal Res 24:62–66
189. Bubenik GA (2008) Thirty-four years since the discovery of gastrointestinal melatonin. J Physiol Pharmacol 59(Suppl 2):33–51
190. Messner M, Hardeland R, Rodenbeck A, Huether G (1998) Tissue retention and subcellular distribution of continuously infused melatonin in rats under near physiological conditions. J Pineal Res 25:251–259
191. Bubenik GA, Brown GM (1997) Pinealectomy reduces melatonin levels in the serum but not in the gastrointestinal tract of rats. Biol Signals 6:40–44
192. Bubenik GA, Ball RO, Pang SF (1992) The effect of food deprivation on brain and gastrointestinal tissue levels of tryptophan, serotonin, 5-hydroxyindoleacetic acid, and melatonin. J Pineal Res 12:7–16
193. Chik CL, Ho AK, Brown GM (1987) Effect of food restriction on 24-h serum and pineal melatonin content in male rats. Acta Endocrinol (Copenh) 115:507–513
194. Dubbels R, Reiter RJ, Klenke E, Goebel A, Schnakenberg E, Ehlers C, Schiwara HW, Schloot W (1995) Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. J Pineal Res 18:28–31
195. Hattori A, Migitaka H, Iigo M, Itoh M, Yamamoto K, Ohtani-Kaneko R, Hara M, Suzuki T, Reiter RJ (1995) Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. Biochem Mol Biol Int 35:627–634
196. Reiter RJ, Manchester LC, Tan DX (2005) Melatonin in walnuts: influence on levels of melatonin and total antioxidant capacity of blood. Nutrition 21:920–924
197. Bubenik GA, Pang SF (1994) The role of serotonin and melatonin in gastrointestinal physiology: ontogeny, regulation of food intake, and mutual serotonin-melatonin feedback. J Pineal Res 16:91–99
198. Bubenik GA, Hacker RR, Brown GM, Bartos L (1999) Melatonin concentrations in the luminal fluid, mucosa, and muscularis of the bovine and porcine gastrointestinal tract. J Pineal Res 26:56–63
199. Tan DX, Manchester LC, Reiter RJ, Qi W, Hanes MA, Farley NJ (1999) High physiological levels of melatonin in the bile of mammals. Life Sci 65:2523–2529
200. Manchester LC, Poeggeler B, Alvares FL, Ogden GB, Reiter RJ (1995) Melatonin immunoreactivity in the photosynthetic prokaryote *Rhodospirillum rubrum*: implications for an ancient antioxidant system. Cell Mol Biol Res 41:391–395
201. Tan DX, Hardeland R, Manchester LC, Paredes SD, Korkmaz A, Sainz RM, Mayo JC, Fuentes-Broto L, Reiter RJ (2010) The changing biological roles of melatonin during evolution: from an antioxidant to signals of darkness, sexual selection and fitness. Biol Rev Camb Philos Soc 85:607–623
202. Bubenik GA, Pang SF, Cockshut JR, Smith PS, Grovum LW, Friendship RM, Hacker RR (2000) Circadian variation of portal, arterial and venous blood levels of melatonin in pigs and its relationship to food intake and sleep. J Pineal Res 28:9–15
203. Iriti M, Varoni EM, Vitalini S (2010) Melatonin in traditional Mediterranean diets. J Pineal Res 49:101–105
204. Peuhkuri K, Sihvola N, Korpela R (2012) Dietary factors and fluctuating levels of melatonin. Food Nutr Res 56

205. Paredes SD, Korkmaz A, Manchester LC, Tan DX, Reiter RJ (2009) Phytomelatonin: a review. Exp Bot 60:57–69
206. Boccalandro HE, Gonzalez CV, Wunderlin DA, Silva MF (2011) Melatonin levels, determined by LC-ESI-MS/MS, fluctuate during the day/night cycle in *Vitis vinifera* cv. *Malbec*: evidence of its antioxidant role in fruits. J Pineal Res 51:226–232
207. Ramakrishna A, Giridhar P, Sankar KU, Ravishankar GA (2012) Melatonin and serotonin profiles in beans of Coffea species. J Pineal Res 52:470–476
208. Poon AM, Chow PH, Mak AS, Pang SF (1997) Autoradiographic localization of 2[125I]iodomelatonin binding sites in the gastrointestinal tract of mammals including humans and birds. J Pineal Res 23:5–14
209. Menendez-Pelaez A, Poeggeler B, Reiter RJ, Barlow-Walden L, Pablos MI, Tan DX (1993) Nuclear localization of melatonin in different mammalian tissues: immunocytochemical and radioimmunoassay evidence. J Cell Biochem 53:373–382
210. Acuña-Castroviejo D, Pablos M, Menéndez Peláez A, Reiter RJ (1993) Melatonin receptors in purified cell nuclei of liver. Res Commun Chem Pathol Pharmacol 82:253–256
211. Lucchelli A, Santagostino-Barbone MG, Tonini M (1997) Investigation into the contractile response of melatonin in the guinea-pig isolated proximal colon: the role of 5-HT4 and melatonin receptors. Br J Pharmacol 121:1775–1781
212. Pozo MJ, Gómez-Pinilla PJ, Camello-Almaraz C, Martin-Cano E, Pascua P, Rol MA, Acuña-Castroviejo D, Camello PJ (2010) Melatonin, a potential therapeutic agent for smooth muscle-related pathological conditions. Curr Med Chem 17:4150–4165
213. Tsukamoto K, Ariga H, Mantyh C, Pappas TN, Yanagi H, Yamamura T, Takahashi T (2007) Luminally released serotonin stimulates colonic motility and accelerates colonic transit in rats. Am J Physiol Regul Integr Comp Physiol 293:R64–R69
214. Bubenik GA (1986) The effect of serotonin, *N*-acetylserotonin, and melatonin on spontaneous contractions of isolated rat intestine. J Pineal Res 3:41–54
215. Harlow HJ, Weekley BL (1986) Effect of melatonin on the force of spontaneous contractions of in vitro rat small and large intestine. J Pineal Res 3:277–284
216. Barajas-Lopez C, Peres AL, Espinosa-Luna R, Reyes-Vazquez C, Prieto-Gomez B (1996) Melatonin modulates cholinergic transmission by blocking nicotinic channels in the Guinea-pig submucous plexus. Eur J Pharmacol 312:319–325
217. Lee PP, Shiu SY, Chow PH, Pang SF (1995) Regional and diurnal studies of melatonin and melatonin binding sites in the duck gastro-intestinal tract. Biol Signals 4:212–224
218. Lewinski A, Rybicka I, Wajs E, Szkudlinski M, Pawlikowski M (1991) Influence of pineal indoleamines on the mitotic activity of gastric and colonic mucosa epithelial cells in the rat: interaction with omeprazole. J Pineal Res 10:104–108
219. Flemstrom G, Sjoblom M, Jedstedt G, Akerman KE (2003) Short fasting dramatically decreases rat duodenal secretory responsiveness to orexin A but not to VIP or melatonin. Am J Physiol Gastrointest Liver Physiol 285:G1091–G1096
220. Sjoblom M, Flemstrom G (2004) Central nervous alpha1-adrenoceptor stimulation induces duodenal luminal release of melatonin 2. J Pineal Res 36:103–108
221. Flemstrom G, Sjoblom M (2005) Epithelial cells and their neighbors. II. New perspectives on efferent signaling between brain, neuroendocrine cells, and gut epithelial cells. Am J Physiol Gastrointest Liver Physiol 289:G377–G380
222. Sommansson A, Saudi WS, Nylander O, Sjoblom M (2013) Melatonin inhibits alcohol-induced increases in duodenal mucosal permeability in rats in vivo. Am J Physiol Gastrointest Liver Physiol 305:G95–G105
223. Sommansson A, Nylander O, Sjoblom M (2013) Melatonin decreases duodenal epithelial paracellular permeability via a nicotinic receptor-dependent pathway in rats in vivo. J Pineal Res 54:282–291
224. Konturek PC, Brzozowski T, Konturek SJ (2011) Gut clock: implication of circadian rhythms in the gastrointestinal tract. J Physiol Pharmacol 62:139–150
225. Reiter RJ, Rosales-Corral S, Coto-Montes A, Boga JA, Tan DX, Davis JM, Konturek PC, Konturek SJ, Brzozowski T (2011) The photoperiod, circadian regulation and chronodisruption: the requisite interplay between the suprachiasmatic nuclei and the pineal and gut melatonin. J Physiol Pharmacol 62:269–274
226. Chen CQ, Fichna J, Bashashati M, Li YY, Storr M (2011) Distribution, function and physiological role of melatonin in the lower gut. World J Gastroenterol 17:3888–3898
227. El MS, Guerin P, Menezo Y (1999) Expression of genes encoding antioxidant enzymes in human and mouse oocytes during the final stages of maturation. Mol Hum Reprod 5:720–725
228. Heubner O (1898) Tumor der glandula pinealis. M Tsch Med Wschr 10:146–166
229. Lane EA, Moss HB (1985) Pharmacokinetics of melatonin in man: first pass hepatic metabolism. J Clin Endocrinol Metab 61:1214–1216
230. Messner M, Huether G, Lorf T, Ramadori G, Schworer H (2001) Presence of melatonin in the human hepatobiliary-gastrointestinal tract. Life Sci 69:543–551
231. Lissoni P, Barni S, Crispino S, Tancini G, Fraschini F (1989) Endocrine and immune effects of melatonin therapy in metastatic cancer patients. Eur J Cancer Clin Oncol 25:789–795
232. Qi W, Tan DX, Reiter RJ, Kim SJ, Manchester LC, Cabrera J, Sainz RM, Mayo JC (1999) Melatonin reduces lipid peroxidation and tissue edema in cerulein-induced acute pancreatitis in rats. Dig Dis Sci 44:2257–2262
233. Muñoz A, Rodríguez T, Molina A, Martínez JJ, Ruiz C, Acuña-Castroviejo D (1992) Melatonin concentration in the umbilical artery and vein in human preterm and term neonates and neonates with acute fetal distress. J Pineal Res 13:184–191
234. Jaldo Alba F, Muñoz-Hoyos A, Molina-Carballo A, Molina Font JA, Acuña-Castroviejo D (1993) Light deprivation increases plasma levels of melatonin during the first 72 h of life in human infants. Acta Endocrinol 129:442–445
235. Muñoz-Hoyos A, Jaldo Alba F, Molina-Carballo A, Rodríguez Cabezas T, Molina Font JA, Acuña-Castroviejo D (1993) Absence of plasma melatonin circadian rhythm during the first 72 hours of life human infants. J Clin Endocrinol Metab 77:699–703
236. Dollins AB, Zhdanova IV, Wurtman RJ, Lynch HJ, Deng MH (1994) Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance. Proc Natl Acad Sci USA 91:1824–1828
237. Aizawa S, Tokura H, Morita T (2002) The administration of exogenous melatonin during the daytime lowers the thermoregulatory setpoint in humans. J Therm Biol 27:115–119
238. Nogueira K, Liberman B, Pimentel-Filho FR, Goldman J, Silva ME, Vieira JO, Buratini JA, Cukiert A (2002) hCG-secreting pineal teratoma causing precocious puberty: report of two patients and review of the literature. J Pediatr Endocrinol Metab 15:1195–1201
239. Hoffman RA, Reiter RJ (1965) Pineal gland: influence on gonads of male hasmters. Science 148:1609–1611
240. Reiter RJ (1980) The pineal and its hormones in the control of reproduction in mammals. Endocr Rev 1:109–131
241. Revel FG, Masson-Pevet M, Pevet P, Mikkelsen JD, Simonneaux V (2009) Melatonin controls seasonal breeding by a network of hypothalamic targets. Neuroendocrinology 90:1–14
242. Reiter RJ, Tan DX, Manchester LC, Paredes SD, Mayo JC, Sainz RM (2009) Melatonin and reproduction revisited. Biol Reprod 81:445–456

243. Turek FW, Desjardins C, Menaker M (1976) Melatonin-induced inhibition of testicular function in adult golden hamsters. Proc Soc Exp Biol Med 151:502–506
244. Chemineau P, Guillaume D, Migaud M, Thiery JC, Pellicer-Rubio MT, Malpaux B (2008) Seasonality of reproduction in mammals: intimate regulatory mechanisms and practical implications. Reprod Domest Anim 43(Suppl 2):40–47
245. Brzezinski A, Seibel MM, Lynch HJ, Deng MH, Wurtman RJ (1987) Melatonin in human preovulatory follicular fluid. J Clin Endocrinol Metab 64:865–867
246. Ronnberg L, Kauppila A, Leppaluoto J, Martikainen H, Vakkuri O (1990) Circadian and seasonal variation in human preovulatory follicular fluid melatonin concentration. J Clin Endocrinol Metab 71:492–496
247. Cohen M, Roselle D, Chabner B, Schmidt TJ, Lippman M (1978) Evidence for a cytoplasmic melatonin receptor. Nature 274:894–895
248. Niles LP, Wang J, Shen L, Lobb DK, Younglai EV (1999) Melatonin receptor mRNA expression in human granulosa cells. Mol Cell Endocrinol 156:107–110
249. Nakamura Y, Tamura H, Takayama H, Kato H (2003) Increased endogenous level of melatonin in preovulatory human follicles does not directly influence progesterone production. Fertil Steril 80:1012–1016
250. Itoh MT, Ishizuka B, Kuribayashi Y, Amemiya A, Sumi Y (1999) Melatonin, its precursors, and synthesizing enzyme activities in the human ovary. Mol Hum Reprod 5:402–408
251. Klein DC, Roseboom PH, Coon SL (1996) New light is shining on the melatonin rhythm enzyme: the first postcloning view. Trends Endocrinol Metab 7:106–112
252. Itoh MT, Ishizuka B, Kudo Y, Fusama S, Amemiya A, Sumi Y (1997) Detection of melatonin and serotonin *N*-acetyltransferase and hydroxyindole-*O*-methyltransferase activities in rat ovary. Mol Cell Endocrinol 136:7–13
253. Tijmes M, Pedraza R, Valladares L (1996) Melatonin in the rat testis: evidence for local synthesis. Steroids 61:65–68
254. Tinajero JC, Fabbri A, Dufau ML (1992) Regulation of corticotropin-releasing factor secretion from Leydig cells by serotonin. Endocrinology 130:1780–1788
255. Vera H, Tijmes M, Ronco AM, Valladares LE (1993) Melatonin binding sites in interstitial cells from immature rat testes. Biol Res 26:337–340
256. Tamura H, Nakamura Y, Terron MP, Flores LJ, Manchester LC, Tan DX, Sugino N, Reiter RJ (2008) Melatonin and pregnancy in the human. Reprod Toxicol 25:291–303
257. Tamura H, Takasaki A, Miwa I, Taniguchi K, Maekawa R, Asada H, Taketani T, Matsuoka A, Yamagata Y, Shimamura K, Morioka H, Ishikawa H, Reiter RJ, Sugino N (2008) Oxidative stress impairs oocyte quality and melatonin protects oocytes from free radical damage and improves fertilization rate. J Pineal Res 44:280–287
258. Tamura H, Takayama H, Nakamura Y, Reiter RJ, Sugino N (2008) Fetal/placental regulation of maternal melatonin in rats. J Pineal Res 44:335–340
259. Tamura H, Takasaki A, Taketani T, Tanabe M, Kizuka F, Lee L, Tamura I, Maekawa R, Aasada H, Yamagata Y, Sugino N (2012) The role of melatonin as an antioxidant in the follicle. J Ovarian Res 5:5
260. Jahnke G, Marr M, Myers C, Wilson R, Travlos G, Price C (1999) Maternal and developmental toxicity evaluation of melatonin administered orally to pregnant Sprague Dawley rats. Toxicol Sci 50:271–279
261. Okatani Y, Okamoto K, Hayashi K, Wakatsuki A, Tamura S, Sagara Y (1998) Maternal-fetal transfer of melatonin in pregnant women near term. J Pineal Res 25:129–134
262. Fernández B, Montero A, Del Aguila CM, Malde JL, Castillo JL, Acuña-Castroviejo D (1992) Relationship between pineal activity and the hypotalamus-hypophysis-gonadal axis in human pregnancy and in the cord blood of term Infants. II. Melatonin and protein hormones. Steroid Biochem (Life Sci Adv) 11:97–103
263. Fernández B, Malde JL, Castillo JL, Montero A, Del Aguila CM, Acuña-Castroviejo D (1992) Relationship between pineal activity and the hypotalamus-hypophysis-gonadal axis in human pregnancy and in the cord blood of term infants. I. Melatonin and steroid hormones. Steroid Biochem (Life Sci Adv) 11:89–96
264. Fernández B, Montero A, Malde JL, Acuña-Castroviejo D (1990) Relationship between renal clearance rates and plasma levels of FSH, bhCG, DHAs and melatonin during normal pregnancy. Med Sci Res 18:713–714
265. Iwasaki S, Nakazawa K, Sakai J, Kometani K, Iwashita M, Yoshimura Y, Maruyama T (2005) Melatonin as a local regulator of human placental function. J Pineal Res 39:261–265
266. Lanoix D, Beghdadi H, Lafond J, Vaillancourt C (2008) Human placental trophoblasts synthesize melatonin and express its receptors. J Pineal Res 45:50–60
267. Schlabritz-Loutsevitch N, Hellner N, Middendorf R, Muller D, Olcese J (2003) The human myometrium as a target for melatonin. J Clin Endocrinol Metab 88:908–913
268. Lanoix D, Ouellette R, Vaillancourt C (2006) Expression of melatoninergic receptors in human placental choriocarcinoma cell lines. Hum Reprod 21:1981–1989
269. Handwerger S (1991) Clinical counterpoint: the physiology of placental lactogen in human pregnancy. Endocr Rev 12:329–336
270. Bronson FH (1995) Seasonal variation in human reproduction: environmental factors. Q Rev Biol 70:141–164
271. Rojansky N, Benshushan A, Meirsdorf S, Lewin A, Laufer N, Safran A (2000) Seasonal variability in fertilization and embryo quality rates in women undergoing IVF. Fertil Steril 74:476–481
272. Kauppila A, Kivela A, Pakarinen A, Vakkuri O (1987) Inverse seasonal relationship between melatonin and ovarian activity in humans in a region with a strong seasonal contrast in luminosity. J Clin Endocrinol Metab 65:823–828
273. Berga SL, Mortola JF, Yen SS (1988) Amplification of nocturnal melatonin secretion in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab 66:242–244
274. Bellastella A, Sinisi AA, Criscuolo T, De BA, Carella C, Iorio S, Sinisi AM, Parlato F, Venditto T, Pisano G (1995) Melatonin and the pituitary-thyroid axis status in blind adults: a possible resetting after puberty. Clin Endocrinol (Oxf) 43:707–711
275. Vaughan GM, Meyer GG, Reiter RJ (1978) Evidence for a pineal-gonadal relationship in humans. In: Reiter RJ (ed) The pineal and reproduction. Karger, Basel, pp 191–223
276. Debus OM, Lerchl A, Bothe HW, Bremer J, Fiedler B, Franssen M, Koehring J, Steils M, Kurlemann G (2002) Spontaneous central melatonin secretion and resorption kinetics of exogenous melatonin: a ventricular CSF study. J Pineal Res 33:213–217
277. Longatti P, Perin A, Rizzo V, Comai S, Giusti P, Costa CV (2007) Ventricular cerebrospinal fluid melatonin concentrations investigated with an endoscopic technique. J Pineal Res 42:113–118
278. Leston J, Harthe C, Brun J, Mottolese C, Mertens P, Sindou M, Claustrat B (2010) Melatonin is released in the third ventricle in humans. A study in movement disorders. Neurosci Lett 469:294–297

279. Tricoire H, Locatelli A, Chemineau P, Malpaux B (2002) Melatonin enters the cerebrospinal fluid through the pineal recess. Endocrinology 143:84–90
280. Rizzo V, Porta C, Moroni M, Scoglio E, Moratti R (2002) Determination of free and total (free plus protein-bound) melatonin in plasma and cerebrospinal fluid by high-performance liquid chromatography with fluorescence detection. J Chromatogr B Anal Technol Biomed Life Sci 774:17–24
281. Silva SO, Ximenes VF, Livramento JA, Catalani LH, Campa A (2005) High concentrations of the melatonin metabolite, N1-acetyl-N2-formyl-5-methoxykynuramine, in cerebrospinal fluid of patients with meningitis: a possible immunomodulatory mechanism. J Pineal Res 39:302–306
282. Seifman MA, Adamides AA, Nguyen PN, Vallance SA, Cooper DJ, Kossmann T, Rosenfeld JV, Morganti-Kossmann MC (2008) Endogenous melatonin increases in cerebrospinal fluid of patients after severe traumatic brain injury and correlates with oxidative stress and metabolic disarray. J Cereb Blood Flow Metab 28:684–696
283. Mayo JC, Sainz RM, Tan DX, Hardeland R, Leon J, Rodriguez C, Reiter RJ (2005) Anti-inflammatory actions of melatonin and its metabolites, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK), in macrophages. J Neuroimmunol 165:139–149
284. Zhou JN, Liu RY, Kamphorst W, Hofman MA, Swaab DF (2003) Early neuropathological Alzheimer's changes in aged individuals are accompanied by decreased cerebrospinal fluid melatonin levels. J Pineal Res 35:125–130
285. Yaga K, Reiter RJ, Richardson BA (1993) Tryptophan loading increases daytime serum melatonin levels in intact and pinealectomized rats. Life Sci 52:1231–1238
286. Zee PC, Mehta R, Turek FW, Blei AT (1991) Portacaval anastomosis disrupts circadian locomotor activity and pineal melatonin rhythms in rats. Brain Res 560:17–22
287. Montagnese S, Middleton B, Mani AR, Skene DJ, Morgan MY (2010) On the origin and the consequences of circadian abnormalities in patients with cirrhosis. Am J Gastroenterol 105:1773–1781
288. Vining RF, McGinley RA, Symons RG (1983) Hormones in saliva: mode of entry and consequent implications for clinical interpretation. Clin Chem 29:1752–1756
289. Morineau G, Boudi A, Barka A, Gourmelen M, Degeilh F, Hardy N, al-Halnak A, Soliman H, Gosling JP, Julien R, Brerault JL, Boudou P, Aubert P, Villette JM, Pruna A, Galons H, Fiet J (1997) Radioimmunoassay of cortisone in serum, urine, and saliva to assess the status of the cortisol-cortisone shuttle. Clin Chem 43:1397–1407
290. Swinkels LM, van Hoof HJ, Ross HA, Smals AG, Benraad TJ (1992) Low ratio of androstenedione to testosterone in plasma and saliva of hirsute women. Clin Chem 38:1819–1823
291. Groschl M (2009) The physiological role of hormones in saliva. BioEssays 31:843–852
292. Bubenik GA (1980) Immunohistological localization of melatonin in the salivary gland of the rat. Adv Biosci 29:95–112
293. Withyachumnarnkul B, Wongprapairot P, Trakulrungsi W (1987) Dynamic uptake of radioactive substance in rat salivary gland following 3H-melatonin administration. J Pineal Res 4:169–175
294. Shimozuma M, Tokuyama R, Tatehara S, Umeki H, Ide S, Mishima K, Saito I, Satomura K (2011) Expression and cellular localization of melatonin-synthesizing enzymes in rat and human salivary glands. Histochem Cell Biol 135:389–396
295. Cevik-Aras H, Ekstsrom J (2008) Melatonin-evoked in vivo secretion of protein and amylase from the parotid gland of the anaesthetised rat. J Pineal Res 45:413–421
296. Laakso ML, Porkka-Heiskanen T, Alila A, Stenberg D, Johansson G (1990) Correlation between salivary and serum melatonin: dependence on serum melatonin levels. J Pineal Res 9:39–50
297. McIntyre IM, Norman TR, Burrows GD, Armstrong SM (1987) Melatonin rhythm in human plasma and saliva. J Pineal Res 4:177–183
298. Cevik-Aras H, Godoy T, Ekstrom J (2011) Melatonin-induced protein synthesis in the rat parotid gland. J Physiol Pharmacol 62:95–99
299. Maestroni GJM, Sulli A, Pizzorni C, Villaggio B, Cutolo M (2002) Melatonin in rheumatoid arthritis. Ann N Y Acad Sci 966:271–275
300. Straub RH, Cutolo M (2007) Circadian rhythms in rheumatoid arthritis: implications for pathophysiology and therapeutic management. Arthritis Rheum 56:399–408
301. Mitchell MD, Sayers L, Keirse MJ, Anderson AB, Turnbull AC (1978) Melatonin in amniotic fluid during human parturition. Br J Obstet Gynaecol 85:684–686
302. Kivela A, Kauppila A, Leppaluoto J, Vakkuri O (1989) Serum and amniotic fluid melatonin during human labor. J Clin Endocrinol Metab 69:1065–1068
303. Ciesla W (1998) Low ACTH and high melatonin concentrations in amniotic fluid as hormonal markers of high risk of fetal abnormalities. Preliminary studies. Prenat Diagn 18:980–983
304. Xu DX, Wang H, Ning H, Zhao L, Chen YH (2007) Maternally administered melatonin differentially regulates lipopolysaccharide-induced proinflammatory and anti-inflammatory cytokines in maternal serum, amniotic fluid, fetal liver, and fetal brain. J Pineal Res 43:74–79
305. Illnerova H, Buresova M, Presl J (1993) Melatonin rhythm in human milk. J Clin Endocrinol Metab 77:838–841
306. Cubero J, Valero V, Sanchez J, Rivero M, Parvez H, Rodriguez AB, Barriga C (2005) The circadian rhythm of tryptophan in breast milk affects the rhythms of 6-sulfatoxymelatonin and sleep in newborn. Neuroendocrinol Lett 26:657–661
307. Cohen EA, Hadash A, Shehadeh N, Pillar G (2012) Breastfeeding may improve nocturnal sleep and reduce infantile colic: potential role of breast milk melatonin. Eur J Pediatr 171:729–732
308. Pontes GN, Cardoso EC, Carneiro-Sampaio MM, Markus RP (2006) Injury switches melatonin production source from endocrine (pineal) to paracrine (phagocytes)—melatonin in human colostrum and colostrum phagocytes. J Pineal Res 41:136–141
309. Jan JE, Wasdell MB, Freeman RD, Bax M (2007) Evidence supporting the use of melatonin in short gestation infants. J Pineal Res 42:22–27
310. Arslanoglu S, Bertino E, Nicocia M, Moro GE (2012) WAPM Working Group on Nutrition: potential chronobiotic role of human milk in sleep regulation. J Perinat Med 40:1–8
311. Sanchez-Barcelo EJ, Mediavilla MD, Reiter RJ (2011) Clinical uses of melatonin in pediatrics. Int J Pediatr 2011:892624
312. Muñoz-Hoyos A, Amorós-Rodriguez I, Molina-Carballo A, Uberos-Fernández J, Acuña-Castroviejo D (1996) Pineal response after pyridoxine test in children. J Neural Transm 103:833–842
313. Reiter RJ, Tan D, Kim SJ, Manchester LC, Qi W, Garcia JJ, Cabrera JC, El-Sokkary G, Rouvier-Garay V (1999) Augmentation of indices of oxidative damage in life-long melatonin-deficient rats. Mech Ageing Dev 110:157–173
314. Reagan-Shaw S, Nihal M, Ahmad N (2008) Dose translation from animal to human studies revisited. FASEB J 22:659–661
315. Barchas J, DaCosta F, Spector S (1967) Acute pharmacology of melatonin. Nature 214:919–920
316. Seabra ML, Bignotto M, Pinto LR Jr, Tufik S (2000) Randomized, double-blind clinical trial, controlled with placebo, of the toxicology of chronic melatonin treatment. J Pineal Res 29:193–200

317. Reiter RJ, Korkmaz A (2008) Clinical aspects of melatonin. Saudi Med J 29:1537–1547
318. Sanchez-Barcelo EJ, Mediavilla MD, Tan DX, Reiter RJ (2010) Clinical uses of melatonin: evaluation of human trials. Curr Med Chem 17:2070–2095
319. Nordlund JJ, Lerner AB (1977) The effects of oral melatonin on skin color and on the release of pituitary hormones. J Clin Endocrinol Metab 45:768–774
320. Lerner AB, Nordlund JJ (1979) Melatonin: clinical biochemistry. J Neural Transm 13:339–347
321. De Bleecker JL, Lamont BH, Verstraete AG, Schelfhout VJ (1999) Melatonin and painful gynecomastia. Neurology 53:435–436
322. Calvo JR, Guerrero JM, Osuna C, Molinero P, Carrillo-Vico A (2002) Melatonin triggers Crohn's disease symptoms. J Pineal Res 32:277–278
323. Terry PD, Villinger F, Bubenik GA, Sitaraman SV (2009) Melatonin and ulcerative colitis: evidence, biological mechanisms, and future research. Inflamm Bowel Dis 15:134–140
324. Poeggeler B, Hardeland R (1994) Detection and quantification of melatonin in a dinoflagellate, *Gonyaulax polyedra*: solutions to the problem of methoxyindole destruction in non-vertebrate material. J Pineal Res 17:1–10
325. Macias M, Rodriguez-Cabezas MN, Reiter RJ, Osuna A, Acuna-Castroviejo D (1999) Presence and effects of melatonin in *Trypanosoma cruzi*. J Pineal Res 27:86–94
326. Hardeland R, Balzer I, Poeggeler B, Fuhrberg B, Uria H, Behrmann G, Wolf R, Meyer TJ, Reiter RJ (1995) On the primary functions of melatonin in evolution: mediation of photoperiodic signals in a unicell, photooxidation, and scavenging of free radicals. J Pineal Res 18:104–111