Chapter 2

How Can Molecular Pharmacology Help Understand the Multiple Actions of Melatonin: 20 Years of Research and Trends

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Additional information is available at the end of the chapter

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Abstract

Melatonin actions are so numerous that a naive reader might become suspicious at such wonders. In a systematic way, we would like to summarize the various approaches that led to what is scientifically sounded in terms of molecular pharmacology: where and how melatonin is acting as a molecule, what can be its action as an antioxidant per se, and its side effects at a molecular level not as a drug or in vivo. Finally, the nature of the relationship between melatonin and mitochondria should be decrypted as well. The road we took from 1987 up to now, and particularly after 1995, will be mentioned with special considerations to the receptors from various species and our goals beyond that; the synthesis and catabolism of melatonin and their link to other enzymes; the discovery of the MT_3 binding sites, and what's left to understand on this particularly interesting target; and the search for agonists that occulted part of the potential discovery of true and potent antagonists, a situation quite unique among the G-protein-coupled receptors.

Keywords: melatonin, molecular pharmacology, MT_1, MT_2, catabolism

1. Introduction

Melatonin is a neurohormone synthesized by the pineal gland at night. The longer the night, the higher the concentration of melatonin in the blood. Even if new information is modulating this basic principle, this rhythmicity has been the basis of many published observations linking melatonin to many physiological features of animals, including human. This comprises the daily changes in light and the way the body understands the successions of dark and clear

periods but also the circannual rhythmicity, during which animals prepare for the harsher winter period during which access to food is more difficult. By "measuring" the length of melatonin synthesis periods (directly proportional to the length of the nights), animals start to modify their physiology in order to prepare the time to come: accumulation of fat (fat storing) for some, food storing for others, and preparing the bodies to reproduction for all animals at the best period to avoid the exposure of the newborns to cold and difficult conditions. Apparently, humans have lost this advanced capacity in our ever lit-up society.

New evidences recently caught our attention and challenge our ways of understanding the melatonin actions and pathways, whether because it seems that light can be "seen" by the body without the relay of melatonin or because one finds that melatonin is synthesized in mitochondria in all parts of the brain, as opposed to the pivotal and ancient statement: melatonin is synthesized by the pineal gland only. Nowadays, one can also see many publications claiming that melatonin helps cure cancer as well as so many other diseases (see a non-exhaustive selection of such actions since 2015 in **Table 1**). All these information should be treated with respect, integrated inside our decade-old knowledge and carefully evaluated as a contribution to a bigger picture. The basics on melatonin can be found in some recent reviews [1–5]. The present chapter concentrates on the molecular pharmacology of melatonin. This small molecule, derived from tryptophan, has a limited number of recognized targets. It is synthesized and catabolized by a limited and well-known number of enzymes that have been described in the past (see **Figure 1** for a simplistic summary). The core of the discussion seems always to be the same: how this molecule can be active on so many pathological events?

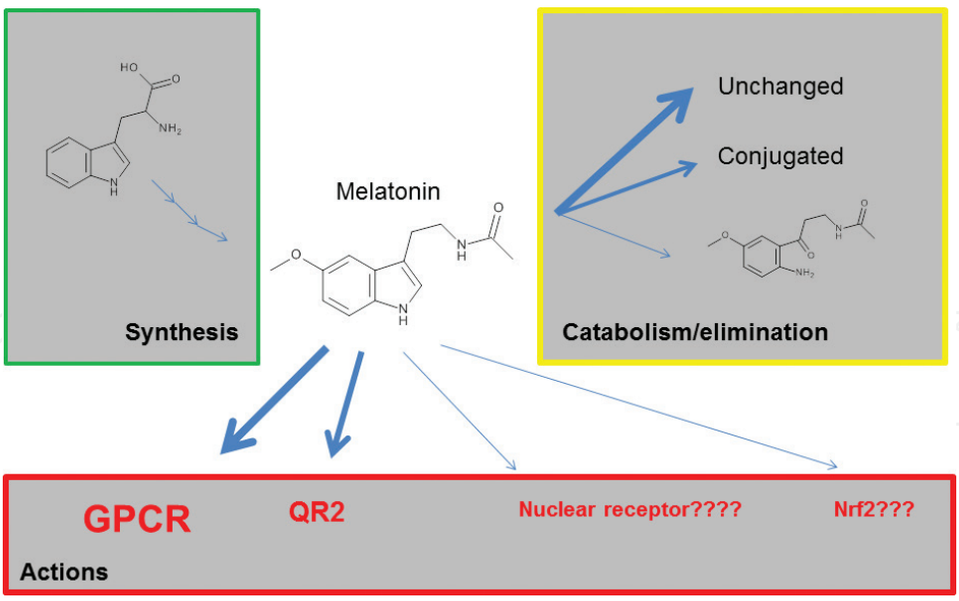

Figure 1. A simplistic summary of melatonin-related proteins. Melatonin is synthesized from tryptophan by a series of enzyme the the limiting step of which is catalyzed by Arylalkylamine N-acetyl transferase (green box); melatonin is excreted mainly unchanged from mammalian bodies or conjugated either by UDP-glucuronosyltranserases or by sulfotranferases, but it is also catabolized by indoleamine-2,3-dioxygenase, myeloperoxidase or cytochrome c (yellow box). At the molecular level, the targets of melatonin are mainly: Its two melatonin receptors, MT_1 and MT_2, QR2 (formerly known as MT_3). Furthermore, putative targets might exist such as nuclear receptors and particularly Nrf2 that might explain some of the anti-oxidant capacities of melatonin (red box).

How antioxidant this molecule is and why? What makes it so special? Some of these points are reviewed in the present chapter based on two decades of research in this area.

Beyond these biochemical features, melatonin has two unique features: it is very soluble but seems to travel freely through biological membranes, and its possible toxicity is extremely low (although some human cases of undesirable effects were reported), permitting scientists to give on many models very large amounts of the compound in cellulo and in vivo without apparent associated major toxicity. It was thus obvious that in many cases, the activity at those "pharmacological" dosages led to surprisingly numerous therapeutic properties of this molecule. Furthermore, the discovery that melatonin had, in cellulo and in vivo, antioxidant properties added to the multiple possibilities of use of melatonin, leading to this apparent paramount of therapeutic properties.

2. Melatonin synthesis

Melatonin is mostly synthesized starting from tryptophan in the pineal gland by a series of enzymes, the limiting one being arylalkylamine *N*-acetyltransferase (AANAT) also known as the timezyme [6]. Many studies have been performed on this enzyme and its requirements in terms of substrate specificity and inhibitor research, in particular in human [7, 8]. Over the years, several groups hypothesized and reported the possibility that melatonin was also synthesized in mitochondria (see also below, Section 6.4), suggesting that the antioxidant properties of the molecule would confer a strong resistance of mitochondria to the generation of ROS, a common feature of those subcellular organelles. Indeed, if the dogma was, in the 1950s and later on, that melatonin was mainly synthesized in the pineal gland, a fact that was clearly confirmed by surgical removing of the gland would lead to a major reduction of circulating melatonin and to the loss of some of the circadian and circannual rhythms; several papers co-indicated that such local synthesis that does exist should be taken into consideration (see, for instance, Stehle et al. [9]). What is more troubling is the recent precise description of melatonin synthesis in mitochondria, at least in brain-derived organelles [10] as well as the presence of a functional GPCR (MT_1). Intuitively, many previous strong knowledge would go against the finding that melatonin is synthesized in mitochondria, even if that was recently restricted to brain-derived mitochondria [11]. But melatonin is also known to "travel" freely inside membranes. Thus in order to stay inside mitochondria, it should be sequestered inside them in order to prevent the damages of ROS production—a common and key feature of the respiratory chain—thanks to the antioxidant properties of melatonin (see below, Section 6.1.2, for further discussion).

No revolution, nevertheless, occurred in the way melatonin is synthesized. It comes from several steps. All those enzymes have been cloned and studied, including from human origin. The particular case of AANAT catalyzing the limiting step of the synthesis has been at the source of the seminal work of David Klein's laboratory (see, for instance [6, 7]). This enzyme is destroyed during the day and active only at night. The way it is regulated is different according to species, but it seems a formidable waste of energy to handle it that way (synthesis and immediate destruction for "nothing") [12, 13], strongly suggesting by the way this pathway is regulated that it is of major

importance for physiology. The enzyme was cloned in our laboratory, and a thorough study of its substrate and co-substrate specificities was reported [8]. It was attempting, at one stage, to try to find specific inhibitors of the enzyme, in order to better understand in in situ situations the roles of melatonin at various locations. Several publications including ours reported those efforts [14–18]. If one particular point should be stressed, it is the elegant ways analogues of an intermediary state of the substrate/co-substrate complex permitted to turn molecules into powerful inhibitors, although overall fragile ones [19], as well as the way that the incorporation of an exotic amino acid in place of a serine permitted to stabilize the enzyme, rendered insensitive to proteolysis [20–22].

3. Melatonin receptor molecular pharmacology

3.1. Melatonin receptors

To somewhat summarize the situation, there were two GPCRs (MT_1 and MT_2) found throughout the animal kingdom, an elusive binding site (MT_3) that turned out to be an enzyme—quinone reductase 2 (QR2) [23] (see below, Section 6.3), another receptor (Mel1c) that was present in fishes, birds, and reptilians and that evolved to a GPR50 in mammals [24], with the curious property to have lost the recognition of melatonin, with a single exception (in platypus [25]), and finally the elusive nuclear receptovr, first described by Becker-André et al. [26] and then retracted [27]. Several other research papers [28] pointed at nuclear receptor(s) to explain the circadian rhythm of some key metabolism enzymes that could logically be dependent on the circadian rhythm of melatonin (see discussion in Jan et al. [29]).

What are the most characterized in the melatonin field, besides the multiple functions of the molecule itself (see below), are the binding characteristics at its receptors. The receptors were first discovered and cloned/characterized by Reppert's group [30] from both hamster and human. This première was followed by a series of cloning, including the discovery that hamster had only one functional melatonin receptor [31], to the contrary of most of the mammals that possess two (MT_1 and MT_2): human, rat, mice, sheep (although it was long believed to possess also a single receptor form [32]), etc. Cloning was also reported for other species, including birds and fishes [33, 34] and probably insects [35]. This led to the possibility to establish the binding pharmacology of those receptors in several laboratory species—mouse [36], rat [37], and sheep [32]—as well as in human [38]. For years, then, our goal has been to synthesize analogues of melatonin and use them to better understand the MT_1 and MT_2 roles, as well as to be able to somehow modulate them. It is not the place, here, to review the chemistry that has been explored around melatonin, but recent reviews could be looked at: Mor et al. [39], Garrat and Tsotinis [40], Rivara et al. [41], and Zlotos et al. [42]. The field would have benefit from a quest of specific and stable in vivo binders, particularly antagonists, permitting to explore and understand the limit of the melatonin actions, at least through these particular targets.

Incidentally, one must point out that there are still no antibodies against the receptors. We and many others tried over the last decades to produce such tools with a general negative endpoint. This, of course, has been an obstacle to a better understanding of those receptors.

Nevertheless, the repartition of the receptors in various organs, and particularly throughout the brain, has been nicely reviewed by Ng et al. [43] with some precisions of their respective functions: these seem to be as follows—improvement of neurogenesis (hippocampus with a memory maintenance via the inhibition of long-term potentiation by MT_2 receptor); MT_1 would regulate the REM sleep; MT_1 would also confer a protection against Huntington disease. In terms of melatonin receptor actions, those are the strongest information available. It seems clear, for example, that most of the protection offered by melatonin in multiple pathological situations (as summarized in **Table 1**) are not mediated by its receptors.

It would be very complicated to be exhaustive in terms of characterization of those binding sites, as the data is scattered throughout the literature. What is "easy" is that we and others using the binding assay developed around Vakkuri et al.'s 2-[^{125}I] iodomelatonin [44] for establishing the basic molecular pharmacology of the MT_1 and MT_2 receptors from several key laboratory species and from humans, but basic data can be found in Jockers et al. [45].

In the next sections, three aspects must be covered: the binding, heterodimerization, and structure of the melatonin receptors.

3.2. Binding, functionality, and heterodimerization

Initially, several reports were done using [^{3}H]melatonin, but the specific activity of the tracer was not sufficient to gain robust information on the receptors. It is only recently that heavily labeled [^{3}H]melatonin became available. This radiotracer permitted to better understand the various states of the receptors and their behavior in that context [46]. Historically the binding studies were largely facilitated by the use of the super-agonist, 2-[^{125}I]-iodomelatonin [44]. Not only this compound is easy to synthesize, but its sensitivity counteracted the very high affinity of melatonin for its receptors, as well as the paucity of the expression of these receptors in relevant tissues. Almost all the laboratories involved in melatonin research have been using this radiotracer. It must be pointed out, though, that attempts to use alternative ligands have been reported, mainly in the spirit of using specific ligands of one or the other of melatonin receptors [47]. Unfortunately, only ligands specific of MT_2 have been reported, so far. MT_1-specific binders have been elusive, despite the wide variety of melatonin analogues that have been synthesized. As stated elsewhere in the present essay, the main focus of chemistry research in this melatonin domain over the last decades was to find alternative ligand agonists at the receptors with strong stability in vivo.

Functionality of seven-transmembrane domain receptors is a complex science, providing daily new data. The number of coupling pathways at receptors in general is important, and more are discovered often. An excellent review has been published very recently [48], and the contribution of the same authors to the IUPHAR compendium on melatonin receptor functionality [45] should be considered as reference documents to understand the various pathways, at least as of today.

Nevertheless relatively few publications address the functionality of ligands in a global way. Indeed, if some functional data has been produced around a series of chemical analogues completing the classical binding displacement approach, rather few address the global and "standardized" characterizations of a series of ligands on MT_1 and/or MT_2. There are cases

where given compounds were characterized as partial agonists that turned out to be inverse agonists instead [49], leading to a yet another level of complexity of melatonin receptor pharmacology. We recently embarked in such a task, by screening the agonist/antagonist behavior of a series of compounds (Legros & Boutin, in preparation) after assessing the various methods [50]. We also extended these coupling measurements to a small series of potential antagonists specific of MT_2 [51], to conclude that the compounds were not antagonists but rather partial agonists. As stated and described by Kenakin [52], one should further dig the concept of biased ligands. Indeed, it seems clear, now, that some at least of the downstream pathways of melatonin receptors are elicited by some agonist ligands while not by other ones. This concept has been a bias of the approach to melatonin research. Indeed, while seeking for tools to understand these pathways and their implications in various pathological models, we never had access to real, stable, and potent antagonists, despite past claims for such compounds [53, 54]. Even when large-scale screening campaigns were attempted [55], the poor affinity (compared to the already existing compounds: low μM affinity in the best cases *versus* low nM already available ones) of those newly discovered compounds was not in favor of trying to develop series of chemicals around those hits. A similar situation occurred when we attempted to find peptide ligands at melatonin receptors [56].

After the first evidences that crystallogenesis of membrane proteins would be a challenge, we thought we would continue to search for ligands with a trial-and-error approach as we did for years, without the help of the visualization of the compounds in the protein as it became "mundane" these last years concerning co-crystallizations of compounds in their soluble protein targets. By multiplying the number of ligands in attempts to better describe the topography of the melatonin-binding site, even using mutagenesis [57–60], we also multiplied the assays on the functionality of the receptors, because we more and more discovered the ways the receptors were transferring their message to the cell. The simplistic view that a handful of such pathways between the receptors and the inner cell existed became obsolete. The by-product of such variety was that we found biased ligands that activated one but not the other(s) signaling pathways downstream melatonin receptor, as it is briefly discussed elsewhere.

Then, another new aspect came up: receptors can dimerize, a feature that was known for quite some time (see Rodbell [61], and see seminal review by Bockaert and Pin, [62]). Even though it was often believed to be an artifact of the purification attempts, the reality of such structures in situ was evidenced when one realized and demonstrated heterodimerisation between various types of such receptors: heterodimers between isoforms of a receptor, GABA [63], heterodimers between two unrelated GPCRs or even between GPCR, and another type of protein [64]. A paramount of examples were published, and their studies were made possible using the BRET technology [65]. In brief, engineering two receptors to make each of them fused with a carefully chosen fluorescent protein leads to a system in which the excitation of one of them results in the emission of fluorescent in the region exciting the other one. This cannot occur if the proteins are not physically in a very close vicinity of one another. Melatonin receptors have been also shown to be able to dimerize with serotonin 5HT2c receptor [66], as well as between MT_1 and MT_2 [67] or between MT_1 and GPR50 [68], the melatonin-related receptor (evolved from Mel1c [24] and that has lost its property to bind melatonin [69]). More recently, the heterodimerisation of GPR50 and the transforming growth factor-β receptor [70] potentially open

interesting routes toward the role of this orphan receptor as well as its implication in cancer development. Obviously many control experiments should be run in these explorations, as it would be attempting to conclude that any receptor can dimerize—and thus regulate the signaling pathway of—any other receptor (see Damian et al. [71] for a counter example, among some others).

3.3. Purification/structure

Attempts to crystallize GPCRs have been done for years with various successes. Beside several reports of models of the receptors [72–74], that turned to be more or less disappointing because they poorly brought new information—somewhat as expected. Thus, several lines of strategies were further explored. One of them led to a pure, functional MT_1 receptor, after several years of technical challenges: expression, stabilization, purification, and functionality measurement [75, 76]. We embarked several years ago in an approach that attempted to be original: as a first step in this purification/crystallization of melatonin receptor(s) project, we cloned melatonin receptors from as many and as various species as possible. Many such melatonin receptors have been reported in the literature, such as various sheep strains, buffalo, fishes, and even coral, many of which has been deposited in GenBank but not described in a publication. The rationale behind this Noah-ark type of research was to systematically use melatonin receptors from those variously evolved species (birds, reptilians, mammals from various environments, some harsh insects, etc.) that have in common their capacity to recognize melatonin—by definition. We aimed at comparing their thermal stability once they were stably expressed in CHO cells. We would choose the most resistant one and use that as a model in the process of purification/reconstitution established previously by Logez et al. in our laboratory [75, 76]. Despite a few success [25], we terminated the program for resource limitation reasons.

4. Melatonin catabolism

Melatonin catabolism has been described and discussed in-depth by Hardeland throughout the living kingdom [77]. In short, the main route of melatonin elimination (from the body) is not catabolism but rather conjugation and excretion via the urine. Thus there are three ways to consider: (i) the unchanged melatonin that one can find in urine; (ii) the conjugates, mostly glucuronides and sulfates; and (iii) the catabolism itself. Catabolism means that the molecule is transformed into something quite different from the original molecule. For example, in melatonin case, several reports demonstrated the opening of the indol ring. This opening possibly occurred through cytochrome c [78] or through 1,2-dioxygenase [79]. This was of importance because not too many compounds bear a formyl moiety such as the one produced during the cleavage of the indol cycle by either of these enzymes. This catabolism process would generate several products including N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK) [80]. The same paper, though, pointed out the absence of such metabolite(s) in human urine, strongly suggesting that the main catabolic route of melatonin would rather be through conjugation, even after oxidative stress.

5. The melatonin paradox

The field suffered from two paradoxes: safety and high affinity to natively poorly expressed receptors. First, melatonin is a very safe molecule, as far as we know; there is no report of human toxicity for overdose, and in mice the lethal dose is superior to 800 mg/kg [81]. Nevertheless, the French Agency for Food, Environmental and Occupational Health & Safety (www.anses.fr) emitted a report—in French—pointing at several cases of toxicity linked to melatonin consumption. Although they were ~100 cases in France reported during a 30-year period survey (i.e., a relatively modest number of cases, some of which have not been univocally linked to melatonin intake), the literature on clinical trials of melatonin is large enough to consider melatonin as reasonably safe [82], with the usual cases of deliberate overdose. In any case, it is not unusual to find reports in the literature where the dosage of melatonin in vivo or in cellulo is important. It was reported at several occasions—even if it probably depends on the cell type—cells treated with 1 mM of melatonin without apparent cell toxicity and even, in some cases, with beneficial effects [83–87]. Why is it a flaw? Because one can treat almost anything with this compound, at almost whatever dosage, and observe something, including relevant benefits for the situation (see **Table 1** and further examples in Boutin [88]). Furthermore, melatonin has a friendly behavior in terms of pharmacokinetics. When compared to another multi-card compound, resveratrol, it seems that unlike it, melatonin circulates in the blood after oral consumption at a fairly high concentration, while only 1 to 2% of resveratrol was found at the peak after ingestion of 25 mg/kg of resveratrol [89].

Finally, as stated elsewhere in the present essay, the affinity of melatonin for its receptors is in the low nanomolar range [45]. Many strict analogues have been synthesized with similar high affinities for the receptors. Thus, it has been complicated and sometimes impossible to start new chemical programs ad initio, or at least starting from molecules issued from high-throughput screening campaigns, for example, from which hits are rather in the high micromolar range. Therefore, new compounds with unexpected structures have been slowly emerged in the field. As a representative example, D600 (hydroxyl-verapamil) is one of the few compounds showing strong specificity for MT_1 [90]. No chemical program to date has been published to explore this observation and to deliver a specific ligand at MT_1 receptor with some pharmacological properties (and specificity) rendering it amenable to in cellulo or in vivo experiments.

6. Melatonin actions

6.1. Overall

Melatonin is the core master of rhythms. This part of the story is beyond any doubt. It translates the succession of days (light) and night (darkness) to the body. In the absence of light, the pineal gland (and more particularly the AANAT, the limiting step of melatonin biosynthesis)

synthesizes melatonin. Nevertheless, a report [91] shows that, at least in the European hamster, the circannual rhythm could be maintained even after pinealectomy, thanks to light action in an accelerated photoperiodic regime, demonstrating the hypothalamic integration of the photoperiodic signal even in pinealectomized animals and, thus, in the absence of pineal gland synthesis of melatonin.

Melatonin circadian rhythm can be measured in the blood from healthy volunteers and is clearly linked to the successions of days and nights. The timezyme (AANAT) is the master key of this process: active during dark periods and inactive during day (as the enzyme is destroyed by the light-induced proteasome).

6.1.1. Foreword

Melatonin exerts protective actions far beyond mammals, as several reports showed the role of melatonin in protecting yeast [92], bacteria [93], zebrafish [94], and plants [95] from various types of insult. For a discussion of melatonin throughout evolution, see also Tan et al. [96]. For decades, melatonin has been described as a compound able to fight almost any pathological situations. Tekbas et al. [97] even seriously considered melatonin as an antibiotic and Anderson et al. as an anti-Ebola virus agent [98]. A sample of those numerous actions can be found in Boutin [88], up to 2015. **Table 1** of the present essay is the follow-up of that particular list of beneficial properties. Many of those properties of melatonin seem to be linked to the capacity of the compound to limit reactive oxygen species (ROS) actions. ROS have been first documented as an "infamous" group of highly reactive molecules responsible for oxidative stress. In an enlightening review, Roy and coworkers [99] defined the field of reactive oxygen species, by starting to recall that ROS are also regulating signaling pathways in physiological situations. They also emphasized the fact that treatments with so-called antioxidants failed to show efficacy or/and positive effects in the prevention of diseases or health complications coming from oxidative stress. Nevertheless, it seems that according to a common belief, melatonin falls outside that particular category and is the ultimate scavenger/antioxidant molecule that has multiple capacities to prevent almost any diseases.

It is sometimes complicated to find common sense in such a plethora of actions. **Table 1** lists some of these many actions, as published between 2015 and today. There is no way to be able to understand why melatonin has been reported for so many years in so many pathological situations. And the purpose of the present essay is not to do so. It is rather to make a compendium of those actions and to let the community know that such papers exist and that the reason why melatonin is so ubiquitously active remains a mystery.

It is attempting, though, to make a rapid survey of those publications and to conclude that the common factor is the production of ROS. Then, we can hypothesize that most of these beneficial actions were due to a capacity of melatonin to induce antioxidant enzymatic defenses. To conclude on this working hypothesis, one will have to identify the nuclear receptor mediating this property. Nuclear factor erythroid 2-related factor 2 (Nrf2) might be a good candidate, but a wishful thinking is certainly not a proof of fact.

Authors	Date	Protection against	Targets	Amount	Species	Ref
Abdel-Moneim et al.	2015	*Naja naja* venom toxicity	—	10 mg/kg	Rat	[100]
Allagui et al.	2015	Aluminum-induced toxicity	/	10 mg/kg	Rat	[101]
Al-Olayan et al.	2015	Aluminum-induced injury	Neurons	10 mg/kg	Rat	[102]
Al-Rasheed et al.	2016	CCl4-induced toxicity	Liver	20 mg/kg	Rat	[103]
Amin et al.	2015	Diabetes-induced apoptosis	Heart	10 mg/kg	Rat	[104]
Asghari et al.	2017	Aluminum phosphide toxicity	Heart	20–50 mg/kg	Rat	[105]
Banaei et al.	2016	Ischemia–reperfusion injury	Kidney	10 mg/kg	Rat	[106]
Barberino et al.	2017	Cisplatin-induceddamage	Ovaries	5–20 mg/kg	Mouse	[107]
Berkiks et al.	2017	Ccognitive disorders	Brain	5 mg/kg	Rat	[108]
Cao et al.	2017	Subarachnoid hemorrhage	Brain	150 mg/kg	Rat	[109]
Cebi et al.	2018	Radioiodine treatment	Testicles	12 mg/kg/day	Rat	[110]
Chang et al.	2016	Ischemia–reperfusion injury	Kidney	90 mg	Rat	[111]
Chang et al.	2015	Ischemia/reperfusion injury	Adipose stem cells	120 mg/kg	Rat	[112]
Chen et al.	2016	Endoplasmic reticulum stress	Pancreas	0.5–2 mM	Rat	[113]
Chen et al.	2017	Neuropathic pain	/	???	Rat	[114]
Czechowska et al.	2015	Thioacetamide-induced fibrosis	Liver	10 mg/kg	Rat	[115]
Das et al.	2017	Mitochondrial dysfunction	Hepatocytes	10–20 mg/kg/day	Mouse	[116]
Ding et al.	2018	Post-traumtic cardiac function	Heart	100 μM	Rat	[117]
Ding et al.	2015	Traumatic injury-induced apoptosis	Brain	10 mg/kg	Mouse	[118]
Dos Santos et al.	2018	Lupus nephritis injury	Kidney	10 mg/kg/day	Mouse	[119]
Drag-Kozak et al.	2018	Cadmium-induced toxicity	Reproductive organ	4 mg/L	Carp	[120]
Ewida et al.	2016	Metabolic syndroma	Kidney	5 mg/kg	Rat	[121]
Favero et al.	2017	Fibromyalgia-related alterations	Muscle	2.5–5 mg/kg	Rat	[122]
Fernandez-Gil et al.	2017	Radiotherapy-iondued toxicity	Intestine	45 mg/day	Rat	[123]
Galley et al.	2017	Paclitaxel-induced dysfunction	Mitochondria	5–50 mg/kg	Rat	[124]

Authors	Date	Protection against	Targets	Amount	Species	Ref
Ghaznavi et al.	2016	Gentamicin-induced toxicity	Kidney	15 mg/kg/day	Rat	[125]
Ghosh et al.	2017	Copper ascorbate-induced damage	Heart mitochondria	1 μM	Goat	[126]
Goc et al.	2017	Sodium nitroprusside toxicity	Organs	10 mg/kg	Mouse	[127]
Goudarzi et al.	2017	Cyclophophamide-induced stress	Kidneys	5–20 mg/kg	Mouse	[128]
Hermoso et al.	2016	Steatosis	Liver	10 mg/kg	Rat	[129]
Hsu et al.	2017	Trauma-induced hemorrhage	Liver	2 mg/kg	Rat	[130]
Hu et al.	2017	BBB damage	BBB	15 mg/kg	Rat	[131]
Ji et al.	2017	Sepsis-associated encephalopathy	Brain	10 mg/kg	Mouse	[132]
Jiang et al.	2016	Diabetic-induced inflammation	Retina	10 mg/kg/day	Rat	[133]
Jin et al.	2016	Non-alcoholic fatty liver disease	Liver	/	Mouse	[134]
Karaer et al.	2015	Radiation damage	Inner ear	5 mg/kg	Rat	[135]
Karimfar et al.	2015	Cryopreservation stress	Sperm	0.001–1 mM	Human	[136]
Khaksar et al.	2017	Fluoxetine-induced tissue injury	Organs	1 mg/kg	Rat	[137]
Khalil et al.	2015	Zonisamide-induced toxicity	/	10 mg/kg	Rat	[138]
Koc et al.	2016	Apoptosis	Olfactive neurons	10 mg/kg/day	Rat	[139]
Lebda et al.	2018	Thioacetamide-induced fibrosis	Liver	5 mg/kg/day	Rat	[140]
Lee et al.	2016	H_2O_2-mediated cell death	Keratinocytes	2.5–10 μM	Human	[141]
Li et al.	2016	Cadmium-induced toxicity	Testicles	1 mg	Mouse	[142]
Li et al.	2015	Maturation defect	Oocyte	0.001–1 μM	Pig	[143]
Lopez et al.	2017	MPTP-toxicity	Brain	10 mg/kg	Mouse	[144]
Lv et al.	2018	Cr(VI) toxicity	Testicles	25 mg/kg	Mouse	[145]
Ma et al.	2018	Oxidative injury	Heart	100 μM	Rat	[146]
Ma et al.	2017	Tripterygium glycosides toxicity	Ovaries	20 mg/kg/day	Mouse	[147]
Ma et al.	2015	Adriamicyn-toxicity	Breast cancer cells	10 mg/kg/day	Rat	[148]
Mehrzadi et al.	2016	Gentamicin-induced toxicity	Kidney	20 mg/kg/day	Rat	[149]

Authors	Date	Protection against	Targets	Amount	Species	Ref
Mirhoseini et al.	2017	Torsion/detorsion model	Testicles	25 μg/kg	Rat	[150]
Montasser et al.	2017	Methotrexate-induced toxicity	Liver	10 mg/kg	Rat	[151]
Mukherjee et al.	2015	Isoproterenol-induced damage	Heart mitochondria	0.125–4 μM	Goat	[152]
Munoz et al.	2017	Cumene peroxide-induced stress	Pineal gland	10 mg/kg/day	Rat	[153]
Naseri et al.	2017	Irradiation-induced toxicity	Brain	100 mg/kg	Rat	[154]
Naskar et al.	2015	MPTP-induced Parkinsonism	Brain	10–30 mg/kg	Mouse	[155]
O'Neal-Moffitt et al.	2015	Alzheimer neiuropathology	/	Ad libitum???	Mouse	[156]
Ortiz et al.	2015	Radiation-induced mucositis	Mouth	45 mg/kg/day	Rat	[157]
Othman et al.	2016	Bisphenopl A-induced toxicity	Testicles	10 mg/kg	Rat	[158]
Ozsoy et al.	2016	Mitochondrial dysfunction	Liver	10 mg/kg	Rat	[159]
Ozsoy et al.	2015	6-hydroxydopamine stress	Neurons	10 mg/kg	Rat	[160]
Pal et al.	2016	Stress-induced behavior changes	/	10–100 mg/kg	Rat	[161]
Pang et al.	2016	Frozen–thawed cycles	Sperm	0.01–1 mM	Bovine	[162]
Patino et al.	2016	o2 & Glucose deprivation	Brain slices	10–30 μM	Rat	[163]
Paul et al.	2018	Oxidative stress	Substancia nigra	10–30 mg/kg	Rat	[164]
Rajput et al.	2017	Alcohol-induced stress	Brain	20 mg/kg	Mouse	[165]
Sadek and Khattab	2017	Arginine-induced pancreatis	Pancreas	50 mg/kg	Rat	[166]
Sarihan et al.	2015	TCDD-induced injury	Heart	5 mg/kg/day	Rat	[167]
Scheuer et al.	2016	UVR-induced erythrema	Skin	0.5–12.5%	Human	[168]
Shahrokhi et al.	2016	Ischmia/reperfusion-oxidative stress	Estomac	10 mg/kg	Rat	[169]
Shang et al.	2016	Colitis-induced neuron damage	Colon	2.5 mg/kg/day	Rat	[170]
Shao et al.	2015	LPS-induced mastitis	Breast	5–20 mg/kg	Mouse	[171]
Shokri et al.	2015	Pilocarpine-induced epilesy	Brain	5–20 mg/kg	Rat	[172]
Shokrzadeh et al.	2015	Cyclophosphamide toxicity	Lung	2.5–20 mg/kg	Mouse	[173]
Sinha et al.	2018	Hypoxy/Ischemy	Brain	10 mg/kg	Mouse	[174]
Tanabe et al.	2015	Oxidative stress	???	100 μg/kg	Mouse	[175]
Tang et al.	2017	Abdominal aortic aneurysm	Aorta	10 mg/kg/day	Rat	[176]
Tas et al.	2015	Ischemia/reperfusion injury	Intestine	50 mg/kg	Rat	[177]

Authors	Date	Protection against	Targets	Amount	Species	Ref
Torabi et al.	2017	Cyclophosphamide-induced toxicity	Testicles	10 mg/kg/day	Rat	[178]
Uygur et al.	2016	As-induced apoptosis	Testicles	25 mg/kg/day	Rat	[179]
Vazan et al.	2015	Epinephrine-induced injury	Heart	50 μM	Rat	[180]
Vinod et al.	2016	Aging-induced NO rhythm loss	Brain	30 μg/kg/day	Rat	[181]
Wang et al.	2018	Intracerebral Hemorrahge	Brain	???	Rat	[182]
Wang et al.	2016	Smoke-induiced vascular injury	Blood samples	10 mg/kg	Rat	[183]
Wang et al.	2016	Smoke-induiced vascular injury	Blood samples	3 mg/day	Human	[183]
Xue et al.	2017	Kainic-induced cell death	Brain	20 mg/kg	Mouse	[184]
Yang et al.	2018	Subarachnoid hemorrhage	Brain	0.1–10 μM	Mouse	[185]
Yi et al.	2017	Stress-induced inflammation	Macrophages	50–100 mg/kg	Mouse	[186]
Yildirim et al.	2016	Ureteral obstruction-induced injury	Kidney	10 mg/kg	Rat	[187]
Yu et al.	2018	Ischemia–reperfusion injury	Heart	10 mg/kg	Rat	[188]
Yu et al.	2018	MEHP-induced meiosis defect	Oocytes	???	Pig	[189]
Yu et al.	2015	Ischemia/reperfusion injury	Heart	10 mg/kg/day	Rat	[190]
Yu et al.	2015	Ischemia/reperfusion injury	Heart	20 mg/kg/day	Rat	[191]
Zasada et al.	2015	Nitrobenzene-induced peroxidation	Thyroids	0.001–10 mM	Pig	[192]
Zhai et al.	2017	Pathological cardiac hypertrophy	Heart	20 mg/kg/day	Mouse	[193]
Zhang et al.	2017	Bisphenol A-induced toxicity	Oocytes	30 mg/kg	Mouse	[194]
Zhang et al.	2017	Diabetic cardiomyopathy	Heart	20 mg/kg/day	Mouse	[195]
Zhang et al.	2017	Arsenic-induced injury	Liver	5–20 mg/kg	Rat	[196]
Zhang et al.	2016	β-amyloid-induced damages	Brain	50 μM	Rat	[197]
Zhao et al.	2017	NaF-induced injury	Embryos	50–100 μM?	Mouse	[198]
Zhou et al.	2017	Ischemia/reperfusion injry	Heart	20 mg/kg	Mouse	[199]
Zhu et al.	2018	Oxidative stress	Heart endothelium	10 μM	Rat	[200]
Plants						
Kobylinska et al.	2017	Lead-induced cell death	Tobacco cells	200 nM	Plant	[201]
Wang et al.	2017	Drought stress	Arabidopsis	???	Plant	[202]

Authors	Date	Protection against	Targets	Amount	Species	Ref
Xu et al.	2016	Thermotolerance	Tomato plants	10 μM	Plant	[203]
Zheng et al.	2017	Salt-stress	Plant cells	—	Plant	[204]
Cells						
Baburina et al.	2017	Aging	Mitochondria	7 mg/kg/day	Rat	[205]
Bardak et al.	2017	2-ethylpyridine-induced stress	ARPE-19 cells	200 μM	Human	[206]
Charao et al.	2015	Paraquat-induced toxicity	A549 cells	10 μg/mL	Human	[207]
Chen et al.	2015	Bile acid-induced oxidative stress	L02 cells	1 μM	Human	[208]
Fu et al.	2017	Chloranil-induced toxicity	PC12 cells	25–200 μM	Mouse	[209]
Gurer-Orhan et al.	2016	b-amyloid-induced damage	Cells	10–100 μM	Hamster	[210]
Han et al.	2017	Obesity-associated stress	Oocytes	30 mg/kg/day	Mouse	[211]
Janjetovic et al.	2017	UVB-induced damage	Melanocytes	50 μM	Human	[212]
Ji et al.	2016	Angiotensin-II-indued injury	Podocytes	0.1–1 mM	Mouse	[83]
Jumnongprakhon et al.	2015	Methamphetamine-toxicity	C6 cells	1–100 nM	Rat	[213]
Liu et al.	2015	Hypoxia-induced	N2a cells	5 μg/mL	Mouse	[214]
Lu et al.	2015	LPS-induced hypertrophy	Myocardial cells	1.5–6 mg/mL	Rat	[215]
Maarman et al.	2017	Uric acid-induced toxicity	C2C12 myotubes	10 nM	Mouse	[216]
Miao et al.	2018	benzo(a)pyrene meiotic failure	Oocytes	1 nM–1 mM	Pig	[84]
Mehrzadi et al.	2017	H2O2-induced toxicity	MSC	10 nM–1 mM	Human	[85]
Ozerkan et al.	2015	CCl4-induced cytotoxicity	HepG2 & Hep3B	10 nM	Human	[217]
Pang et al.	2017	Early apoptosis	Oocytes	1 nM	Bovine	[218]
Sagrillo-Fagundes et al.	2016	Hypoxia-reoxygenation toxicity	Trophoblasts	1 mM	Human	[86]
Sanchez-Bretano et al.	2017	H_2O_2-induced cell death	661 W cells	0.1–1 μM		[219]
Song et al.	2015	LPS-induced inflammation	Stem cells	100 nM	Mouse	[220]
Tan et al.	2016	Oxidative stress-induced cell death	Adipocytes	100 μM	Human	[221]
Waseem et al.	2017	Oxaliplatin-induced toxicity	SHSY-5Y cells	10 μM	Human	[222]
Wongprayoon et al.	2017	Methamphetamine-induced stress	SH-SY5Y cells	0.01–1 μM	Human	[223]
Xie et al.	2015	Hypoxia-induced hypertrophy	Cardiomycyte cell line	1 mM	Rat	[87]

Authors	Date	Protection against	Targets	Amount	Species	Ref
Xue et al.	2017	Kainic-induced cell death	N2a cells	50–100 μM	Mouse	[184]
Yang et al.	2017	Iron overload senescence	MSCstem cells	10 nM–100 μM	Mouse	[224]
Yang et al.	2017	Glucocorticoid-induced impairment	Isolated knee joints	1 μM	Mouse	[225]
Yu et al.	2017	Ischemia–reperfusion injury	H9c2	10 μM	Rat	[226]
Zhao et al.	2018	Ab-induced neurotoxicity	Primary neurons	0.1–100 μM	Mouse	[227]
Zhou et al.	2018	rotenone-induced cell death	SH-SY5Y cells	50–500 μM	Human	[228]
Zhu et al.	2015	Myocardial infarction	Adipose stem cells	5 μM	Rat	[229]

Table 1. Some of the actions of melatonin observed in various pathophysiological situations.

6.1.2. Melatonin as an antioxidant molecule

Forman et al. in two seminal papers explained that the notion of hydroxyl radical scavengers is an extreme case of wishful thinking [230, 231]. Later on, he and his coworkers clearly showed that a unique molecule could not be a scavenger of superoxides, hydrogen peroxides, or other hydroperoxides or hydroxyl radicals. Indeed, all chemicals inside a cell react chemically with radical species, that is, proteins, lipids, nucleic acids, etc. Thus, because all organic compounds react with radicals with rate constants approaching the diffusion limitation, no compound can be better than the sum of the others to scavenge those ROS [231]. This can apply to melatonin. Like many other chemicals, whether indol-based or not, this compound, even at large concentrations, cannot be, per se, a scavenger. Therefore claims that melatonin is a super scavenger, with many advantages over other similar naturally occurring compounds, must be taken with extreme caution, despite several in-depth reviews, such as the one by Galano et al. [232]. Even the use of "direct" detection methods of radicals (to prove this hypothesis) should be handled with much caution [233]. Nevertheless, melatonin sustains antioxidant properties (see Rodriguez et al. [234] for review). Indeed, it can increase the expression of antioxidant enzymes (see, e.g., Mahrzadi et al. [149] and references therein). Melatonin can also act as a potent antiapoptotic agent in many cells [235], maybe through an antioxidant type of activity, as a relationship between ROS and apoptosis and autophagy has been well documented. How can melatonin induce those antioxidant defenses?

6.1.3. Melatonin as a ligand of Nrf2?

At the time (2003) Rodriguez et al. wrote their review [234] on antioxidant capacities of melatonin, Nrf2 was not really an identified and recognized partner in this process. The relationship between melatonin actions and the role of nuclear factor erythroid 2-related factor 2 (Nrf2) has been reported more than 50 times in the literature these last years, starting around 2009 [236].

Nrf2 is a key factor in the induction of antioxidant protein defenses of the cell. It binds to a region called EpRE—also known as ARE [230]. This transcription factor (belonging to the huge family of Cap'n'collar transcription factors) is neutralized in cellulo by another factor, Kelch-like ECH-associated protein 1 (Keap1). The heterodimer is directed to the proteasome where the proteins are destroyed. Upon some conditions, including pharmacological ones (for instance, sprout-derived chemicals [237]), the dimer is open, and the free Nrf2 migrates to the nucleus of the cell where it associates with the EpRE region. This translates by the induction of several key proteins of the antioxidant cellular armada, such as heme oxygenase 1, quinone reductase 1, glutathione S-transferase π1, etc., but also enzymes from the phase 2 drug metabolism, such as UDP glucuronosyltransferases. There is a large literature indicating that melatonin induces Nrf2 expression and/or its separation with its corepressor, Keap1 (about 50 publications reported at least the induction of Nrf2 by melatonin). Furthermore, it has been shown several times that upon melatonin treatment, the cytosolic Nrf2 migrates to the nucleus where it can exert its inductive function. One question remains unanswered, though; it is the possibility that Nrf2 was the elusive nuclear factor described at several occasions [26]? Unfortunately, the tridimensional structure of Nrf2 and/or of its complex with Keap1 has not been reported. It seems that Nrf2 has no a priori structure and is only adopting define 3D shape either once linked to Keap1 (a complex that is then directed to the proteasome) or when in complex with a ligand. Much more need to be done to understand this relationship that might enlighten part of the observation of **Table 1**.

6.2. Through MT_1/MT_2

The specificity of actions linked to the binding of melatonin to one of its receptors, MT_1 and MT_2, is still a matter of debate. Indeed, a thorough survey of its action is not possible in vivo in wildtype animals, because we are still lacking reliable and isoform-specific antagonists (see discussion in Jockers et al. [45]). It is possible, though, to study the role of one or the other of the receptors using either natural KO animals [such as the Siberian hamster, but not the European hamster (Gautier & Boutin [281])] or, alternatively, MT_1 or MT_2 (or both) KO animals, which have been engineered [238–240], but results are slow to be issued [241–243] (see also discussion in Jockers et al. [45]). Nevertheless, general conclusions can be drawn from accumulated data, as reviewed by the same authors [45]. It is difficult, as of today, without drowning in the 3970 available reviews on melatonin, to clearly segregate between the subtype roles. Among the clearest facts, mice lacking MT_1 receptors exhibit higher mean blood glucose levels than wildtype mice [244]. Those KO animals tend to be more glucose intolerant and insulin resistant than their wildtype counterparts. Through many different parameters, both MT_1 and MT_2 receptors seem to have a role in the phase shift of circadian rhythms, as demonstrated by several lines of indications, including knockout animals, the use of specific MT_2 antagonists (luzindole, 4P-PDOT), as well as ex vivo experiments. Melatonin can activate an immune response. Remarkably, that was proposed as early as 1926 by Berman. This activity seems to depend on the MT_1 receptor [245], but opposite claims have also been published [246]. Liu et al. showed that it was MT_2 that was the receptor implicated in axogenesis and the formation of functional synapses [247].

Nevertheless, it seems to me improbable that even only some of the actions in **Table 1** were through the binding of melatonin onto its receptors.

6.3. Through QR2

As stated previously, it was rapidly discovered that two melatonin-binding sites were GPCR in mammals and an extra one, Mel1c, in reptilians and birds. The group of Dubocovich also pointed at a binding site, ML2 [248, 249], with rather unconventional properties (particularly with very fast exchange) baptized MT_3. In 1999 we embarked in an attempt to clone this particular receptor, after having obtained similar results for the pharmacological description of this particular "receptor" [250]. We had the chance to identify it by using a series of inverse pharmacology techniques, comprising an analogue of a specific MT_3 ligand, MCA-NAT, on which affinity chromatography succeeded. The binding site was an enzyme with a peculiar story, quinone reductase 2 (QR2 a.k.a. NQO2) [23]. The activity of this enzyme was first described in the early 1960s as a reductase using unconventional donors as co-substrates, such as *N*-benzyl, *N*-methyl, or *N*-ribosyl dihydronicotinamides, and Talalay's group established that the enzyme was the enzyme once described by Liao et al. [251]. Interestingly, they clearly established the nature of the enzyme and particularly its incapacity to recognize NADH or NAD(P)H as co-substrates, as well as its sensitivity to some chemical, in an orthogonal way to QR1. For instance, QR2 is insensitive to the reference QR1 inhibitor, dicoumarol. When we discovered that QR2 was indeed MT_3, we had to reinforce this observation by generating KO cell lines [252], KO mouse strain [253] and various tools that would help to understand the potential role of this enzyme (see Vella et al. [254] and references there in). Although the enzyme was identified during a pure melatonin-related program, it turned out to have nothing in common, a priori, with the melatoninergic systems. Indeed, while able to bind melatonin with a rather strong affinity—in the nM range—QR2 is only poorly inhibited by melatonin, in the 50 μM range, suggesting that melatonin regulation was not a player in the QR2 game. Indeed, as often in the drug metabolism area, enzymes from both phases I and II, such as cytochrome P450, UGTs, or glutathione S-transferases, are often enzymes with enough plasticity in their catalytic sites in order to accommodate xenobiotics that are, by definition, molecules of various chemical structures issued from the environment at large.

Nevertheless, I suggested that QR2 inhibition at high dose of melatonin could be an explanation for melatonin exerting its antioxidant capacities [88].

6.4. Through mitochondria

Incidentally, a couple of papers reported not only the synthesis of melatonin in mitochondria but also the presence in these organelles—at least those isolated from the brain—of a measurable binding, signing the presence of MT_1 receptors. Again, as long as the mitochondrial DNA is not reported for genes encoding for these GPCRs, it seems possible to hypothesize that those binding sites were a leftover from the brain preparation of mitochondria, a possibility reinforced by the difficulty of preparing "pure" mitochondria from these lipid- and membrane-rich organs. Beyond these hypothetical technical considerations lays also the fact that our laboratory had experienced "very" often cells with no binding activity, suggesting that mitochondria would express melatonin receptors only in melatonin receptor-rich organs—such as the brain—an indirect suggestion that the presence of those receptors in these organelles might be a "simple" signature of a difficult

separation between all the kinds of membranes present in a neuronal cell. There were several reports over the last decade showing a protective effect of melatonin onto mitochondria functions (see **Table 2**). Then several reviews suggested that melatonin was synthesized by mitochondria (see, for instance, Manchester et al., 2015 [255], Reiter et al., 2017 [256] and 2018 [257]). Particularly interesting is the fact that Cellular and Molecular Life Science published a special issue in 2017 (volume 74, issue 21) dealing with melatonin and mitochondria, emphasizing the interest of the community for these observations and their consequences. A reason for this hypothesis was given: mitochondria, like chloroplasts in plants, evolved from bacteria. Because originally cyanobacteria were subjected to heavy exposition to toxic free radicals, they evolved in keeping melatonin as an antioxidant, scavenging these radicals and thus preserving their integrity. Because this happened about 3 billion years ago, melatonin has been selected to protect and defend those microorganisms.

Of course, when bacteria colonized eukaryotic cells, the trait was maintained throughout evolution, including in mammals. Thus, no matter how high or low the blood melatonin concentration is, this particular intra-mitochondria concentration remains constant (not depending on the circadian rhythm), protecting mitochondria from the never ending production of free radicals that is the signature of sane mitochondria. An impressive series of publications were issued in these last few years (see **Table 2**) dealing with situations where toxicity was prevented by melatonin. This can be further extended to the protection afforded by mitochondria-synthesized melatonin to oocytes [278]. Finally, one can also add the observation that mitochondria melatonin protects plants from drought episodes [202]. Particularly interesting was the last one in which Suofu et al. [10] demonstrated the presence of the main melatonin synthesis enzymes, arylalkylamine *N*-acetyltransferase (AANAT) and acetylserotonin *O*-methyltransferase (HIOMT), in mitochondria matrix, as well as the high concentration of melatonin inside those mitochondria matrix. Furthermore, they showed the presence of MT_1 receptor and the actual coupling of this receptor, turning this observation into a major progress in the domain, as rare are the receptors signaling in the mitochondria. This observation was challenged by Ahluwalia et al. [279] (replied by Suofu et al. [11]) that was able to show the presence of the melatonin receptors in muscle fibers, but not in mitochondria thereof. It is clear that this breakthrough information will be better understood after the observation will be confirmed independently. Of course, questions remain in the skeptical reader mind: if the melatonin system evolved from bacteria over several billion years, then the genetic material should have evolved together with it, meaning that the mitochondrial DNA should encode for MT_1, AANAT, and ASMT, which does not seem to be the case. This observation would also lead to an extra complexity involving the protein importation system (TOM, Tim, etc.) and the *ad hoc* addressing sequence(s) onto those proteins, all of which have not been seen so far. Furthermore, the discovery and description of an inward transport of melatonin in mitochondria [280] are not fitting an in situ synthesis. For those of us who have been working with subcellular organelles, it is very hard to assess the purity of those organelles because of the continuum that exists between all the membranes from cells. One should also add to this the particular complexity of the brain tissue that is by essence very lipid-rich, leading to an extra difficulty in preparing pure membranes or pure subcellular organelles. Nevertheless, the several evidences on the melatonin actions at the level of mitochondria cannot be doubted and change our view of its role and of the role of MT_1, as MT_2 seems to be absent from the organelle.

Protection against	Authors	Year	Reference
Doxorubicin	Xu and Ashraf	2002	[258]
Oxidative stress	Jou et al.	2004	[259]
NO synthase induced dysfunction	Escames et al.	2006	[260]
Apoptosis	Han et al.	2006	[261]
Ischemia-Reperfusion	Petrosillo et al.	2006	[262]
Apoptosis	Luchetti et al.	2007	[263]
Oxidative stress	Jou et al.	2007	[264]
UV exposition	Fischer et al.	2008	[265]
Aging	Petrosillo et al.	2008	[266]
Oxidative stress	Hibaoui et al.	2009	[267]
Permeability transition	Jou et al.	2010	[268]
Permeability transition	Jou et al.	2011	[269]
Bisphenol A	Anjum et al.	2011	[270]
CCl_4	Chechshevik et al.	2012	[271]
Isoproterenol	Mukherjee et al.	2012	[272]
Ischemia-Reperfusion	Yang et al.	2013	[273]
UV exposition	Canonico et al.	2013	[274]
Demyelination induced stress	Kashani et al.	2014	[275]
Cd	Guo et al.	2014	[276]
Isoproterenol	Mukherjee et al.	2015	[272]
Ischemic-Stroke	Yang et al.	2015	[277]
Lipid toxicity	Ozsoy et al.	2016	[159]
Aging	Baburina et al.	2017	[205]
Lipid toxicity	Das et al.	2017	[116]
Paclitaxel	Galley et al.	2017	[124]
Copper	Ghosh et al.	2017	[126]

Table 2. Melatonin protects mitochondria against various stresses.

7. Future paths?

Trying to summarize the literature on subjects like melatonin is obviously impossible. One will give his/her view on some of the points that are the most attractive to him/her. It was thus vain to attempt to solve issues with such an essay on this neurohormone. The future will tell if melatonin is an exceptional molecule with many capacities. What is clear, as of today, is that melatonin has been described on a plethora of situations with beneficial endpoints. If melatonin is an antioxidant—but the concept behind this word is different from one author

to another—it is not as a scavenger of radical oxygen species, but most probably through its capacity to induce cellular defenses against oxidative stress. Melatonin has different known targets; two, MT_1 and MT_2, are well described, but these receptors bring more unexpected novelties over the years, an enzyme—QR2—the study of which could be part of an explanation for the antioxidant properties of melatonin, and, finally, a pathway, linked to Nrf2 that seems to be another part of the explanation for these properties. There are many routes still to explore to understand what is behind this molecule, and the spectacular associated with it should be concealed and mastered until beyond (and despite) our hopes; facts will be revealed.

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