

INVITED REVIEW

Melatonin and the pathophysiology of cellular membranes

Russel J. Reiter¹, Lorena Fuentes-Broto¹, Sergio D. Paredes¹, Dun-Xian Tan¹, Joaquin J. Garcia²

ABSTRACT: The ability of melatonin to influence the physiology of cell membranes is reviewed in this report. Publications related to this field from 1993 – present. Melatonin is a ubiquitously acting indoleamine which is associated with a variety of important functions within both unicellular and multicellular organisms. By virtue of its ability to protect lipids from free radical damage, melatonin is remarkably beneficial in preserving the morphological and functional integrity of cell membranes. In doing so, it reduces the quantity of oxidized lipids in membranes and maintains them at optimal fluidity, i.e., prevents them from becoming rigid. This contributes significantly to the function of proteins (receptors, channels, pores, etc.) in the cell membranes and helps in preserving the normal physiology of the cells. In addition to these indirect effects of melatonin on membrane function, there is evidence that this indoleamine also may act directly on channels assisting membranes in maintaining proper ion gradients and current. The role of melatonin in the functioning of membrane channels and pores is an area of research that should be experimentally exploited.

KEY WORDS: Melatonin, cell membranes, lipid peroxidation, membrane channels, membrane pores, cell membrane receptors

AFFILIATIONS

¹Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, Texas, USA

²Department of Pharmacology and Physiology, University of Zaragoza, Zaragoza, Spain

CORRESPONDENCE

Russel J. Reiter, Ph.D., Dr.
E-mail: reiter@uthscsa.edu

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INTRODUCTION

The discovery of melatonin as an antioxidant (1) has stimulated a large number of studies related to the ability of this molecule to protect lipids, proteins and DNA from oxidative damage (2-6). Indeed, a literature search in PubMed indicates more publications related to the free radical scavenging and antioxidative actions of melatonin than in any other area of research on this molecule.

A remarkable feature of melatonin is the variety of actions it utilizes to reduce oxidative stress, i.e., the damage resulting from the oxidation of molecules by free radicals and related reactants. Besides its ability to scavenge the highly toxic hydroxyl radical ($\cdot\text{OH}$), melatonin is also effective in neutralizing the peroxynitrite anion (ONOO^-), hydrogen peroxide, the superoxide anion radical (O_2^-), singlet oxygen as well as other reactants (1, 2, 7-16).

When melatonin functions as a free radical scavenger, it generates other metabolites that are likewise capable of detoxifying radical species and/

or their molecular derivatives. These metabolites include cyclic 3-hydroxymelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), and N1-acetyl-5-methoxykynuramine (AMK) among others (4, 13, 14, 17, 18); the relation of these metabolites is outlined in Figure 1.

Besides radical scavenging, melatonin has indirect antioxidative actions by means of its ability to stimulate antioxidative enzymes (19-22). These enzymes remove radicals and their precursors, thus, reducing the likelihood of oxidative damage. Moreover, melatonin, or its metabolite, AMK, inhibits one pro-oxidative enzyme, i.e., inducible nitric oxide synthase (iNOS) (23). Inhibition of this enzyme reduces the formation of nitric oxide ($\text{NO}\cdot$) which has the capability of coupling with the O_2^- to form the ONOO^- . Hence, by reducing the activity of iNOS melatonin limits formation of the ONOO^- , a non-radical reactant which is equally toxic to $\cdot\text{OH}$.

One aspect of melatonin's ability to forestall oxidative damage and limit cellular death due to free radical-mediated apoptosis seems to have

TABLE 1. Representative reports in which melatonin was found to reduce the level of induced lipid peroxidation. There are a very large number of publications documenting the ability of melatonin to reduce the oxidation of membrane lipids in many different species and induced by many different means.

Species	Means used to induce oxidative stress	Lipid peroxidation parameter lowered by melatonin	Reference
Human	Sepsis in premature newborns	Blood MDA	(37)
Human	Asphyxiation in newborn	Blood MDA	(38)
Human	Major vascular surgery	Blood MDA	(39)
Rat	Kianic acid	Brain MDA	(40)
Rat	Paraquat	Respiratory MDA	(41)
Rat	Excessive exercise	Muscle MDA	(42)
Rat	Phenobarbital	Hepatic MDA	(43)
Rat	Ischemia-reperfusion	Hepatic MDA	(44)
Rat	Carbon tetrachloride Peroxides	Hepatic lipid	(45)
Rat	Dinitrobenzene sulfonic acid	Colon MDA	(46)
Rat	Streptozocin-induced diabetes	Brain, liver and renal MDA	(47)
Rat	Danorubicin or doxorubicin	Renal MDA+ 4-HDA	(48)
Rat	Gentamicin	Blood MDA	(49)
Rat	Alendronat	Stomach MDA	(50)
Rat	Pressure organ MDA	Skin and remote	(51)
Rat	Diquat	Plasma, liver	(52)
Mouse	Diquat and kidney isoprostanes	Plasma, liver	(53)
Mouse	High-LET Fe ⁵⁶ particle irradiation	Cerebellar MDA	(54)
Dove	Latex bead	Blood MDA	(55)

MDA=malondialdehyde; 4-HDA=4-hydroxylalkenals.

been somewhat overlooked. In 1999, Urata and colleagues reported that melatonin elevated the intracellular levels of another critically important antioxidant, glutathione (GSH), by stimulating its synthesis at the level of its rate limiting enzyme gamma-glutamylcysteine synthase (now known as glutamylcysteine ligase) (24). This finding did not receive much attention nor interest although the observation was later confirmed by Winiarska et al (25). Recently, however, Sainz and co-workers reported that the incubation of either LNCap or PC3 prostate cells with melatonin almost doubled the already high concentrations of GSH and protected the cells from ionizing radiation, a treatment associated with excessive free radical generation (26). In the same study, depletion of GSH levels by inhibition of its synthesis greatly increased the vulnerability of these cells to radiation-mediated damage and apoptosis. Collectively, the results of this study and of the two other published reports in this field suggest that the ability of melatonin to incrementally change intracellular GSH levels may be a more important aspect of the indole's antioxidant activity than originally envisaged.

Another feature that contributes to melatonin's efficiency in reducing oxidative stress is its ability to curtail electron leakage from the complexes of the respiratory chain in the mitochondria. As electrons are shunted between the complexes of

the electron transport chain (ETC), some of them escape and interact with nearby oxygen molecules to generate the O₂⁻. Melatonin theoretically works at the level of Complex I and Complex III of the ETC to increase the efficiency of electron transfer and reduce electron leakage (27-30); this action has come to be known as the radical avoidance action of melatonin (13, 29).

The combination of the actions of melatonin and its metabolites described herein obviously makes it an optimal molecule for resisting damage to critical cellular organelles and molecules that are normally a consequence of free radicals and related reactants which are persistently generated within cells because of their usage of oxygen as the basis of their metabolism. Importantly, melatonin has been shown to function within membranes, mitochondria, cytosol and within the nucleus to resist free radical mutilation (31). In doing so, it functions as a critical factor in reducing cellular loss due to apoptosis (32). As a result, melatonin may have a critical function in limiting organ deterioration that accompanies toxin exposure, ionizing radiation, prescription drug usage, and the process of aging.

Membrane structure

Membranes in cells, i.e., the plasma membrane as well as those of subcellular organelles, are similar in structure, i.e., the phospholipid bilayer is universally the basic construct of cellular membranes. Phospholipids are amphiphilic molecules with a hydrophobic and a hydrophilic portion. The essential physical forces for organizing biological membranes are the hydrophobic interactions between the fatty acyl chains of the lipid molecules. These interactions allow the formation of the phospholipid bilayer, with the polar heads facing the surrounding aqueous surfaces while the fatty acyl positions form a continuous hydrophobic interior. Each phospholipid layer is referred to as a leaflet.

All membranes contain the following lipids: phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingomyelin and phosphatidylinositol. Many membranes also contain cholesterol, a molecule particularly abundant in the plasma membranes of mammalian cells. Another lipid, cardiolipin (diphosphatidylglycerol), is found only in the inner mitochondrial membrane.

Besides lipids, membranes also contain proteins. The ratio of proteins to lipids varies widely among membranes from different structures. Thus, the inner mitochondrial membrane is roughly 75% protein while the myelin membrane is composed of only 18% protein. Examples of proteins in cellular membranes include pores, channels, and receptors for hormones and neurotransmitters.

Additionally, carbohydrates are essential constituents of many membranes. They are bound either to proteins as components of glycoproteins or to lipids as constituents of glycolipids. Carbohydrates are particularly abundant in the plasma membrane of cells of eukaryotes but they are absent from the inner mitochondrial membranes.

Melatonin and membrane lipid peroxidation

Lipids, as compared to most other molecules, are readily damaged by free radicals. As a result, because of their high phospholipid content, cellular membranes are often rendered less

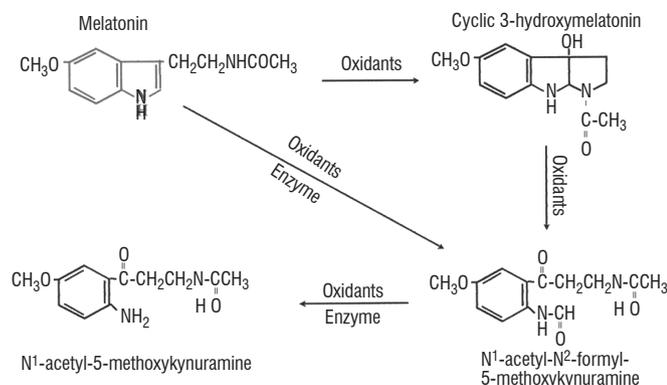


FIGURE 1. Melatonin, when it functions as a scavenger, is metabolized to cyclic 3-hydroxymelatonin or N¹-acetyl-N²-formyl-5-methoxykynuramine (AFMK). Like the parent molecule, these metabolites are likewise effective free radical scavengers. In doing so, cyclic 3-hydroxymelatonin is metabolized to AFMK while AFMK is converted to N¹-acetyl-5-methoxykynuramine (AMK). The sequential scavenging of radicals by melatonin and its metabolites is referred to as the antioxidant cascade of melatonin

than optimally functional. This negatively impacts not only their physiology but that of the entire cell.

The process of membrane lipid peroxidation is a result of an attack upon a lipid by any species that is sufficiently reactive to abstract a hydrogen molecule from a methylene (-CH₂-) group. Fatty acids with no or one double bond are relatively more resistant to hydrogen extraction; however, polyunsaturated fatty acids (PUFA) are highly vulnerable. The process of initiation of lipid peroxidation is readily achieved by the highly reactive ·OH and by the ONOO·. Peroxidation of PUFA eventually gives rise of lipid peroxy radicals (LOO·) which are also capable of abstracting a hydrogen from an adjacent fatty acid molecule. Thus, the peroxidation of lipids in propagated by a molecule formed in the breakdown of lipids; this propagation state ensures that the process becomes a chain reaction. Theoretically, if not interrupted, once underway the peroxidation of a single fatty acid could result in the breakdown of all the lipids in a cell or in a tissue. Fortunately, the chain reaction can be interrupted by peroxy radical scavengers, i.e., the so-called chain breaking antioxidants. Vitamin E is an excellent chain breaking antioxidant.

The ability of melatonin to limit or prevent the peroxidation of membrane lipids has been frequently investigated (33-36). The usual indices for estimation of the degree of lipid breakdown are the measurements of malondialdehyde and 4-hydroxyalkenals (MDA+4-HDA). The biochemical assays for these molecules are not particularly sensitive but they are simple and widely used. There are a very large number of reports related to the ability of melatonin to reduce tissue levels of MDA, 4-HDA or both (Table 1).

From the findings summarized in the table and the literature publications it is obvious that melatonin is highly effective in reducing the oxidation of membrane lipids. This protective action of melatonin is independent of the technique used to estimate levels of damaged lipid products, of the species in which melatonin's efficacy was investigated or the method/toxin

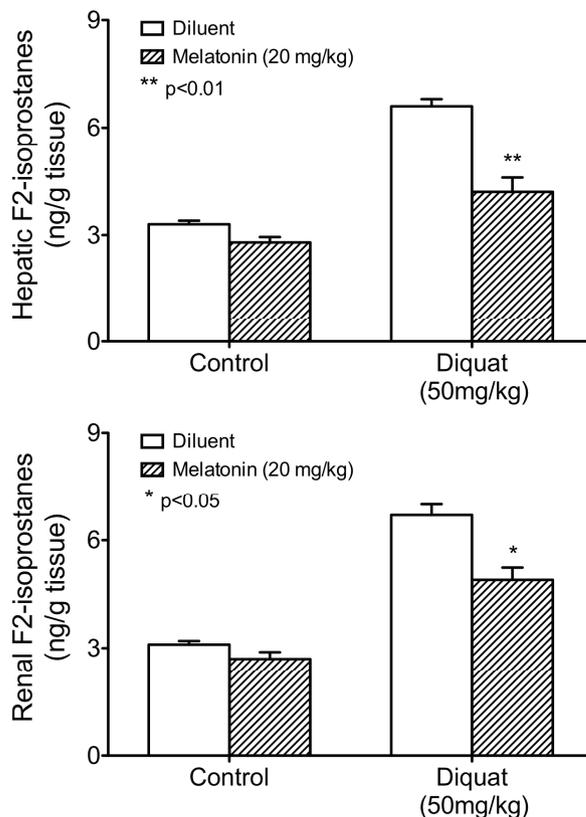


FIGURE 2. Hepatic and renal levels of F₂-isoprostanes, a sensitive index of lipid peroxidation, in mice at 6 hours after an intraperitoneal injection of either saline (Control) or the toxic bipyridyl herbicide, diquat. Half of the mice in each group were given an intraperitoneal injection of melatonin 30 minutes prior to either the saline or diquat injections. F₂-isoprostanes were measured using gas chromatography/negative chemical ionization mass spectrometry. Data are means ± SEM.

used to induce lipid peroxidation. Moreover, melatonin has this capability in every organ, a fact that documents its ability to cross all morphophysiological barriers. When compared to vitamin E, considered to be the premier lipid antioxidant, melatonin actually was found to be more effective in limiting lipid membrane destruction (47).

One particular class of toxic agents generated during lipid peroxidation is the isoprostanes. These are prostaglandin-like molecules formed during the peroxidation of arachidonic acid as well as from eicosapentaenoic (EPA) and docosahexanoic (DHA); collectively they are referred to as the F₂-isoprostanes. These agents are highly useful markers of lipid peroxidation and can be measured in tissue, blood and urine. F₂-isoprostanes are highly stable molecules and are usually quantified using gas chromatography/mass spectrometry (GC/MS).

Zhang *et al.*, (52) and Xu and colleagues (53) used the level of isoprostanes to evaluate melatonin's ability to protect against damage to lipids in hepatic cellular membranes following the treatment of animals with the bipyridyl herbicide, diquat. This highly toxic molecule is widely used throughout the world and its ingestion can cause serious damage to lipids and even death. When Fischer 344 rats were injected with 40 mg/kg di-

quat (40mg/kg), a rapid rise in plasma and hepatic isoprostane levels were measured with peak values being achieved within 3 (liver) or 6 hours (plasma), respectively (52). Rats given melatonin (20mg/kg) concomitantly with diquat had significantly attenuated rises in hepatic and plasma (Fig. 2) isoprostane levels documenting the protective actions of melatonin against lipid peroxidation. Evidence that liver function was also partially preserved when melatonin was given to diquat-treated animals was documented by the fact that the indole reduced the rise in blood alanine aminotransferase (ALT) levels that resulted when hepatic tissue was destroyed by the herbicide.

Xu *et al.*, also used melatonin to abrogate hepatic and renal lipid peroxidation in mice given diquat. In both tissues as well as in the plasma, melatonin reduced isoprostane levels enhanced as a consequence of diquat toxicity (53). Moreover, melatonin reduced the 24 hour death rate of diquat-treated mice from 44% to 9%. This latter finding is important given that thousands of deaths occur annually due to the accidental or intentional ingestion of diquat and the fact that currently there is no known antidote or treatment for individuals suffering from the toxicity of this herbicide. The results also document, based on the highly sensitive GC/MS assay for isoprostanes, that melatonin is a potent protector against lipid peroxidation.

As noted above, lipid peroxidation is a self-propagating process since the peroxy radical, which is generated during the breakdown of lipids, is sufficiently reactive that it can damage a by-stander lipid. Antioxidants that are capable of preventing the propagation of lipid peroxidation by virtue of their ability to scavenge the peroxy radical are known as chain breaking antioxidants; the best known is vitamin E. Whether melatonin scavenges the peroxy radical is debated. More likely, melatonin reduces the oxidation of lipids and preserves membrane integrity due to its ability to scavenge the radicals or related molecules that initiate the process, particularly the $\cdot\text{OH}$ and the $\text{ONOO}\cdot$.

Melatonin and membrane fluidity

There is general agreement that the peroxidation of lipids in membranes makes them more rigid (56, 57). Accumulated free radical damage to the molecules constituting membranes is used to explain the lower fluidity of cellular membranes in aged animals. Changes in the optimal fluidity of membranes generally has a negative effect on their function, *e.g.*, membrane-associated enzymes function less efficiently as do membrane receptor-mediated signal transduction processes (58). Moreover, changes in the fluidity of cellular membranes have been implicated in aging as well as in disease processes (59-61).

Several methods are available to determine membrane fluidity, the two most common of which are electron paramagnetic resonance spectroscopy and fluorescence spectroscopy. Studies that have examined the influence of melatonin on membrane fluidity have used exclusively the latter method to estimate the fluid nature of membranes. Antioxidants, *e.g.*, vitamin E, are generally capable of influencing the fluidity of membranes (62).

Given that melatonin is an inhibitor of lipid peroxidation, it was assumed that, in doing so, it would also maintain cell

membranes in a state of optimal fluidity. When this was experimentally tested, it was in fact found to be the case. Thus, when melatonin limited lipid peroxidation in cells it also prevented their membranes from becoming rigid. The first study in this area was that of Garcia *et al.* (63). In this investigation, hepatic microsomes were exposed to a combination of FeCl_3 , ADP and NADPH to induce the oxidation of lipids; half of the samples were also treated with melatonin. In a dose-response manner, melatonin reduced the accumulated levels of MDA+4-HDA and likewise prevented membrane rigidity, measured by fluorescence spectrometry. The association between the biophysical characteristics of membranes and the number of phospholipids that are oxidized is consistent with the published literature (64). In this study, melatonin only changed membrane fluidity when the microsomes were exposed to oxidative stress; this indicates that the preservation of membrane fluidity was very likely due to the free radical scavenging actions of the indole.

The same group tested the combination of tamoxifen, a synthetic antiestrogen used as a treatment to inhibit breast cancer, and melatonin in reducing membrane rigidity and curtailing the oxidative breakdown of lipids (65). Again, microsomes were exposed to oxidizing agents to induce lipid peroxidation. Tamoxifen and melatonin, alone or in combination, reduced the oxidation of membrane lipids and resisted the changes in membrane fluidity. In combination, the two agents had additive effects on both parameters. Also, when the drugs were combined, they reduced basal membrane rigidity as well as further lowering MDA+4-HDA levels.

Ionizing radiation, due to the fact that it induces free radical generation, mutilates all major molecules in cells. Hepatic membranes recovered from the liver of rats subjected to whole body ionizing radiation exhibited increased rigidity while the DNA of the hepatic cells had elevated levels of 8-hydroxy-2-deoxyguanosine, a product that results from free radical damage to the genome. Both the elevated membrane rigidity as well as the quantity of damaged DNA was reduced when melatonin had been administered prior to the exposure of the rats to the ionizing radiation (66). Since membranes are not exclusively composed of lipids but also contain proteins, damage to these molecules could have accounted, at least in part, to the elevated rigidity of the membranes after ionizing radiation exposure. Melatonin is also known to prevent free radical damage to proteins (67).

Cholesterol is a major determinant of the fluidity of some membranes. Cholesterol, because of its hydrophobicity, does not form a sheet structure on its own; rather cholesterol intercalates among the phospholipids that make up membranes. The polar hydroxyl group of the cholesterol molecule is normally in contact with the aqueous solution in the vicinity of the polar heads of the phospholipids while the steroid ring interacts with and immobilizes the fatty acyl chains of the phospholipids. In general, elevated concentrations of cholesterol in membranes tend to make them less fluid (more rigid).

That melatonin might actually position itself within the cell membrane was suggested by Ceraulo and co-workers (68). Using lecithin reverse micelles, they deduced that in the presence of domains from apolar organic solvent to surfactant to water, melatonin would locate in the micellar phase with a preferen-

tial location in the surfactant polar head group domain. In this position, the authors surmised that melatonin could readily scavenge radicals in both the aqueous as well as the lipid phases. These findings suggest that melatonin shares both hydrophilic and lipophilic characteristics (69) and that it may have some positional advantages as a radical scavenger.

Physiological implications

The plasma membrane surrounds the cytoplasm and provides a physical barrier that separates molecules within the cell from the extracellular environment. Given that the intracellular milieu is markedly different from that outside the cell, it is obviously important that the integrity and optimal physiology of the plasma membrane of any cell is critical to its survival. Besides serving as a barrier that is selectively permeable and capable of influencing what enters or exists the cell, the plasma membrane has a role in anchoring the internal cytoskeleton which provides shape to the cell as well as supplying attachments to the extracellular matrix (for most cells) assisting them in the formation of a discrete tissue/organ (70).

The movement of constituents across the plasma membrane can be either passive, i.e., requiring no energy expenditure by the cell, or active where the cell must use energy to move the solute. This movement of substances also allows the membrane to maintain the cell membrane potential.

Besides serving a barrier function, embedded in the bilayered lipid plasma membrane are a variety of proteins which aid cells in communicating with one another. Moreover, protein receptors are found throughout the membranes of all cells and function in the reception of information from signaling molecules in the extracellular environment. Finally, there are surface proteins on the outside of the plasma membrane that identify the cell to other cells and solutes. These features allow for cell-to-cell communication.

The deterioration of the plasma membrane due to the oxidation of its lipid and protein constituents has devastating consequences on the function and survival of cells. The destructive effects of these oxidative processes are readily apparent when cells containing oxidatively damaged molecules in their plasma membrane are visualized using phase contrast (32) or fluorescence microscopy (70). Excessive damage to the plasma membrane, which is invariably accompanied by destruction of internal organellar membranes as well, leads to implosion of the cell via apoptosis or necrosis.

Clearly, anything that damages the cell membrane compromises the physiology and survival of the cell (71, 72). This damage changes the dynamics of channels, pores and receptors; it renders the cell less efficient in transducing messages received from the outside and changes the transport of substances through the membrane. Thus, oxidation in the cell membrane makes the cell function suboptimally, making it vulnerable to being killed by molecules it may otherwise be capable of resisting.

Besides reducing cellular changes that occur due to the peroxidation of lipids within membranes, melatonin may also have some direct effects on membrane channel function. High-voltage activated calcium channels (HVACC) and intracellular free Ca^{2+} concentrations were investigated in dorsal root gan-

glion neurons using whole cell patch clamping and fluorescence imaging techniques (73). Melatonin was found to inhibit HVACC in a dose-dependent manner. Thus, melatonin inhibited the entrance of Ca^{2+} into the cell which was dependent on extracellular Ca^{2+} levels.

Alterations in cellular calcium homeostasis and smooth muscle contractility are consequences of acute cholecystitis. This combination of effects leads to severe gallbladder dysfunction, a condition for which there is no current effective treatment. Gomez-Pinilla and co-workers investigated the potential beneficial actions of melatonin on these processes in gallbladder smooth muscle tissue obtained from the guinea pig (74). The efficacy of melatonin was tested under two different experimental conditions, one in which the common bile duct had been ligated for two days and a second in which the duct had been initially ligated and then deligated for two days. Both these conditions were associated with inflammatory responses in the gallbladder.

Under these conditions, the inflammation-mediated malfunction of Ca^{2+} responses to cholecystokinin or caffeine were reversed by melatonin treatment; the indole also reduced the detrimental effects of AC on Ca^{2+} influx through both L-type and capacitative Ca^{2+} channels and, moreover, it preserved the pharmacological phenotype of the channels. Inflammation of the gallbladder was also associated with elevated oxidative stress in this tissue as evidenced by the elevated MDA levels and depressed GSH concentrations; both these changes were likewise reversed by melatonin. Additionally, melatonin lowered cyclooxygenase-2 (COX-2) levels in the inflamed gallbladder. The authors concluded, and the results support this conclusion, that melatonin lowers the degree of oxidative damage and reduces inflammation which presumably leads to an improvement of Ca^{2+} channel physiology. In glial cells stimulated with the excitotoxin, glutamate, melatonin protected the cells from oxidative stress and also Ca^{2+} influx and reduced cell death (75).

Melatonin's effect on K^{1+} current in rat cerebellar granule cells has also been investigated (76). Using a conventional patch-clamp technique two types of K^{1+} current, both a transient outward current and a delayed rectifier K^{1+} current were recorded. A 78% increase in the delayed rectifier current was recorded upon the application of melatonin; the increase was reversible and presented no desensitization upon the repeated application of melatonin. Since the effect of melatonin on the K^{1+} current could be duplicated by adding the melatonin receptor agonist, iodomelatonin, the authors concluded the effect was receptor-mediated (77).

The results of Hou *et al* (78) contrast with those of Huan *et al* (76). The former workers examined the effect of melatonin on the delayed rectifier K^{1+} current in CA1 pyramidal neurons in hippocampal slices using the patch clamp technique. In a concentration dependent manner, melatonin caused a reduction in the K^{1+} current, a response that was not blocked by luzindole, an antagonist of the two best known membrane melatonin receptors, the MT1 and MT2 receptor. Hou *et al* believed, as a consequence of their findings, that melatonin influences K^{1+} currents via its interaction with intracellular indole-related domains on potassium channels (78). The differences between the effects of melatonin on K^{1+} currents in cerebellar granule

cells and pyramidal cells of the hippocampus currently remain unexplained.

Using cultured cerebellar granule cells, Lax performed a detailed examination of the effects of melatonin on nicotine-evoked currents (79). With a combination of electrophysiological and Ca^{2+} -imaging methodologies, he identified some granule cells in which nicotine caused both intracellular Ca^{2+} transients and inward whole-cell currents. Judging from their sensitivity to nicotine and the time constant of the current decay, it was surmised that the responses were mediated by the neuronal acetylcholine receptor. With the use of melatonin levels as low as 1 pM, Lax showed that the indole attenuated the amplitude dose-dependently but was without influence on the receptor's apparent affinity or on the current's rise or decay time (79). The inhibitory effect of melatonin was suppressed by luzindole, a competitive MT1 and MT2 melatonin receptor antagonist. Given that melatonin's actions were mediated by physiologically relevant concentrations of the indole, this author feels the evidence provides a means by which the circadian rhythm of circulating melatonin may be operative in modulating cholinergic activity, at least on cerebellar granule neurons.

The influence of melatonin on the water channel aquaporin-1 (AQP-1) was examined in a model of spinal cord injury by Nescic *et al* (80). AQP-1 is particularly abundant in the small diameter sensory neurons of the dorsal horn of the spinal cord. Contusion injury of the spinal cord of rats was found to be associated with a four to five-fold elevation in the number of AQP-1 channels at the level of thoracic cord injury with delayed increases in pore levels eventually being observed in the cervical and lumbar cord. Melatonin reduced the AQP-1 increases in the injured spinal cord thereby limiting neuronal and astrocyte swelling. The inhibition of the rises in the water pore following injury to the spinal cord was associated with a significant decrease in mechanical allodynia, i.e., pain related

to mechanical stimulation. The implication of these findings is that by reducing AQP-1, melatonin prevents cellular swelling and pain associated with injury to the spinal cord.

Obviously, much needs to be learned regarding the effects of melatonin on the physiology of the plasma membrane of cells. Due to its ability to reduce oxidative damage in this structure, it is virtually certain that melatonin at least secondarily influences the function of cell membranes and, therefore, the integrity of cells. Additionally, however, melatonin may have direct receptor-mediated or receptor-independent actions on channels and pores in the cell membrane. The evidence in this field is clearly sparse and an area is ripe for investigation.

Concluding remarks

Intact and optimally functional plasma membranes of cells are critical for them to efficiently carry out their prescribed functions. By virtue of melatonin's ability to function as a direct free radical scavenger and indirect antioxidant, it is a major molecule in protecting membrane constituents from oxidative mutilation. In doing so, melatonin also optimizes the physiology of membrane receptors, channels and pores as well as maintaining the shape of the cell. Secondly, melatonin is reported to have some direct effects on channels and pores within all membranes. Although this latter field is not yet well exploited, it seems likely that research in this area will flourish within the next decade.

In general, melatonin functions in all parts of all cells and improves physiological infrastructure. As a result, it enhances cell function and optimizes the ability of cells to survive in a hostile environment. Among many apparent functions that melatonin has, it seems likely that its ability to preserve the morphological and functional aspects of the cell membrane may be among its most important actions.

Melatonin ve hücresel membranların patofizyolojisi

ÖZET: Bu derlemede melatoninin hücre membranlarının fizyolojisindeki katkısı değerlendirildi. 1993'den itibaren bu konuda mevcut literatürleri kapsamaktadır. Melatonin gerek tek hücrelilerde gerekse çok hücrelilerde bulunan çeşitli önemli fonksiyonları olan, yaygın dağılım gösteren bir indolamindir. Serbest radikal hasarına karşı lipidler üzerindeki koruyucu etkisi ile melatonin hücre membranlarının morfolojik ve fonksiyonel bütünlüğünün korunmasında çok önemlidir. Bu etkisiyle melatonin membranlardaki okside lipid miktarını azaltarak membranın optimal akışkanlığını korur; rijid hale gelmesini önler. Bu etki hücre membranlarındaki proteinlerin (reseptörler, kanallar ve porlar gibi) fonksiyonları için çok önemlidir ve hücrenin normal fizyolojisinin korunmasına katkıda bulunur. Melatoninin sözü edilen indirekt etkisine ilave olarak, bulgular bu indolaminin membran kanalları üzerinde direkt etkisiyle iyon gradiyentinin ve akımının uygun olarak sürdürülmesini sağladığını göstermiştir. Melatoninin membranın kanal ve por fonksiyonlarına etkileri deneysel olarak incelenmesi gereken bir alandır.

ANAHTAR KELİMELE: Melatonin, hücre membranları, lipid peroksidasyon, membran kanalları, membrane porları, hücre membrane reseptörleri

REFERENCES

1. Tan DX, Chen LD, Poeggeler B, Manchester LC, Reiter RJ. Melatonin: a potent, endogenous hydroxyl radical scavenger. *Endocrine J*, 1: 57-60, 1993.
2. Reiter RJ. Functional pleiotrophy of the neurohormone melatonin: antioxidant protection and neuroendocrine regulation. *Front Neuroendocrinol*, 16: 383-415, 1995.
3. Reiter RJ, Tan DX, Kim SJ, Qi W. Melatonin as a pharmacological agent against oxidative damage to lipids and DNA. *Proc West Pharmacol Soc*, 41: 229-236, 1998.
4. Manda K, Ueno M, Anzai K. AFMK, a melatonin metabolite, attenuates X-ray-induced oxidative damage to DNA, proteins and lipids in mice. *J Pineal Res*, 42: 386-393, 2007.
5. Maldonado MD, Murillo-Cabezas F, Terron MP, Flores LJ, Tan DX, Manchester LC, Reiter RJ. The potential of melatonin in reducing morbidity-mortality after craniocerebral trauma. *J Pineal Res*, 42: 1-11, 2007.
6. Tengattini, S, Reiter RJ, Tan DX, Terron MP, Rodella LF. Cardiovascular diseases: protective effects of melatonin. *J Pineal Res*, 44: 16-25, 2008.
7. Tan DX, Manchester LC, Reiter RJ, Plummer BF, Limson J, Weintraub ST, Qi W. Melatonin directly scavenges hydrogen peroxide: a potentially new metabolic pathway of melatonin biotransformation. *Free Radic Biol Med*, 29: 1177-1185, 2000.
8. Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derivatives: a never ending interaction of melatonin with reactive oxygen and nitrogen species? *J Pineal Res*, 42: 28-42, 2007.
9. Pappolla MA, Chyan YJ, Poeggeler B, Frangione B, Wilson G, Ghiso J, et al. An assessment of the antioxidant and anti-amyloidogenic properties of melatonin: implications for Alzheimer's disease. *J Neural Transm*, 107: 203-231, 2000.
10. Reiter RJ, Tan DX, Manchester LC, Qi W. Biochemical reactivity of melatonin with reactive oxygen and nitrogen species: a review of the evidence. *Cell Biochem Biophys*, 34: 237-256, 2001.
11. Reiter RJ, Tan DX, Manchester LC, El-Sawi MR. Melatonin reduces oxidative damage and promotes mitochondrial respiration: implications for aging. *Ann NY Acad Sci*, 959: 238-250, 2002.
12. Allegra M, Reiter RJ, Tan DX, Gentile C, Tesoriere L, Livrea MA. The chemistry of melatonin's interaction with reactive species. *J Pineal Res*, 34:1-10, 2003.
13. Hardeland R. Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. *Endocrine*, 27: 119-130, 2005.
14. Hardeland R, Backhaus C, Fadavi A. Reactions of the NO redox forms NO⁺, .NO and HNO (protonated NO⁻) with the melatonin metabolite N1-acetyl-5-methoxykypuramine. *J Pineal Res*, 43: 382-388, 2007.
15. Zavodnik IB, Domanski AV, Lapshina EA, Bryszewska M, Reiter RJ. Melatonin directly scavenges peroxy and alkoxy radicals generated in red blood cells and a cell-free system: chemiluminescence measurements and theoretical calculations. *Life Sci*, 79: 391-400, 2006.
16. Peyrot N, Ducrocq C. Potential role of tryptophan derivatives in stress responses characterized by the generation of reactive oxygen and nitrogen species. *J Pineal Res*, 45: 235-246. 2008.
17. Tan DX, Reiter RJ, Manchester LC, Yan MT, El-Sawi M, Sainz RM, Mayo JC, Kohen R, Allegra M, Hardeland R. Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. *Curr Topics Med Chem*, 2: 181-198, 2002.
18. Tan DX, Manchester LC, Sainz RM, Mayo JC, Alvarez F, Reiter RJ. Antioxidant strategies in protection against neurodegenerative disorders. *Expert Opin Ther Patents*, 13: 1513-1542, 2003.
19. Pablos MI, Agapito MT, Gutierrez R, Recio JM, Reiter RJ, Barlow-Walden L, Acuña-Castroviejo D, Menendez-Pelaez A. Melatonin stimulates the activity of the detoxifying enzyme glutathione peroxidase in several tissues of chicks. *J Pineal Res*, 19: 111-115, 1995.
20. Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress: a review. *J Biomed Res*, 7: 444-458, 2000.
21. Rodriguez C, Mayo JC, Sainz RM, Antolín I, Herrera F, Martín V, Reiter RJ. Regulation of antioxidant enzymes: a significant role for melatonin. *J Pineal Res*, 36: 1-9, 2004.
22. Tomas-Zapico C, Coto-Montes A. A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. *J Pineal Res*, 39: 99-104, 2005.
23. Leon J, Escames G, Rodriguez MI, López LC, Tapias V, Entrena A, Camacho E, Carrión MD, Gallo MA, Espinosa A, Tan DX, Reiter RJ, Acuña-Castroviejo D. Inhibition of neuronal nitric oxide synthase activity by N1-acetyl-5-methoxykypuramine, a brain metabolite of melatonin. *J Neurochem*, 98: 2023-2033, 2006.
24. Urata Y, Honma S, Goto S, Todoroki S, Iida T, Cho S, Honma K, Kondo T. Melatonin induces gamma-glutamyl-cysteine synthase mediated by activator protein-1 in human vascular endothelial cells. *Free Radic Biol Med*, 27: 838-847, 1999.
25. Winiarska K, Fraczyk T, Molinska D, Drozak J, Bryla J. Melatonin attenuates diabetes-induced oxidative stress in rabbits. *J Pineal Res*, 40: 168-176, 2006.
26. Sainz RM, Reiter RJ, Tan DX, Roldan F, Natarajan M, Quiros I, Hevia D, Rodriguez C, Mayo JC. Critical role of glutathione in melatonin enhancement of tumor necrosis factor and ionizing radiation-induced apoptosis in prostate cancer cells in vitro. *J Pineal Res*, 45: 258-270, 2008.
27. Okatani Y, Wakasuki A, Reiter RJ. Hepatic mitochondrial dysfunction in senescence-accelerated mice: correction by long-term, orally administered physiological levels of melatonin. *J Pineal Res*, 33: 127-133, 2002.
28. Martin M, Macias M, Escames G, Reiter RJ, Agapito MT, Ortiz GG, Acuña-Castroviejo D. Melatonin-induced increased activity of the respiratory complexes I and IV can prevent mitochondrial damage induced by ruthenium red in vivo. *J Pineal Res*, 28: 242-248, 2000.
29. Leon J, Acuna-Castroviejo D, Escames G, Tan DX, Reiter RJ. Melatonin mitigates mitochondrial malfunction. *J Pineal Res*, 38: 1-9, 2005.
30. Reiter RJ, Paredes SD, Korkmaz A, Jou MJ, Tan DX. Melatonin combats molecular terrorism at the mitochondrial level. *Interdisc Toxicol*, 1: 137-149, 2008.
31. Jou MJ, Peng TI, Yu PZ, Jou SB, Reiter RJ, Chen JY, Wu HY, Chen CC, Hsu LF. Melatonin protects against common deletion of mitochondria DNA-augmented mito-

- chondrial oxidative stress and apoptosis. *J Pineal Res*, 43: 389-403, 2007.
32. Jou, MJ, Peng TI, Reiter RJ, Jou SB, Wu HY, Wen ST. Visualization of the antioxidative effects of melatonin at the mitochondrial level during oxidative stress-induced apoptosis of rat brain astrocytes. *J Pineal Res*, 37: 55-70, 2004.
 33. Cervantes M, Morali G, Letechipia-Vallejo G. Melatonin and ischemia-reperfusion injury. *J Pineal Res*, 45: 1-7, 2008.
 34. De Filippis D, Iuvone T, Esposito G, Steardo L, Arnold GH, Paul AP, De Man Joris G, De Winter Benedicte Y. Melatonin reverses lipopolysaccharide-induced gastrointestinal mobility disturbances through the inhibition of oxidative stress. *J Pineal Res*, 44: 45-51, 2008.
 35. 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. Oxidative stress impairs oocyte quality and melatonin protects oocytes from free radical damage and improves fertilization rate. *J Pineal Res*, 44: 280-287, 2008.
 36. Gitto E, Pelligrino S, Gitto P, Barberi I, Reiter RJ. Oxidative stress of the newborn in the pre-and post-natal period and the clinical utility of melatonin. *J Pineal Res*, 46: 128-139, 2009.
 37. Gitto E, Karbownik M, Reiter RJ, Tan DX, Cuzzocrea S, Chiurazzi P, Cordaro S, Corona G, Trimarchi G, Barberi I. Effects of melatonin treatment in septic newborns. *Pediatr Res*, 50: 756-760, 2000.
 38. Fulia F, Gitto E, Cuzzocrea S, Reiter RJ, Dugo L, Gitto P, Barberi S, Cordaro S, Barberi I. Increased levels of malondialdehyde and nitrite/nitrate in the blood of asphyxiated newborns: reduction by melatonin. *J Pineal Res*, 31: 343-349, 2001.
 39. Kucukakin B, Lykkesfeldt J, Nielsen HJ, Reiter RJ, Rosenberg J, Gogenur I. Utility of melatonin to treat surgical stress after major vascular surgery - a safety study. *J Pineal Res*, 44: 426-431, 2008.
 40. Melchiorri D, Reiter RJ, Sewerynek E, Chen LD, Nistico G. Melatonin reduces kainate-induced lipid peroxidation in homogenates of different brain regions. *FASEB J*, 9:1205-1210, 1995.
 41. Melchiorri D, Reiter RJ, Sewerynek E, Hara M, Chen LD, Nistico G. Paraquat toxicity and oxidative damage: reduction by melatonin. *Biochem Pharmacol*, 51: 1095-1099, 1996.
 42. Hara M, Abe M, Suzuki T, and Reiter RJ. Tissue changes in glutathione metabolism and lipid peroxidation by swimming are partially prevented by melatonin. *Pharmacol Toxicol*, 78: 308-312, 1996.
 43. Sewerynek E, Abe M, Reiter RJ, Barlow-Walden LR, Barlow-Walden LR, Chen L, McCabe TJ, Roman LJ, Diaz-Lopez B. Melatonin administration prevents lipopolysaccharide-induced oxidative damage in phenobarbital-treated animals. *J Cell Biochem*, 58: 336-344, 1995.
 44. Sewerynek E, Reiter RJ, Melchiorri D, Ortiz GG, Lewinski A. Oxidative damage in liver induced by ischemia-reperfusion: protection by melatonin. *Hepatogastroenterology*, 43: 898-905, 1996.
 45. Ohta Y, Kongo M, Sasaki E, Nishida K, Ishiguro I. Therapeutic effect of melatonin on carbon tetrachloride-induced acute liver injury in rats. *J Pineal Res*, 28: 119-126, 2000.
 46. Cuzzocrea S, Mazzson E, Serraino I, Lepore V, Terranova ML, Ciccolo A, Caputi AP. Melatonin reduces dinitrobenzene sulfonic acid-induced colitis. *J Pineal Res*, 2001; 30:1-12.
 47. Baydas G, Canatan H, Turkoglu A. Comparative analysis of the protective actions of melatonin and vitamin E on streptozocin-induced diabetes mellitus. *J Pineal Res*, 32: 225-230, 2002.
 48. Dziegiel P, Suder E, Surowiak P, Jethon Z, Rabczyński J, Januszewska L, Sopol M, Zabel M. Role of exogenous melatonin in reducing the nephrotoxic effect of daunorubicin and doxorubicin in the rat. *J Pineal Res*, 33: 95-100, 2002.
 49. Sener G, Sehirli O, Altunbas HZ, Ersoy Y, Paskaloglu K, Arbak S, Ayanoglu-Dulger G. Melatonin protects against gentamicin-induced nephrotoxicity in rats. *J Pineal Res*, 32: 231-236, 2002.
 50. Sener G, Goren FO, Ulusoy NB, Ersoy Y, Arbak S, Dulger GA. Protective effect of melatonin and omeprazole against alendronat-induced gastric damaged. *Dig Dis Sci*, 50: 1506-1512, 2005.
 51. Sener G, Sert G, Sehirli A, Arbak S, Gedik N, Avanoğlu-Dulger G. Melatonin protects against pressure ulcer-induced oxidative injury of the skin and remote organs of rats. *J Pineal Res*, 40: 280-287, 2006.
 52. Zhang L, Wei W, Xu J, Min F, Wang L, Wang X, Cao S, Tan DX, Qi W, Reiter RJ. Inhibitory effect of melatonin on diquat-induced lipid peroxidation in vivo as assessed by the measurement of F2-isoprostones. *J Pineal Res*, 40: 326-331, 2006.
 53. Xu J, Sun S, Wei W, Fu J, Qi W, Manchester LC, Tan DX, Reiter RJ. Melatonin reduces mortality and oxidatively-mediated hepatic and renal damage due to diquat treatment. *J Pineal Res*, 42: 166-171, 2007.
 54. Manda K, Ueno M, Anzai K. Melatonin mitigates oxidation damage and apoptosis in mouse cerebellum induced by high-LET 56Fe particle irradiation. *J Pineal Res*, 44: 189-196, 2008.
 55. Terron MP, Marchena JM, Shadi F, Lea RW, Rodriguez AB. Melatonin: an antioxidant at physiological concentrations. *J Pineal Res*, 31: 95-96, 2001.
 56. Debretsov GE, Borschevskaya TA, Petrov VA, Vladimirov YA. The increase of phospholipid bilayer rigidity after lipid peroxidation. *FEBS Lett*. 84: 125-128, 1977.
 57. Schroeder F. Role of membrane asymmetry in aging. *Neurobiol Aging*, 5: 323-333, 1984.
 58. Curtis MT, Gilfor D, Farber JL. Lipid peroxidation increases the molecular order of microsomal membranes. *Arch Biochem Biophys*, 1989; 235:644-649.
 59. McGrath LT, Douglas AF, McLean E, Brown JH, Doherty CC, Johnston GD, Archbold GP. Oxidative stress and erythrocyte membrane fluidity in patients undergoing regular dialysis. *Clin Chem Acta*, 235: 179-188, 1995.
 60. Choe M, Jackson C, Yu BP. Lipid peroxidation contributes to age-related membrane rigidity. *Free Radic Biol Med*, 18: 977-984, 1995.
 61. Choi JH, Yu BP. Brain synaptosomal aging: free radical on membrane fluidity. *Free Radic Biol Med*, 1995; 18:133-139.

- 62.** Miki M, Motoyama T, Takenaka Y, Mino M. Tocopherol behavior and membrane constituents in erythrocytes with oxidant stress. *Basic Life Sci*, 49: 595-604, 1988.
- 63.** Garcia JJ, Reiter RJ, Guerrero JM, Escames G, Yu BP, Oh CS, Muñoz-Hoyos A. Melatonin prevents changes in microsomal membrane fluidity during induced lipid peroxidation. *FEBS Lett*, 408: 297-300, 1997.
- 64.** Kaplan P, Racay P, Lehotsky J, Mezesooa V. Change in fluidity of brain endoplasmic reticulum membranes by oxygen free radicals: a protective effect of stobadine, alpha-tocopherol acetate and butylated hydroxytoluene. *Neurochem Res*, 20: 815-820, 1995.
- 65.** Garcia JJ, Reiter RJ, Ortiz GG, Oh CS, Tang L, Yu BP, Escames G. Melatonin enhances tamoxifen's ability to prevent the reduction in microsomal membrane fluidity induced by lipid peroxidation. *J Membr Biol*, 162: 59-65, 1998.
- 66.** Karbownik M, Reiter RJ, Qi W, Garcia JJ, Tan DX, Manchester LC, Vijayalaxmi. Protective effects of melatonin against oxidation of guanine bases in DNA and decreased microsomal membrane fluidity in rat liver induced by whole body ionizing radiation. *Mol Cell Biochem*, 211: 137-144, 2000.
- 67.** Abe M, Reiter RJ, Orhii PB, Hara M, Poeggeler B. Inhibitory effect of melatonin on cataract formation in newborn rats: evidence for an antioxidative role for melatonin. *J Pineal Res*, 17: 94-100, 1994.
- 68.** Ceraulo L, Ferrugia M, Tesoriere L, Segreto S, Livrea MA, Turco Liveri V. Interactions of melatonin with membrane models: partitioning of melatonin in AOT and lecithin reverse micelles. *J Pineal Res*, 26: 108-112, 1999.
- 69.** Shida CS, Castrucci AML, Lamy-Freund MT. High solubility of melatonin in aqueous medium. *J Pineal Res*, 16: 198-201, 1994.
- 70.** Benitez-King G. Melatonin as a cytoskeleton modulation: implications for cell physiology and disease. *J Pineal Res*, 40: 1-9, 2006.
- 71.** Venkataraman P, Krishnamoorthy G, Vengatesh G, Srinivasan N, Aruldas Mm, Arunakaran J. Protective role of melatonin on PCB (Aroclor 1,254) induced oxidative stress and changes in acetylcholine esterase and membrane bound ATPases in cerebellum, cerebral cortex and hippocampus of adult rat brain. *Int J Dev Neurosci*, 26: 585-91, 2008.
- 72.** Tjong YW, Li MF, Hung MW, Fung ML. Melatonin ameliorates hippocampal nitric oxide production and large conductance calcium-activated potassium channel activity in chronic intermittent hypoxia. *J Pineal Res*, 44: 234-243, 2008.
- 73.** Ayar A, Martin DJ, Ozcan M, Kelestimur H. Melatonin inhibits high voltage activated calcium currents in cultured rat dorsal root ganglion cells. *Neurosci Lett*, 313: 73-77, 2001.
- 74.** Gomez-Pinilla PJ, Camello PJ, Pozo MJ. Protective effect of melatonin on Ca²⁺ homeostasis and contractility in acute cholecystitis. *J Pineal Res*, 44: 250-260, 2008.
- 75.** Das A, Belagvdu A, Reiter RJ, Ray SK, Banik NL. Cytoprotective effects of melatonin on C6 astroglial cells exposed to glutamate toxicity and oxidative stress. *J Pineal Res*, 45: 117-124, 2008.
- 76.** Huan C, Zhou M, Wu M, Zhang Z, Mei Y. Activation of melatonin receptor increases a delayed rectifier K⁺ current in rat cerebellar granule cells. *Brain Res*. 917: 182-190, 2001.
- 77.** Liu LY, Hoffman GE, Fei XW, Li Z, Zhang ZH, Mei YA. Delayed rectifier outward K⁺ current mediates the migration of rat cerebellar granule cells stimulated by melatonin. *J Neurochem*, 102: 333-344, 2007.
- 78.** Hou SW, Zhang P, Sun FY. Melatonin inhibits outward delayed rectifier potassium currents in hippocampal CA1 pyramidal neuron via intracellular indole-related domains. *J Pineal Res*, 36: 242-248, 2004.
- 79.** Lax P. Melatonin inhibits nicotinic currents in cultured rat cerebellar granule neurons. *J Pineal Res*, 44: 70-77, 2008.
- 80.** Nestic O, Lee J, Unabia GC, Johnson K, Ye Z, Vergara L, Hulsebosch CE, Perez-Polo JR. Aquaporin-1 - a novel player in spinal cord injury. *J Neurochem*, 105: 628-640, 2008.