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〈Review〉

Melatonin-induced Calbindin-D9k is Involved in Protecting Cells against Conditions That Cause Cell Death

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ABSTRACT

Melatonin (N-acetyl-5-methoxytryptamine) is the major neurohormone secreted during the night by the vertebrate pineal gland. The circadian pattern of pineal melatonin secretion is related to the biological clock within the suprachiasmatic nucleus (SCN) of the hypothalamus in mammals. The SCN coordinates the body's rhythms to the environmental light-dark cycle in response to light perceived by the retina, which acts mainly on retinal ganglion cells that contain the photopigment melanopsin. Calbindin-D9k (CaBP-9k) is a member of the S100 family of intracellular calcium-binding proteins, and in this review, we discuss the involvement of melatonin and CaBP-9k with respect to calcium homeostasis and apoptotic cell death. In future studies, we hope to provide important information on the roles played by CaBP-9k in cell signal transduction, cell proliferation, and Ca^{2+} homeostasis *in vivo* and *in vitro*.

(Key words : Calbindin-D9k, Melatonin, Apoptosis, Ca^{2+} homeostasis)

INTRODUCTION

Melatonin is known chemically as N-acetyl-5-methoxytryptamine. McCord and Allen first discovered that an extract from the pineal glands of cows lightened the color of frog skin (McCord and Allen, 1917), while Lerner *et al*. isolated and named melatonin and defined its chemical structure in 1958 (Lerner *et al*., 1958). It is known that melatonin is the major neurohormone secreted during the night by the vertebrate pineal gland. Tryptophan, the precursor for melatonin biosynthesis, is converted into melatonin via the synthesis of serotonin. Serotonin is converted into N-acetylserotonin by the enzyme arylalkylamine-N-acetyl transferase (AANAT), and then N-acetylserotonin is metabolized into melatonin by the enzyme hydroxyindole-O-methyltransferase (HIOMT) (Axelrod and Wurtman, 1968). The enzymatically-formed melatonin is released into the blood and, in higher concentrations, into the cerebrospinal fluid (Tricoire *et al*., 2003). It is then rapidly distributed to most of the body's tissues (Cardinali and Pevet, 1998).

The circadian pattern of pineal melatonin secretion is regulated by the biological clock that resides within the suprachiasmatic nucleus (SCN) of the mammalian hypothalamus, and lesions in the SCN abolish the rhythm of pineal melatonin production in mammals (Klein and Moore, 1979). The SCN is linked to the environmental light-dark cycle by light perceived by the retina, acting mainly on a subgroup of retinal ganglion cells that contain the photopigment melanopsin (Berson *et al*., 2002).

The SCN regulates the pineal gland's function through a polysynaptic network, which involves the paraventricular nucleus of the hypothalamus. Polysynaptic fibers, descending from this region, project through the medial forebrain bundle and the reticular formation to the intermediolateral horns of the cervical segments of the spinal cord (Buijs *et al*., 1998). Postganglionic sympathetic fibers from the superior cervical ganglia innervate the pineal gland and regulate melatonin biosynthesis through the presynaptic release of norepinephrine (NE). NE release occurs during the "night" portion of the circadian pacemaker cycle, provided that this occurs in a dark environment.

Activation of the pineal β-adrenergic receptors by NE results in an increase in the concentration of 3',5'-cyclic adenosine monophosphate (cAMP) that promotes the biosynthesis of melatonin (Klein *et al*., 1971). Alpha1-adrenergic receptors potentiate β-adrenergic activity by producing a sharp increase in intracellular Ca^{2+} and the activation of protein kinase C (PKC) and of prostaglandin synthesis (Ho and Klein, 1987; Krause

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and Dubocovich, 1990). Cyclic AMP stimulates AANAT expression and phosphorylation via protein kinase A, and it also allows AANAT to be stabilized by the binding of 14-3-3 proteins (Klein *et al*., 2002; Schomerus and Korf, 2005). Nocturnal exposure to bright light suppresses melatonin production immediately by the degradation of pineal AANAT (Gastel *et al*., 1998). It has now been demonstrated that melatonin is produced by many other organs in addition to the pineal gland, including the retina (Tosini and Menaker, 1998), gastrointestinal tract (Bubenik, 2001), skin (Slominski *et al*., 2005), lymphocytes (Carrillo-Vico *et al*., 2004), and bone marrow (Conti *et al*., 2000).

Pineal melatonin production occurs during the dark phase of the circadian cycle and is strongly suppressed by light, thus the time and duration of the increase in melatonin reflects the length of the environmental night period (Cardinali and Pevet, 1998). Plasma melatonin exhibits a circadian rhythm with high levels at night, and low levels during the day, attaining peak concentrations between 02:00 and 04:00 h. Longer nights are associated with a longer period of melatonin secretion (Cardinali and Pevet, 1998). Hence, melatonin is a signal of darkness that encodes time-of-day and length-of-day information to the brain including the SCN and peripheral organs (Pandi-Perumal *et al*., 2007). In mammals, melatonin is critical for the regulation of seasonal changes for various physiological, neuroendocrine and reproductive functions (Cardinali and Pevet, 1998; Reiter, 1980).

Melatonin is reported to play a role in the sleep-gate (a sudden increase in nocturnal sleepiness) as a time cue and sleep-anticipating signal in the circadian cycle (Dijk and von Schantz, 2005; Zisapel, 2007). Other actions of this hormone include the inhibition of dopamine (DA) release in the hypothalamus and retina (Zisapel, 2001), the aging process (Karasek, 2004; Reiter *et al*., 1998), a reduction in blood pressure control (Grossman *et al*., 2006; Scheer *et al*., 2004), free-radical scavenging (Reiter *et al*., 2007), and an involvement in the immune response (Srinivasan *et al*., 2005; Carrillo-Vico *et al*., 2006). Melatonin production decreases with age, and in several diseases, including certain malignancies, Alzheimer's disease (AD) (Pappolla *et al*., 2000), and cardiovascular disease (Reiter *et al*., 2009). This decrease in melatonin output has been linked to insomnia (Haimov *et al*., 1994; Leger *et al*., 2004) and to a higher prevalence of cancer (Reiter *et al*., 2007; Schernhammer and Hankinson, 2009).

In this review, we will focus our attention on the relationship between melatonin and the S100 calcium ion (Ca^{2+}) binding proteins, including calbindin-D9k (CaBP-9k).

THE RELATIONSHIP BETWEEN MELATONIN, OXIDATIVE STRESS AND APOPTOTIC CELL DEATH AND Ca^{2+} REGULATION *IN VIVO* AND *IN VITRO*

The process of programmed cell death, known as apoptosis, is induced by the triggering of highly specialized pathways. The extrinsic pathway is initiated by the binding of death ligands to their receptors, whereas the intrinsic pathway is activated by various types of cellular damage and involves the Bcl-2 family of proteins (Youle and Strasser, 2008). In addition, it has been shown that, in response to a wide variety of agents and conditions, Ca^{2+} signaling can also lead to apoptosis (Rong and Distelhorst, 2008).

Apoptosis is a genetically programmed cell elimination process involved in physiological and pathological events that are regulated by members of the Bcl-2 family, of which Bax was the first member to be identified as a cell death accelerator. Bax and Bid are pro-apoptotic members of the Bcl-2 gene family and promote cell death, while the anti-apoptotic members of the gene family, Bcl-2 and Bcl-XL, suppress the pro-apoptotic function of Bax and promote cell survival (Smaili *et al*., 2000; Krajewski *et al*., 1994). Bax and Bid, are highly repressed in living cells, while both proteins are increased in cell death (Hsu *et al*., 1997). Bcl-2 expression is associated with the membranes of organelles, including endoplasmic reticulum (ER), mitochondria, and nuclei (Krajewski *et al*., 1994), and Bcl-xL exists as both soluble and membrane-bound forms (Hsu *et al*., 1997). During apoptosis, Bcl-2 remains bound to the membranes, but the cytosolic forms of Bax, Bid and Bcl-xL are redistributed from the cytosol to the organelle membranes, and in particular to the mitochondrial membrane (Hsu *et al*., 1997; Li *et al*., 1998). The mechanisms leading to Bax and Bcl-xL redistribution are still unknown, while Bid is believed to be post-translationally cleaved by caspase-8 leading to Bax transactivation, which is the activation followed by translocation of the protein (Li *et al*., 1998). Overexpression of Bax can accelerate cell death in response to various apoptotic stimuli (Yang and Korsmeyer, 1996). Physiologically, Bax plays an important role in neuronal development and spermatogenesis. Under pathological conditions such as cerebral and cardiac ischemia, the upregulation of Bax has been detected in the afflicted area

of the tissues, leading to the participation of this protein in neuronal and cardiomyocytic cell death (Krajewski *et al*., 1999). In certain cases of human colorectal cancer, mutations were found in the gene encoding Bax, suggesting that inactivation of Bax promotes tumorigenesis by enabling the tumor cells to be less susceptible to cell death (LeBlanc *et al*., 2002).

Recently, it has been suggested that Bcl-2 proteins may regulate and be regulated by cytosolic Ca^{2+} levels, which influence cell death signaling (Scorrano *et al*., 2003; Carvalho *et al*., 2004). The translocation of Bax to the mitochondria is associated with a release of cytochrome c from the mitochondrial intermembrane space and a loss of the mitochondrial membrane potential (Smaili *et al*., 2001). Mitochondria are important Ca^{2+} stores and are in close relation and communication with the ER (Rizzuto and Pozzan, 2006), and the uptake and release of Ca^{2+} from both these organelles modulates intracellular Ca^{2+} signaling (Smaili and Russell, 1999). Interestingly, Bax and other pro-apoptotic members of the Bcl-2 family were shown to modulate ER and mitochondrial Ca^{2+} stores (Nutt *et al*., 2001; Pan *et al*., 2001). It has been reported that Bax depletes Ca^{2+} from the ER store and activates caspase-12 (Zong *et al*., 2003) and, once ER Ca^{2+} stores have been depleted, there is a reduction in Bax-induced apoptotic cell death (Scorrano *et al*., 2003).

During cytosolic Ca^{2+} overload, mitochondria and the ER may take up Ca^{2+}, which causes Ca^{2+} accumulation, a change in the mitochondrial pH, an increase in the production of reactive oxygen species (ROS), and a decrease, or complete loss, in the mitochondrial membrane potential. Several reports have shown that inhibitors of the electron transport chain, such as malonate, 1-methyl-phenylpyridinium (MPP^+) and 3-nitropropionic acid (3NP) induce cell injuries and neuronal degeneration *in vitro* and *in vivo* (Brouillet *et al*., 1993; Smith and Bennett 1997), similar to those present in Huntington's disease (HD) (Beal *et al*., 1993; Brouillet *et al*., 1995). During its development, patients with HD present motor symptoms, psychic disorders and cognitive deficits. HD, as with many neurodegenerative processes, is associated with changes in Ca^{2+} homeostasis and ROS production, and because mitochondria are important for Ca^{2+} homeostasis and signaling, and are also involved in apoptotic cell death, there is a special interest in understanding the relationship between Ca^{2+} and cell death signaling with respect to neurodegenerative processes (Beal *et al*., 1993; Brouillet *et al*., 1995). In mice, transgenic for HD, Smaili *et al.* observed that there is a sustained and significant increase in cytosolic Ca^{2+} after the stimulation of brain slices with glutamate (Smaili *et al*., 2008), which might be related to the increased levels of Ca^{2+} in intracellular stores and the inability of mitochondria to uptake high levels of the ion present in the cytosol.

Aging is a multifaceted process associated with several functional and structural problems, and the brain is one of the organs most affected by chronic and degenerative diseases. Of these, Alzheimer's and Parkinson's diseases are the most prevalent and cause the most severe functional impairments. Therefore, it is necessary to investigate the age-related risk factors, the possible mechanisms involved in brain damage, and the future prospects for prevention and therapy. In addition, it is also known that, in neurodegenerative disorders, there are alterations in Ca^{2+} homeostasis, which contribute to cell death. In different tissues from senescent rats, it has been shown that there is an increase in Ca^{2+} content in intracellular stores such as the ER and mitochondria (Lopes *et al*., 2004; 2006). These data have been corroborated by the discovery of a decrease in Ca^{2+} buffering capacity, as well as an increase in apoptotic cell death in different smooth muscle tissues (Lopes *et al*. 2006; 2007). The HD neurodegenerative process has been associated with members of the apoptotic pathway such as Bax and Bcl-2 (Beal *et al*., 1993; Brouillet *et al*., 1995), and the results showed an increase in Bax expression and a decrease in the level of the Bcl-2 protein. Therefore, these studies indicate that there are changes in Ca^{2+} levels that may affect mitochondrial functions and may contribute to the apoptotic cell death process in aging and neurodegeneration.

It has been shown that melatonin scavenges and/or reduces the formation of ROS produced by mitochondria (Jou *et al*., 2004; 2007). These results suggest that a potent mitochondria-targeted protection mechanism is provided by melatonin and its metabolites, which are capable of reducing free radical damage in the brain. Its actions include the direct detoxification of ROS, promotion of the activities of antioxidant enzymes, a reduction in the formation of ROS and an enhancement in the production of glutathione, an important intracellular antioxidant (Hardeland, 2005; Tan *et al*., 2005). Recent work has demonstrated that melatonin may target mitochondrial permeability transition-dependent/independent mechanisms upon Ca^{2+} stress-induced apoptosis in astrocytes (Jou *et al*., 2009a; 2009b). Melatonin may protect astrocytes, as well as neurons, in the brain from free radical-mediated damage and it has been shown to protect against both hippocampal injury (Hunget *et*

al., 2008)] and spinal cord injury in rats (Samantaray *et al*., 2008). Melatonin prevents the inhibition of mitochondrial respiration in rat liver mitochondria and striatal synaptosomes treated with MPP^+ (Absi *et al*., 2000). Melatonin also inhibits mouse brain apoptosis associated with both acute/chronic and MPTP treatment (Ortiz *et al*., 2001; Ortiz *et al*., 2001; Antolin *et al*., 2002). The administration of melatonin improves a hemi-Parkinson condition in rats caused by the intranigral application of the neurotoxin, 6-OHDA (Dabbeni-Sala *et al*., 2001a). Melatonin also protects against an increase in ROS production and a decrease in glutathione (GSH) levels in cerebellar granule neurons after treatment with kainic acid (Dabbeni-Sala *et al*., 2001b).

Ling *et al*. reported that the protective effect of melatonin against injured cerebral neurons is related to the Bcl-2 protein (Ling *et al*., 1999). Bcl-2 expression is elevated in the striatum and the cortex after kainate injection, suggesting that after the induction of lesions by kainate, melatonin takes on a protective function in the central nervous system (Chuang *et al*., 2001). The direct effect of melatonin on mitochondrial energy metabolism has suggested a new homeostatic mechanism for the regulation of mitochondrial function (Acuna-Castroviejo *et al*., 2001). A subsequent study also demonstrated that the protective effect of melatonin is associated with the induction of Bcl-2 expression, leading to the suppression of apoptotic cell death (Yoo *et al*., 2002). The relationship between melatonin, Ca^{2+} regulation and apoptosis is summarized in Fig. 1.

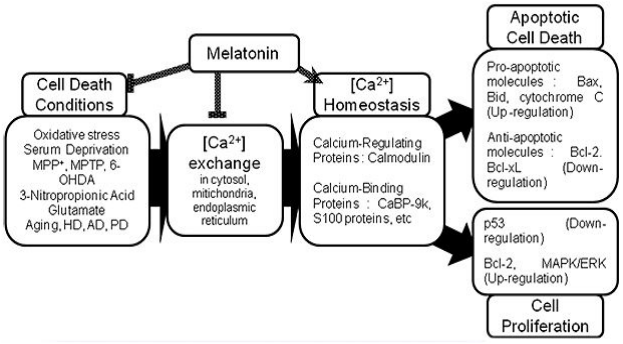

Fig. 1. Interactions between cell death and cell proliferation with respect to calcium ion homeostasis, calcium-regulating/binding proteins, and melatonin. MPP+, 1-methy-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA, 6-hydroxydopamine; HD, Huntington's disease; AD, Alzheimer's disease; PD, Parkinson's disease; CaBP-9k, calbindin-D9k; MAPK/ERK, mitogen-activated protein kinase/extracellular signal-regulated kinase. →, activation; ⊣, inhibition.

THE RELATIONSHIP BETWEEN CABP-9K AND Ca^{2+} *IN VIVO* AND *IN VITRO*

Calcium binding proteins (calbindins), including calmodulin, parvabumin, troponin C and S100 proteins, are members of a large family of intracellular proteins with a high affinity for calcium. The calcium binding protein D-9k (CaBP-9k) is a 9 kDa polypeptide with two calcium binding domains that is expressed in the mammalian intestine (duodenum), kidney, pituitary gland, growth cartilage and female reproductive tissues, such as the placenta, uterus and fetal membranes (Choi *et al*., 2005; Lee *et al*., 2003; Tinnanooru *et al*., 2008). CaBP-9k is involved in both luminal and glandular functions of the endometrial epithelia at the time of implantation (Kim *et al*., 2009), while duodenal and renal CaBP-9k plays a role in the active calcium transport system, and is regulated by vitamin D (Tinnanooru *et al*., 2008). The biological roles of CaBP-9k can be summarized as follows: (1) fetal calcium uptake - CaBP-9k is expressed in epithelial and myometrial cells and in uterine tissue of pregnant rats, but not in non-pregnant rats; (2) uterine contractions - CaBP-9k is present in myometrial fibers, where it is believed to help regulate the strength, duration and frequency of contractions by altering free calcium levels in the cytoplasm; (3) calcification of bone - the localization of CaBP-9k indicates that it plays a role in bone calcification; (4) calcification of teeth; (5) calcium transport and uptake in the mammalian intestine - active calcium transport across the intestine is directly proportional to the levels of CaBP-9k.

The S100 proteins are low-molecular-weight proteins that range in size from 9 to 13 kDa. These calcium-binding proteins are characterized by helix-loop-helix structural domains ("EF-hand type" conformation), and are thought to play a role in mediating calcium signals during cell growth, differentiation and motility (Schäfer and Heizmann, 1996). To date, 17 different proteins have been assigned to the S100 protein family, and most were isolated in screens for mRNAs or proteins whose expression is regulated by cellular growth, transformation, or differentiation, suggesting a direct role for S100 proteins in cell cycle regulation.

CaBP-9k is a member of the S100 family of intracellular calcium-binding proteins. The N-terminal region (amino acids 4 to 48) of rat CaBP-9k is similar to rat and mouse S100B, with approximately 49.5% homology between the N-terminus of

CaBP-9k and S100B (Yoo and Jeung, 2010). The C-terminal portion of CaBP-9k has a more highly conserved EF-hand domain, which confers 100 times greater binding affinity to Ca^{2+} compared to the N-terminal portion (Donato, 1999). The Ca^{2+} binding capability of S100 proteins is thought to be important for their functional activity (Donato, 1999), and no S100 proteins without bound Ca^{2+} have been reported. Following the C-terminal EF-hand region is a stretch of amino acids, referred to as the C-terminal extension, and separating the EF-hand domains is an intermediate region known as the hinge. Together, the hinge and C-terminal extension are the most variable regions found in the different S100 proteins and it has been suggested that they confer a specific biological activity on the individual proteins. The function of S100 proteins in cancer progression is not well known, but significant findings have suggested that S100 proteins can enhance cancer progression by stimulating the signaling pathways known to be important in cell survival (Donato, 2001; Heizmann *et al*., 2002; Most *et al*., 2003).

Among the various intracellular interactions attributed to S100 proteins, one of the more fascinating observations is their interaction with the tumor suppressor protein p53 (Baudier *et al*., 1992; Emberley *et al*., 2004; Mueller *et al*., 2005). In some cases, this interaction prevents p53 from binding to the promoter regions of its target genes, several of which are pro-survival, and thus from activating their transcription. In other cases, the interaction is thought to result in the sequestration of p53 in the cytoplasm, which stops the cell from initiating apoptosis. It is suspected that this process is followed by the selection of mutant-containing tumor cells, which can evade signals that initiate apoptosis (Baudier *et al*., 1992; Emberley *et al*., 2004; Mueller *et al*., 2005). It is likely that these cancer cells thrive because of their growth advantage, resulting in a more aggressive tumor. This hypothesis has several discrepancies, such as the fact that the p53-responsive gene Bax is induced after the addition of S100A4 (Chen *et al*., 2001). This could be due to regulatory factors that acquire a dominant function, or to variations between different cell types and model systems used in different studies. Regardless of the interaction between S100A4 and p53, S100A4 seems to demonstrate a consistent cellular role in promoting/acquiring invasive properties (Chen *et al*., 2001). This is most likely due to an interaction with proteins other than p53. The interactions of S100 proteins with cytoskeletal proteins and an alteration in cytoskeletal dynamics emphasize the fact that S100s can interact with several different proteins in different locations in the cell. In particular, S100B (Baudier *et al*., 1992) and S100A2 (Mueller *et al*., 2005) interact with p53 in the presence of calcium. We have recently demonstrated that CaBP-9k also interacts with p53, suggesting a possible correlation between this interaction and cell proliferation, cell cycle progression, and cell differentiation (Yoo and Jeung, 2010).

S100B stimulates neurite outgrowth (Kligman and Marshak, 1985; Winningham-Major *et al*., 1989) and enhances the survival of neurons during development (Van Eldik *et al*., 1991; Bhattacharyya *et al*., 1992; Whitaker-Azmitia *et al*., 1990; Ueda *et al*., 1995) and in brain injury (Barger *et al*., 1995). In addition, S100B prevents motor neuron degeneration in newborn rats after sciatic nerve section (Iwasaki *et al*., 1997), while the local administration of S100B stimulates the regeneration of injured rat sciatic nerve *in vivo* (Haglid *et al*., 1997). These reports point to a physiological role for secreted S100B as a neurotrophic factor, which could be important during both development and nerve regeneration, and it has been shown that S100B protects neurons against glutamate- and staurosporine-induced damage *in vitro* (Ahlemeyer *et al*., 2000). The pro-survival activity of extracellular S100B and the ability of this protein to stimulate neurite outgrowth depends on the nuclear translocation of NF-κB and the up-regulation of expression of the anti-apoptotic factor, Bcl-2, in target neurons (Alexanian Bamburg, 1999; Huttunen *et al*., 2000). It is worth noting that extracellular S100A4 is reported to activate the extracellular signal regulated protein kinase (ERK1/2) signaling pathway (Novitskaya *et al*., 2000), as extracellular S100B also stimulates the phosphorylation of ERK1/2 in astrocytes (Gonçalves *et al*., 2000), a finding consistent with the stimulatory effect of S100B on astrocyte proliferation.

S100B exerts its neurotoxic effects *in vitro* by inducing apoptosis in neurons (Mariggiò *et al*., 1994; Hu *et al*., 1997). In addition, S100B causes an elevation in ROS, cytochrome C release and activation of the caspase cascade (Huttunen *et al*., 2000). Under these conditions, Bcl-2 was shown to be down-regulated (Huttunen *et al*., 2000), a finding which is in accordance with the observation that the down-regulation of Bcl-2 is necessary for S100B to cause neuronal apoptosis (Wang *et al*., 1999). S100B-induced apoptosis in PC12 cells was also attributed to an S100B-dependent increased conductance in L-type Ca^{2+} channels (Mariggiò *et al*., 1994) and the up-regulation of a set of genes implicated in apoptosis (c-fos, c-jun, bax, bcl-x, p-15, and p-21) (Fulle *et al*., 2000).

In our most recent study, we first demonstrated that CaBP-9k expression is associated with ROS production during cell death induced by H_2O_2 in rat pituitary GH3 cells. We also found that CaBP-9k expression was increased by melatonin treatment during H_2O_2-mediated cell death, suggesting a functional link between increased cell death and the induction of CaBP-9k expression. Under conditions that promoted cell death, Bax expression was increased and Bcl-2 expression was decreased. We also identified a potential role for the mitogen-activated protein kinase (MAPK)/ERK signaling pathway (Yoo and Jeung, 2010). The knockdown of CaBP-9k expression using a small inhibitory RNA (siRNA) resulted in an elevation of H_2O_2-induced cell death, whereas cell survival was increased in cells that overexpressed CaBP-9k, providing additional evidence that the induction of CaBP-9k expression may be associated with survival signaling during H_2O_2-mediated oxidative cell death. Taken together, the data suggest that there may be a role for CaBP-9k interaction in cell proliferation and cell cycle progression (Yoo and Jeung, 2010). The relationship between melatonin, CaBP-9k, Ca^{2+} regulation and apoptosis is summarized in Fig. 1.

CONCLUSION

Melatonin (N-acetyl-5-methoxytryptamine) is the major neurohormone secreted during the night by the vertebrate pineal gland. The circadian pattern of pineal melatonin secretion is related to the biological clock within the suprachiasmatic nucleus (SCN) in mammals. The SCN is coordinated with the environmental light-dark cycle by light perceived by the retina, which acts mainly on retinal ganglion cells that contain the photopigment melanopsin. The changing melatonin rhythm controls seasonal reproduction in photoperiodic animals through the activation of receptors within the hypothalamic-pituitary reproductive axis. In addition, melatonin exerts anticancer effects on different tumor types (Cos *et al*., 2008; Pizarro *et al*., 2008; Sainz *et al*., 2008;), and it also is a broad-spectrum antioxidant (Rodriguez *et al*., 2004) and free radical scavenger (Tan *et al*., 1993; Peyrot and Ducroq, 2008; Hardelland *et al*., 2009). Melatonin interacts with estrogen-signaling pathways through indirect neuroendocrine mechanisms, direct actions at the tumor cell level, and the regulation of enzymes involved in the biosynthesis of estrogens (Cos *et al*., 2008; Reiter *et al*., 2007b). Several studies have demonstrated that melatonin treatment has an anti-apoptotic effect (Yoo *et al*., 2002; Jou *et al*., 2004; Nava *et al*., 2000), with this effect being attributed to its antioxidant properties (Reiter *et al*., 2009). More recently, melatonin has been shown to suppress free radical-mediated ocular disease (Siu *et al*., 2006).

CaBP-9k is a member of the S100 family of intracellular calcium-binding proteins, and is a 9 kDa polypeptide with two calcium binding domains that is expressed in the mammalian intestine (duodenum), kidney, pituitary gland, growth cartilage and female reproductive tissues, i.e., placenta, uterus and fetal membrane (Choi *et al*., 2005; Lee *et al*., 2003; Tinnanooru *et al*., 2008). CaBP-9k is involved in both luminal and glandular epithelial function and implantation (Kim *et al*., 2009). Duodenal and renal CaBP-9k plays a role in the active calcium transport system, and is regulated by vitamin D (Tinnanooru *et al*., 2008). We conclude that melatonin and CaBP-9k expression are involved in calcium homeostasis and apoptotic cell death. In future studies, we hope to provide important information on the functions of CaBP-9k with respect to cell signal transduction, cell proliferation, Ca^{2+} homeostasis, and the regulation of the cell cycle.

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