

# Melatonin, its agonists in pain modulation: clinical application

V. SRINIVASAN<sup>1</sup>, R. ZAKARIA<sup>2</sup>, HARBINDER JEET SINGH<sup>3</sup>,  
D. ACUÑA-CASTROVIEJO<sup>4</sup>

<sup>1</sup> Sri Sathya Sai Medical Educational and Research Foundation Prasanthi Nilayam, 40-Kovai Thirunagar, India; <sup>2</sup> Department of Physiology, School of Medical Sciences, Health Campus Universiti Sains Malaysia, Kelantan, Malaysia; <sup>3</sup> Department of Physiology, Faculty of Medicine, University Technology Mara (UiTM), Selangor, Malaysia; <sup>4</sup> Institute of Biotechnology, Biomedical Research Center, Health Sciences Technology Park, University of Granada, Spain

## ABSTRACT

Melatonin, the hormone of darkness has many physiological functions in the body and also exerts a number of pharmacological effects. Most of these actions of melatonin are mediated through melatonin membrane receptors like  $MT_1/MT_2$  receptors or through nuclear orphan receptors like RZR/ROR receptors or through calcium binding proteins in the cytosol. The finding that pain perception is circadian in nature has prompted many to suggest that “pain modulation” is one of the most important physiological functions of melatonin. By using a number of animal models of pain perception, it has been found that melatonin exerts antinociceptive and antiallodynic effects. Number of studies has shown that melatonin modulates pain perception by acting through opioid receptors, NMDA receptors and G-protein, and they have been analyzed using specific antagonists like naloxone or NMDA-G protein receptor antagonists. Recently it has been shown that melatonin exerts its antinociceptive effects through  $MT_1$  and  $MT_2$  melatonergic receptors located in the dorsal region of the spinal cord as well as in various parts of the brain concerned with pain modulation. Evidences for this have been obtained by using common melatonergic receptor antagonist like luzindole or specific  $MT_2$  receptor antagonist like 4P-PDOT or K-185. In a few clinical studies undertaken during surgery, melatonin has been shown to have analgesic effects. Melatonin is emerging as a new analgesic drug with a novel mechanism of actions and has the potential to be used as a natural pain killer in inflammatory, neuropathic pain conditions and also during surgical procedures.

## Key words

Melatonin • Pain • Analgesia • Antinociception • Antiallodynia • Luzindole • Opioid

## Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone secreted by the pineal gland of all animals as well as in human beings. It is not only synthesized in the pineal gland but also many other organs like eye (Cardinali and Rosner, 1971), gastro-intestinal tract (Bubenik, 2002), skin (Slominiski, 2005), lymphocytes (Carrillo-Vico et al., 2004), thymus (Naranjo et al., 2007) etc. Besides being acting as a hormone regulating reproductive functions of the body (Srinivasan et

al., 2008), it participates in many other important functions of the body like immune regulation (Maestroni et al., 1988; Srinivasan et al., 2008), prevention of cancer (Hill and Blask, 1988), control of circadian rhythms (Armstrong, 1989) and sleep regulation (Tzischinsky et al., 1994; Zhdanova et al., 1995). Since melatonin is secreted at high concentrations during dark periods of the night-hours it is referred as “the chemical code of darkness” (Reiter, 1991).

Number of animal studies has shown that pain perception is circadian in nature (Pilcher et al., 1982;

Pickhard, 1987) and since the circadian secretion of melatonin has been shown to influence pain perception, melatonin is suggested to play a “physiological role” in the mechanism of pain perception and the evidences for this has been reviewed earlier (Srinivasan et al., 2010). This review presents studies on the possible role of melatonin and its receptors in pain modulation and its possible application to human diseases.

## Melatonin, its physiology and metabolism

Melatonin was first identified and isolated by Lerner et al. (1958). Its synthesis begins with tryptophan, obtained from the blood and converted into serotonin. Serotonin is then acetylated to form N-acetyl-serotonin by the enzyme arylalkylamine-N-acetyltransferase (AANAT). It is then methylated to form N-acetyl-5-methoxytryptamine or melatonin by the enzyme hydroxyindole-O-methyl transferase (HIOMT), now called N-acetylserotonin-O-methyl transferase (ASMT). (Axelrod and Wurtman, 1968). The entire biosynthetic pathway is presented in Fig. 1. N-acetyltransferase (NAT) serves as the rate limiting enzyme in this biosynthetic pathway. Once formed in the pineal gland, melatonin is not stored in the gland but is released immediately into the circulation or CSF. The half life of melatonin ranges from 20 to 30 minutes. The enzymes involved in melatonin biosynthesis have been identified in the retina (Cardinali and Rosner, 1971), human lymphocytes (Carrillo-Vico et al., 2004), thymus gland (Naranjo et al., 2007) and many other tissues in the body. Although melatonin is synthesized by many tissues, circulating melatonin is derived only from the pineal gland.

Melatonin is metabolized mainly in the liver by hepatic cytochrome P450 (CYP) monooxygenases and conjugated to form 6-sulfatoxymelatonin (aMT6s) which is the main urinary metabolite of melatonin. In the brain, the primary metabolite is formed by oxidative cleavage and the product is known as N1-acetyl-N2-formyl-5-methoxytryptamine (AFMK). This is demethylated either by arylamine formamidase or hemoperoxidases to form N1-acetyl-5-methoxykynuramine (AMK) (Hira et al., 1974). The other metabolite of

melatonin is 3-hydroxymelatonin (Tan et al., 2007). While the pharmacokinetics of exogenous melatonin, its half life and clearance are relatively uniform in laboratory rodents, in humans it has a highly variable pharmacokinetic profile with low availability.

## Regulation of melatonin biosynthesis

Melatonin synthesis and secretion exhibits a circadian rhythm with low level of secretion during day time and high levels during night time. This circadian rhythm of melatonin synthesis and secretion is regulated by a complex neural circuit with fibres originating from the retina and pass via the retino-hypothalamic tract to the suprachiasmatic nucleus (SCN) of the hypothalamus (Moore, 1996). The cells that innervate the SCN are the special ganglion cells that contain the photopigment, melanopsin (Brainard et al., 2001; Berson et al., 2002). Projections from the SCN pass via the paraventricular nucleus to the medial forebrain bundle and reticular formation, and then make synaptic connections with the cells of the intermediolateral horn cells of the spinal cord from where preganglionic sympathetic fibres to superior cervical ganglion cells arise (Moore, 1996). The postganglionic sympathetic fibres from the SCN reach the pineal gland and stimulate it to release norepinephrine (NE), from their nerve endings. NE in turn activates the  $\beta$ -adrenergic receptors of the pinealocytes to activate the adenylyl cyclase-cyclic AMP system and promotes melatonin biosynthesis (Fig. 2) (Klein et al., 1971). During day-time NE released from the postganglionic sympathetic nerve fibres is suppressed by increased electrical signals of the SCN. At night time with the suppression of the SCN activity, release of NE from the sympathetic fibres is increased, stimulating adenylyl cyclase-cyclic AMP to promote the synthesis of melatonin by the pineal gland (Klein et al., 1992).

## Melatonin receptors

Melatonin's physiological and pharmacological effects in the body are mostly brought out through activation of melatonin receptors present in various tissues of the body. Two different types of melatonin receptors namely,  $MT_1$  and  $MT_2$  have been identified at the membrane level (Reppert et al., 1994,

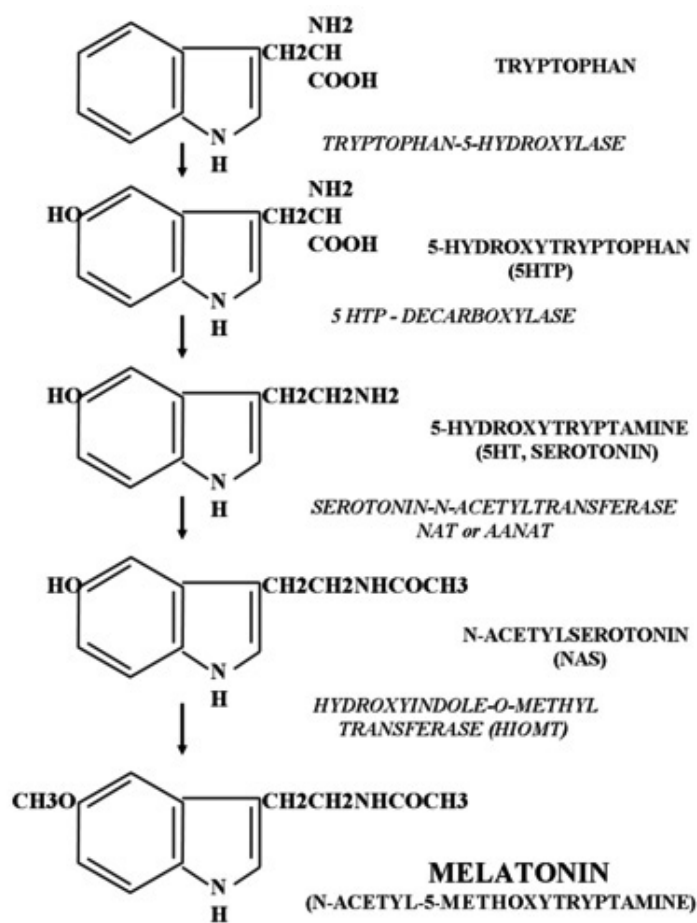


Fig. 1. - Melatonin Biosynthesis.

1995). These receptors belong to the super family of G-protein coupled receptors that have typical seven transmembrane domains (Dubocovich et al., 2005). A third melatonin receptor known as  $MT_3$  which has been later identified as quinone reductase has also been identified (Nosjean et al., 2000). This enzyme belongs to a group of reductases that participate in the antioxidative mechanisms by preventing electron transfer reactions of quinones.

Melatonin also activates nuclear receptors that belong to RZR/ROR orphan receptors that include three subtypes ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) and four splicing variants of the  $\alpha$ -subtype (Becker-Andre et al., 2003). Melatonin also interacts with intracellular proteins such as calmodulin which can antagonize calcium binding to calmodulin (Benetez-King et al., 1993) and calreticulin, which can extrude nuclear receptors to the cytosol such as steroid receptors (Macías et al., 2003).

The signal transduction pathways of melatonin receptors vary among different tissues and cells. In general  $MT_1$  receptor activation causes stimulation of a large variety of G proteins;  $G_{i\alpha 2}$ ,  $G_{i\alpha 3}$ ,  $G_{\alpha q}$  whereas in some tissues melatonin has inhibitory effects on the cAMP signal transduction cascade (Brydon et al., 1999).  $MT_2$  receptors are coupled to a number of signal transduction pathways that includes phosphoinositide activation mechanism, inhibition of adenylyl cyclase and guanylyl cyclase pathways (Boutin et al., 2005).

### Distribution of melatonin receptors for pain transmission

The presence and distribution of melatonin receptors involved in nociceptive transmission has been identified in different parts of the nervous sys-

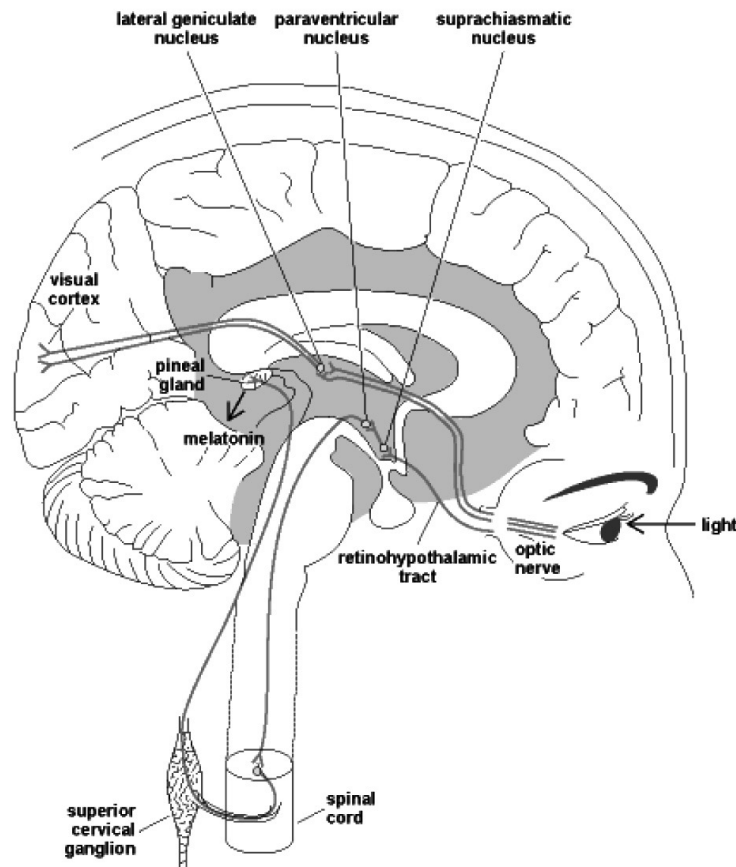


Fig. 2. - Regulation of Melatonin Biosynthesis. Two anatomically distinct pathways which convey light into the human brain. One pathway extends from the retina to the visual cortex and supports the sensory capacity of vision. The other pathway extends from the retina to the pineal gland for the circadian regulation of melatonin production (adapted from George C. Brainard, "Pineal gland", in AccessScience, ©McGraw-Hill Companies, 2008, <http://www.accessscience.com>).

tem. Autoradiographic studies have localized these receptors in the hypothalamus, thalamus, anterior pituitary, dorsal horn of the spinal cord, spinal trigeminal tract and trigeminal nucleus (Weaver et al., 1989; Wan and Pang, 1994; Williams et al., 1995). Receptor binding sites for melatonin are located throughout the spinal cord in the dorsal horn regions with high density being found in the superficial lamina. Both mRNAs and protein for  $MT_1$  and  $MT_2$  melatonin receptors have been found distributed in the dorsal horn regions showing thereby their localization in second order neurons (Wan and Pang, 1994; Wan et al., 1996). Specifically these melatonin receptors have been identified in lamina I-V and X of both ventral and dorsal horns of the lumbar and thoracic segments of the spinal cord, which are the main regions involved in pain transmission (Zahn et al., 2003). As spinal melatonin enhanced

the antinociceptive effect of morphine both melatonin and their receptors  $MT_1$  and  $MT_2$  present in the sensory regions of the spinal cord play a role in modulation of pain transmission (Zahn et al., 2003). Behavioural electrophysiological studies reveal that although melatonin's actions are complex in nature, their effects on spinal nociception have been found to be predominantly inhibitory in nature (Laurido et al., 2002; Nosedá et al., 2004).

## Nociception

Pain elicitation induced by various chemical, mechanical and thermal stimuli induces a constellation of autonomic behaviours that are collectively termed as nociception that includes hyperalgesia (augmented sensitivity to painful stimuli) and allo-

dynia (nociceptive responses to normal innocuous stimuli) (Jarvis et al., 2009; Sandkuhler, 2009). During tissue damage or inflammation, a variety of inflammatory mediators like leukotrienes, PGE<sub>2</sub>, bradykinin, thromboxanes, adenosine and free radicals are released. Emerging evidence indicates that ROS are involved in chronic pain that includes both neuropathic and inflammatory pain (Schmidtko et al., 2009; Tal, 1996). The transmission of pain sensation by primary afferent fibers have their central processes in the dorsal horn of the spinal cord that is influenced by several receptor types like opioid ( $\gamma$  and  $\mu$ ),  $\alpha$ 2-adrenoceptors, N-Methyl-D-aspartate (NMDA) and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and neurotransmitters like substance P, glutamate, calcitonin gene-related polypeptide and neurokinin (Sung et al., 2007; Vanotti et al., 2007). Pharmacological blockade of P2X<sub>4</sub> or p38 MAPK receptors through the use of intrathecal administration of antagonists reversed the mechanical allodynia, indicating that activation of microglial P2X<sub>4</sub> and p38 MAPK receptors are crucial to allodynia and neuropathic pain.

### Melatonin in pain modulation: experimental animal studies

By using several experimental models of acute inflammatory and neuropathic pain in mice and rats, melatonin's antinociceptive effect has been presented (Srinivasan et al., 2010). The first report on melatonin's antinociceptive effects dates back to 1969 publication by Morris and Lutsch in which they postulated that animals are less sensitive to pain during darkness of night time, when plasma melatonin levels are very much higher. This finding was supported by other studies (Lutsch and Morris, 1971; Rosenfield and Rice, 1979). Later studies using models like the tail-flick, hot-plate or hot-water tests and by administering melatonin through i.p or i.c.v, the antinociceptive actions of melatonin has been demonstrated in acute models of pain. By administering melatonin i.p (30 mg/kg) in mice, the antinociceptive effects of exogenous melatonin were first demonstrated in hot-plate model. The long lasting analgesic effect of melatonin observed in this study was shown to be blocked by naloxone,

suggesting the involvement of opioid receptors for the antinociceptive actions of melatonin (Laikin et al., 1981). By using the same hot-plate test models, melatonin's antinociceptive effect (i.p) in mice was confirmed by other investigators (Sugden et al., 1983; Ying and Huang et al., 1990; Golombek et al., 1991; Jeong et al., 2000). The time dependent analgesic effect of intraperitoneal injection of melatonin (20-40 mg/kg) was evaluated using hot-plate model in mice by one of the author's group (DP Cardinali) and it was noted that "melatonin exerted its maximal analgesic effect" when administered in the evening (Golombek et al., 1991). In the same study it was also noted that administration of opiate antagonist naloxone or central benzodiazepine (BZP) antagonist, flumazenil blunted the analgesic effect of melatonin although they did not modify the pain threshold (Golombek et al., 1991). Probably, naloxone blocks the effects of melatonin on flunitrazepam binding in the brain (Gomar et al., 1993), which in turn are modulated by corticotropic peptides (Gomar et al., 1994). The latency of hot-plate responses induced by administration of diazepam was increased by naloxone showing thereby that both central opioid and BZP receptors are involved in the mediation of melatonin's antinociceptive effects. The dose dependent analgesic effects of melatonin was evaluated in mice by using hot-water tail flick test in rats by injecting melatonin through i.c.v route. Three different doses namely 30, 60 or 120 mg/kg were given to rats and it was found that in rats administered with high dose (120 mg/kg), the antinociceptive effects started much earlier (15 minutes after melatonin administration), reached peak in 30 minutes and the analgesic effect lasted for much longer time i.e. 100 minutes (Yu et al., 2000). In this study, naloxone was given (10  $\mu$ g) which antagonized melatonin's (60-120 mg/kg i.p) within 10 minutes after injection and lasted for 45 minutes. This study supported that melatonin's analgesic effect is mediated by central mechanisms (Yu et al., 2000). By using tail-flick studies, the effect of melatonin on tail withdrawal latencies was studied by a number of investigators (Yu et al., 2000; Pekarkova et al., 2001; Li et al., 2005; Wang et al., 2006). In these studies, melatonin when administered in doses 1, 5, or 25 mg/kg (i.p) or 0.25, 0.5, or 1 mg/kg (i.c.v) produced significant increase in tail withdrawal latencies. Administration of either naloxone or luzindole



(melatonin receptor antagonist, i.c.v) antagonized these melatonin induced responses showing thereby that melatonin-induced antinociceptive effects either through central opioid or melatonergic receptors. By using paw withdrawal threshold after application of pinch pressure in rats, it was shown that administration of melatonin 70 mg/kg, with cumulative dose of 210 mg/kg (i.v), increase the paw withdrawal threshold very much (Naguib et al., 2003a). By using response to tail-clamping in rats, the analgesic effects of 2-bromomelatonin was assessed and compared with that of propofol. By using 2-bromomelatonin 38 mg/kg (35-41 mg/kg), it was found that melatonin analog 2-bromomelatonin increased the response to tail-clamping, a sign demonstrating antinociceptive action of 2-bromomelatonin (Naguib et al., 2003b).

### Melatonin's pain modulatory role in inflammatory pain

By using electrical stimulation of the tail test as one of the models for evaluating pain perception, melatonin's antinociceptive effect was tested in carrageenan-induced inflammation in rats (El-Shenawy et al., 2002). Melatonin when injected at doses of 0.5 or 1.0 mg/kg i.p exerted its antinociceptive effect in electrical stimulation of rat tail test by 29.6% and 39.5% respectively. Melatonin (0.5 mg/kg) also potentiated the antinociceptive effects of indomethacin in carrageenan-induced edema in a dose dependent manner thereby demonstrating melatonin's antinociceptive effect in inflammatory pain (El-Shenawy et al., 2002). The mechanism of anti-inflammatory action in carrageenan-induced edema by melatonin was studied earlier in which it was noted that melatonin's anti-inflammatory effect is exerted through inhibition of inducible nitric oxide (iNOS) expression (Cuzzocrea et al., 1997). The mechanism by which carrageenan-induced paw inflammation was elucidated in detail in a study in which carrageenan injection induced time dependent increase in paw oedema, nitrite/nitrate and MDA (the lipid peroxidation product). Increase in paw-volume was noted 4 hrs after carrageenan administration. Treatment with melatonin (5 and 10 mg doses), significantly reduced the paw oedema in a dose dependent manner and this was noted at 1, 2, 3, 4, 5, 6 hr after injection of carrageenan.

Melatonin also caused significant reduction of nitric oxide (NO) and MDA levels suggesting thereby that anti-inflammatory effect of melatonin is mediated through the reduction of NO and lipid peroxidation effects (Billici et al., 2002). In subsequent study of inflammatory pain model, melatonin injection (150-600 µg/paw) caused dose-dependent antinociception that was reversed by antagonism of the NO-cyclic GMP-protein kinase G-K<sup>+</sup> channel pathway suggesting a common mechanism for anti-inflammation and antinociceptive effects of melatonin (Hernandez-Pacheo et al., 2008).

Endotoxin-induced hyperalgesia has been used as one of the models for assessing melatonin's antinociceptive effects in inflammatory type of pain (Kanaan et al., 1996). Intraplantar injection of lipopolysaccharide (LPS) as used in the study caused significant decrease in nociceptive threshold 6 and 10 hr after endotoxin injection. Melatonin injection in 5 or 10 mg/kg doses significantly inhibited LPS-induced hyperalgesia in both time intervals. As melatonin also suppressed TNF-α release from LPS-activated macrophages, suppression of TNF-α is suggested as the mechanism by which melatonin inhibits LPS-induced hyperalgesia (Raghavendra et al., 2000). The antinociceptive and antiallodynic effects of melatonin have been tested in formalin test which also induces inflammatory responses. This pain model produces two nociceptive responses. The first phase occurs within five minutes after formalin injection and is due to activation of C-fibres and release of substance P and bradykinin (Shibata et al., 1989). The second response begins after a short quiescent period and is due to the activation of NMDA receptors and release of histamine, bradykinin and prostaglandins (Ohkubo et al., 1990). By using this formalin model the antinociceptive and antiallodynic effects of melatonin was evaluated in experimentally-induced diabetic rats. Oral administration of melatonin (150 mg/kg) significantly reduced formalin-induced nociception and also produced antiallodynic effects (Arreola-Espino et al., 2007). The antinociceptive effect of melatonin was attributed to its action on MT<sub>2</sub> melatonin receptors in the spinal cord. Using both, melatonin and its analog 6-chloromelatonin, their antinociceptive efficacy was evaluated in another model of inflammatory pain, namely capsaicin-induced hyperalgesia and mechanical allodynia. Prior administration

of melatonin or its analog significantly reduced capsaicin-induced hyperalgesia and allodynia (Tu et al., 2004). This effect was blocked by intrathecal administration of 4P-PDOT (selective  $MT_2$  receptor blocking agent). By using hot-plate latency test and acetic acid-induced abdominal writhes, the effect of pyridomelatonin analogs on antinociceptive effect were evaluated. The activities of the tricyclic pyrrolo-and pyrido-indole derivatives, 5, 9a and 12 were compared with that of melatonin on thermal pain, visceral pain and inflammation. Administration of all these melatonin analog compounds increased the hot-plate latency within 1-2 hr after administration (Elmegeed et al., 2007). Of these compounds, compound 3 and 5 were as effective as melatonin. All these melatonin compounds significantly reduced the number of abdominal writhes caused by injection of acetic acid in mice. Compounds 5 and 12 were most potent in this aspect as they reduced the number of abdominal writhes by 69% and 81% as compared with controls, whereas the other compounds like 3, 9a and melatonin, are less effective as they reduced the number of abdominal writhes by 24%, 41%, and 55% respectively. From this study it was concluded that pyrrole moiety of the compounds 5 and 12 are responsible for their potent antinociceptive effects (Elmegeed et al., 2007).

In animal model of inflammatory pain, superoxide anion radical ( $O_2^-$ ) was administered by subplantar injections into the right hind paw of which interacted with endogenous NO and generated peroxynitrite anion and evoked hyperalgesia. In rats injected with melatonin (25-100 mg/kg) given 30 minutes prior to  $O_2^-$  injection, attenuated the hyperalgesic responses to  $O_2^-$  in dose dependent manner. At 100 mg/kg dose, melatonin significantly improved inflammation and tissue damage by affecting cyclo-oxygenase-2, and inhibitory nitric oxide synthase expression (iNOS) showing the potency of melatonin in reducing the inflammatory type of hyperalgesia (Esposito et al., 2010). Post-inflammatory visceral hyperalgesia is a centrally mediated process that can be studied by an experimental model. In this model, inflammation was induced by injection of nitrobenzenesulfonic acid (TNBS) and visceral motor response (VMR) to graded colorectal distension (CRD) was evaluated. Melatonin was administered at doses of 30, 45, and 60 mg/kg, 20 minutes prior to VMR testing in both naive and TNBS injected rats. The VMR response

to CRD was found increased in TNBS injected rats when compared to naive rats. This increase in VMR and also the response to CRD sensitive spinal neurons was attenuated by melatonin administration (60 mg/kg). The blocking effect of melatonin on hyperalgesia was inhibited either by luzindole or by naltroxone and the authors of this study concluded that melatonin exerts its antinociceptive effect by acting at supraspinal level (Mickle et al., 2010).

### Melatonin's antinociceptive action in neuropathic pain

Nerve injury results in abnormal pain perception known as neuropathic pain associated with hyperalgesia and allodynia. Mice subjected to tight ligation of their sciatic nerve were used for this kind of study. By using this model, the withdrawal latencies, for assessing thermal analgesia and withdrawal threshold for assessing mechanical allodynia were studied. In this study melatonin at highest dose (120 mg/kg, i.v) or (0.1 nmol, i.c.v) reduced the paw withdrawal latencies in response to radiant heat stimulation of the injured hind-paw compared to corresponding values of normal rats. With regard to mechanical allodynia, neither i.p or i.c.v melatonin injection had any effect on the withdrawal thresholds. The findings of this study suggest that melatonin in high doses (both i.p and i.c.v) block thermal hyperalgesia in "*neuropathic mice*" but had no effect on mechanical allodynia. Co-administration of both L-arginine and naloxone reduced the effects of melatonin suggesting that melatonin exerts its antinociceptive effect via the L-arginine-NO-pathway and the opioid system (Ulugol et al., 2006).

The possible role of melatonin and its receptors in mechanical allodynia was assessed in another study by using rat model of neuropathic pain (i.e. ligation of L5/L6 spinal nerves). In this study intrathecal (3-100  $\mu$ g) or oral (37.5-300 mg/kg) administration of melatonin decreased tactile allodynia induced by spinal nerve ligation. Intrathecal administration of luzindole (melatonin receptor antagonist) in doses 1-100  $\mu$ g significantly diminished the antiallodynic effects of melatonin in dose dependent manner. Similarly oral (0.01-1 mg/kg) or intrathecal (0.1-10  $\mu$ g) administration of the highly selective  $MT_2$  melatonin receptor antagonist, 4P-PDOT diminished the

antiallodynic effects of melatonin. These findings reveal that melatonin exerts its antiallodynic effects mainly through  $MT_2$  melatonin receptors (Ambriz-Tututi et al., 2007). The effect of both melatonin and dextromethorphan was assessed on thermal hyperalgesia and mechanical allodynia. Dextromethorphan alone was found to be effective in reducing thermal hyperalgesia at all three different doses tested, (15, 30, 60 mg/kg) but reduced mechanical allodynia only at high doses (30 and 60 mg/kg). But melatonin was effective in reducing thermal hyperalgesia only at the highest dose tested (120 mg/kg) but failed to reverse mechanical allodynia in all the three doses tested (30, 60 and 120 mg/kg). The combined i.v administration of both melatonin (30 mg/kg) and dextromorphan (15 mg/kg) effectively reversed both thermal hyperalgesia and mechanical allodynia suggesting that combined use of melatonin and NMDA receptor antagonist (dextromethorphan) can be more effective in treating neuropathic pain than using either of these drugs alone.

### Clinical studies of melatonin in analgesia

In patients undergoing surgery, they often experience anxiety and pain during surgical manipulation procedures. Systemic analgesia and sedation are required to increase patient's tolerance to surgery. Although the use of preoperative benzodiazepines is the most common practice, it also has its undesirable side effects. To analyze the effects of melatonin on pain in surgery, few studies were carried out. The first investigation of this kind was published in 2007. In this double-blind placebo-controlled study, 33 patients were randomly assigned into two groups, 17 patients receiving melatonin 5 mg (group 1) or 16 patients receiving placebo (group 2) the night before operation and 1 hr before surgery. Mean pain scores were analysed using VAS (visual analog scale) at 6, 12, 18, 24 hr after surgery. Patients treated with melatonin preoperatively had a significant decrease in pain and anxiety at all time during the first 36 hr after surgery. It also improved the recovery of the potency of rest-activity circadian rhythm (Caumo et al., 2007).

The analgesic effect of melatonin has been evaluated in placebo-controlled study on patients who underwent elective hand surgery. Forty patients undergo-

ing elective hand surgery under intravenous regional anaesthesia (IVRA) were randomly assigned into two groups; control group (placebo group) and melatonin (10 mg/d) group. Melatonin premedication reduced the level of anxiety, decreased tourniquet-related pain and enhanced intraoperative and postoperative analgesia without producing clinically significant side effects (Mowafi and Ismail, 2008). The beneficial effect of melatonin on tourniquet-related pain was evident from by lower pain scores. The investigators have chosen tourniquet-related pain since it is the limiting factor for patients undergoing IVRA and this pain is suggested to be due to a number of factors like neuropathic pain induced by stimulation of nerve endings in the cutaneous tissue (Lowrie et al., 1989), skeletal muscle ischemia (Mense, 1993) and local metabolic changes (Kokki et al., 1998). Melatonin was found useful in relieving tourniquet pain, as it depresses the nociceptive discharges of spinal dorsal horn neurons following the stimulation of C-fibres (Laurido et al., 2002). In addition to this, melatonin's action in inhibiting the synaptic potentiation (wind-up) in the spinal cord (Nosedá et al., 2004) is also suggested as the other possible explanation for melatonin's antinociceptive effect seen in this study. Use of melatonin premedication for decreasing pain and anxiety has been tried in another study on patients undergoing cataract surgery. In this study patients were randomly assigned into two groups (melatonin and placebo groups, 20 patients in each group). Melatonin (10 mg/d) or placebo tablet was given orally 90 minutes before surgery. No other sedatives or analgesic premedication were used. Verbal pain score (VPS) and VAS were administered during the time of preoperative visit. After melatonin administration the anxiety score decreased significantly ( $P < 0.05$ ). Pain scores were also significantly lower in melatonin group than control group. Surgeons reported better quality of operative conditions with excellent scores in the melatonin group than control group ( $P = 0.02$ ). The melatonin analgesic effect was clinically evident by lower pain scores and reduction in the intraoperative fentanyl consumption ( $P = 0.007$ ). Melatonin also reduced the intraocular pressure. The conclusions of this study are that melatonin enhanced perioperative analgesia, improved the operative condition during cataract surgery under topical anaesthesia (Ismail and Mowafi, 2009).



## Mechanisms of melatonin's antinociceptive action

Studies on melatonin's antinociceptive actions have pointed out the role of opiate and BZP pathways (Lakin et al., 1981; Sugden, 1983; Golombek et al., 1991). Fluctuations in pain threshold have been correlated with fluctuations of plasma  $\beta$ -endorphin immune reactivity and melatonin release (Barrett et al., 2000). Morphine commonly used in analgesia has been shown to induce melatonin release from rat pineal gland (Esposito et al., 1988). Naloxone administration decreased the nocturnal production of melatonin (Lowenstein et al., 1984). Opioidergic nerve fibres (Coto-Montes et al., 1994) and opioid receptors are found in the pineal gland of several animal species (Govitrapong et al., 2002; Shavali et al., 2005). Melatonin administration induced release of  $\beta$ -endorphin from periaqueductal gray in rats (Yu et al., 2000) and release of  $\beta$ -endorphin from pituitary cells (Shavali et al., 2005). These investigators attribute melatonin's antinociceptive actions to its binding with opioid receptor subtypes and subsequent release of  $\beta$ -endorphins (Shavali et al., 2005). The relationship of melatonin with the opioidergic system is complex and "melatonin-opioid axis" is crucial for melatonin's analgesic effects (Lakin et al., 1981). However the finding that several melatonin agonists and antagonists do not have any affinity for opioid receptors (Shavali et al., 2005) raises doubt over melatonin's analgesic effects through opioid system. It is suggested that melatonin can work through a number of neurotransmitter-receptor systems. In one of the studies conducted on glutamate injection resulted in nociception, melatonin (100 mg/kg i.p) exerted antinociceptive effects. Pretreatment of these animals with opioid receptor antagonist, naloxone (1 mg/kg); 5-HT<sub>2A</sub> receptor antagonist, ketanserin (1 mg/kg); D<sub>2</sub>-dopaminergic receptor antagonist, sulphiride (50 mg/kg); L-arginine (600 mg/kg); or MT<sub>1</sub>/MT<sub>2</sub> receptor antagonist, luzindole (0.15 mg/kg) completely blocked melatonin-induced antinociception (Mantovani et al., 2006). From this study it was concluded that melatonin induces antinociception by interacting with both central and peripheral melatonin receptors and through modulation of opioidergic, serotonergic, dopaminergic, L-arginine-nitric oxide and adrenergic pathways (Mantovani et al., 2006). The interaction of melatonin receptors and

opioid receptors in pain modulation was investigated in a study in which hyperalgesia was induced by injection of FQ/nociceptin (NC), a member of opioid peptide family. When both melatonin (5, 10, 50  $\mu$ g, i.c.v) and NC (10  $\mu$ g, i.c.v) were given, there was a complete absence of hyperalgesic response. This inhibitory effect of melatonin on NC-induced hyperalgesia was completely blocked by injection of either luzindole or naloxone (10  $\mu$ g, i.c.v) (Wang et al., 2006). This study supports the role of both melatonin receptors and opioid receptors in analgesic response induced by melatonin.

## Melatonin modulation of NO dependent pain pathway

Nitric oxide (NO) plays an important role in normal afferent signalling of pain through the dorsal horn of the spinal cord. NO participates in microvascular changes following injury but also has a direct role in axon and myelin breakdown and "clearance" prior to regeneration (Toda et al., 2009; Schmidtko et al., 2009). During such process, NO contributes to the development of neuropathic pain. Low-grade chronic rises in NO contributes to peripheral nerve damage or neuropathy (Schmidtko et al., 2009). The preventing effect of melatonin on NO-induced damage has been studied in number of tissues (Ayodagan et al., 2006). Increased generation of kinins during painful stimuli or activation of AMPA or NMDA receptors during persisting pain generates NO or peroxynitrite, a process that is blocked by melatonin (Gilad et al., 1997). NMDA receptors in the spinal cord play a major role in pain transmission at dorsal horn level of the spinal cord and are involved in the potentiation of nociceptive synaptic transmission in the spinal cord, a phenomenon known as "wind-up" (Jarvis et al., 2009; Schmidtko et al., 2009). In this process there is repetitive increase in the intensity of C-fiber stimulation. Using this C-fiber stimulation for initiating spinal "wind-up" effect, Laurido et al. (2002) found that different doses of melatonin (1.25, 2.5, 5.0, 10 mg/kg) produced dose dependent decreases of spinal "wind-up" effect, with highest dose causing complete suppression of wind-up activity. Melatonin influence on spinal "wind-up" is attributed to its action on NMDA receptor dependent intracellular NO generating pathways (Laurido et al., 2002).

Table I. - Melatonin's antinociceptive actions: Experimental animal studies.

| Animal model used  | Melatonin or its agonist<br>Dose & route   | Effect of melatonin                             | Blocked by                                     | Type of receptors involved                              | References                     |
|--|--|---|--|---|--------------------------------|
| Hot-plate  | 30 mg/kg i.p (melatonin)   | Antinociception                                 | Naloxone                                       | Opiod   | Laikin et al., 1981            |
| Hot-plate  | 20-40 mg/kg i.p (melatonin)  | Antinociception                                 | Naloxone & flumazenil                          | Opiod & BZD   | Golombek et al., 1991          |
| Carrageenan-induced paw inflammation                             | 5 & 10 mg i.p (melatonin)  | Reduction of paw inflammation & antinociception | -  | -   | Cuzzocrea et al., 1997         |
| LPS model  | 5 & 10 mg i.p (melatonin)  | Antinociception                                 | -  | -   | Raghavendra et al., 2000       |
| Hot-water tail flick test  | 30, 60 or 120 mg/kg i.p (melatonin)  | Antinociception                                 | Naloxone                                       | Opiod   | Yu et al., 2000                |
| Electrical stimulation of tail                                   | 0.5 & 1.0 mg i.p (melatonin)   | Antinociception                                 | -  | -   | El-Shenaway et al., 2002       |
| Carrageenan-induced paw inflammation                             | 5 & 10 mg i.p 0.25, 0.5, 1.0 mg i.c.v (melatonin)  | Reduction of inflammation and antinociception   | -  | -   | Billici et al., 2002           |
| Paw-withdrawal threshold   | 70 mg/kg i.v cumulative dose (210 mg/kg) (melatonin)   | Antinociception                                 | Naloxone or luzindole                          | Opiodergic and melatonergic                             | Naguib et al., 2003a           |
| Tail-clamping response   | 38 mg/kg (35-41 mg/kg) (bromomelatonin)  | Antinociception                                 | -  | -   | Naguib et al., 2003b           |
| Capsaicin-induced hyperalgesia                                   | Melatonin 6-chloromelatonin  | Antinociception                                 | 4-P-PDOT                                       | MT <sub>2</sub> melatonin receptors                     | Tu et al., 2004                |
| Ligation of sciatic nerve (neuropathic pain)                     | 120 mg/kg i.v 0.1 nmol i.c.v (melatonin)   | Antinociception                                 | Naloxone                                       | Opiod peptides & I-ARGININE-NO-pathway                  | Ulugol et al., 2006            |
| Ligation of spinal nerves (neuropathic pain)                     | 37.5-300 mg oral 3-100 µg intrathecal (melatonin)  | Antinociception                                 | Luzindole both oral and intrathecal & 4-P-PDOT | MT <sub>1</sub> and MT <sub>2</sub> melatonin receptors | Ambriz-Tututi et al., 2007     |
| Formalin injection model   | 150 mg/kg oral (melatonin)   | Antinociception                                 | 4-P-PDOT                                       | MT <sub>2</sub> melatonin receptors                     | Arreola-Espino et al., 2007    |
| Hot-plate latency test   | 4 mg/kg s.c (melatonin) and 5.16, 5.13, 6.88 & 5.40 mg/kg s.c (melatonin analog, compounds 3, 5, 9a, & 12) | Antinociception                                 | -  | -   | Elmegeed et al., 2007          |
| Inflammatory pain model  | 150-600 µg/paw (melatonin)   | Antinociception                                 |  |   | Hernandez-Pacheco et al., 2008 |
| Post-inflammatory visceral hyperalgesia to colorectal distension | 60 mg/kg (melatonin)   | Antinociception                                 | Naltrexone or luzindole                        | Opiod or melatonin receptors                            | Mickle et al., 2010            |
| Inflammatory type of pain  | 25-100 mg/kg (melatonin)   | Antinociception                                 | -  | -   | Esposito et al., 2010          |

## Role of MT<sub>1</sub> and MT<sub>2</sub> melatonin receptors and analgesia

The role of MT<sub>1</sub>/MT<sub>2</sub> melatonin receptors in the mediation of melatonin's analgesic response was studied by using both melatonin receptor antagonists (common type) luzindole and specific MT<sub>2</sub> receptor antagonist 4-P-PDOT or K-185 (N-butanoyl-2-(5,6,7-trihydro-11-methoxybenzo[3,4]cyclohept[2,1-a]Indol-13-yl)ethanamine). The findings of these studies are already discussed in the earlier paragraphs. The summary of the antinociceptive findings of melatonin and melatonergic agonists and their receptors role are presented in Table I. A special attention is drawn towards the role of MT<sub>2</sub> melatonin receptors in melatonin-induced analgesic mechanisms. In the streptozotocin-induced diabetic rats, it was found that melatonin-induced analgesic response was diminished by prior injection of K-185 (0.2 to 2.0 mg/kg, s.c) showing thereby that MT<sub>2</sub> receptors present in the spinal cord are involved in melatonin's antinociception (Espino et al., 2007). The antiallodynic effect of melatonin in the rat model of neuropathic pain was diminished by intrathecal administration of luzindole (1-100 µg) or 4-P-PDOT (MT<sub>2</sub> receptor antagonist) (0.1 to 10 µg) suggesting that MT<sub>2</sub> receptors in the spinal cord mediate the antiallodynic effects of melatonin (Ambriz-Tututi et al., 2007). The antinociceptive effects of melatonin seen in neuropathic pain models and the localization of MT<sub>1</sub>/MT<sub>2</sub> melatonin receptors in thalamus, hypothalamus, dorsal horn of the spinal cord spinal trigeminal tract and trigeminal nucleus all support that analgesic actions of melatonin are mediated through melatonin receptors (Ambriz-Tututi et al., 2009). Not only melatonin's actions on pain transmission, but melatonin agonist, 2-bromomelatonin effect in decreasing mechanical nociception were blocked by intrathecal injection of either luzindole or naloxone (Onal et al., 2004).

Similarly the effect intrathecal administration of 6-chloromelatonin in reducing capsaicin-induced hyperalgesia was blocked by intrathecal administration of specific MT<sub>2</sub> melatonin receptor antagonist, 4P-PDOT (Tu et al., 2004) confirming the role of MT<sub>2</sub> melatonin receptors in the modulation of pain transmission.

## Melatonin, opioids and G-proteins in pain modulation

Both melatonin and opioids acts through G<sub>i</sub>-mediated decrease in cyclic AMP and modify ion channels. G-protein-coupled receptors action in modifying ion channels has been involved in pain modulation (Marker et al., 2004). G-protein coupled receptor modifies ion channel function either by its direct G<sub>βγ</sub> binding the ion channels or by its indirect ion channel phosphorylation through protein kinases (Dolphin, 2003). G-protein coupled receptor agonists inhibit pain transmission by direct hyperphosphorylation of second-order neurons via activation of G-protein-activated inward-rectifying K(+) (GIRK) channels (Marker et al., 2004) and hyperpolarizing dorsal horn neurons through activation of GIRK channels (Marker et al., 2005). A schematic diagram depicting melatonin's possible mechanisms by which it exerts its antinociceptive action is presented in Fig. 3.

## Conclusions

Melatonin's antinociceptive and antiallodynic effects have been demonstrated in a number of experimental animal models of pain perception. Melatonin has been shown to act at the dorsal horn levels of the spinal cord where a number of receptors like opioid, substance P, NMDA and MT<sub>1</sub>/MT<sub>2</sub> melatonergic receptors are located. Melatonin's antinociceptive actions have been blocked by opioid receptor antagonists, NMDA receptor antagonists, common melatonergic antagonist (luzindole) and specific MT<sub>2</sub> receptor antagonist (4-P-PDOT or K-185) showing thereby that opioid receptors and melatonergic play an important role in the antinociceptive actions of melatonin as well as melatonin agonists 2-bromomelatonin, 6-chloromelatonin and some other melatonergic compounds. These melatonergic agonists also exert their antinociceptive actions by acting through opioid receptors and melatonergic receptors as their actions are blocked by either naloxone or luzindole. Clinical studies on patients undergoing surgery have also shown that melatonin can be used as an effective analgesic drug during surgery.

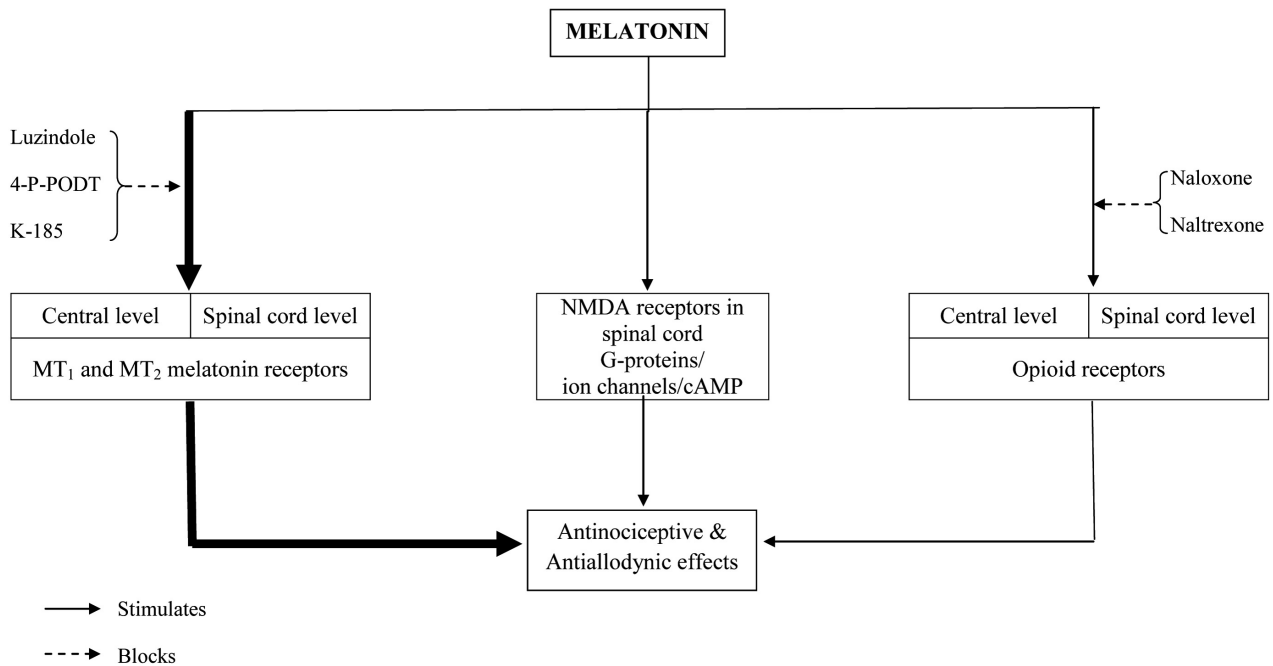


Fig. 3. - Schematic diagram showing melatonin's antinociceptive actions.

## References

- Ambriz-Tututi M. and Granados-Soto V. Oral and spinal melatonin reduces tactile allodynia in rats via activation of MT<sub>2</sub> and opioid receptors. *Pain*, **132**: 273-280, 2007.
- Ambriz-Tututi M., Rocha-Gonzalez H.I., Cruz S.L., Grandos-soto V. Melatonin: A hormone that modulates pain. *Life Sciences*, **84**: 489-498, 2009.
- Armstrong S.M. Melatonin and circadian control in mammals. *Experientia*, **45**: 932-938, 1989.
- Arreola-Espino R., Urquiza-Marin H., Ambriz-Tututi M., Araiza-Saldafia C.I., Caram-Salas N.I., Rocha-gonzalez H.I., Mixicoati-Zecuati T., Granados-soto V. Melatonin reduces formalin-induced nociception and tactile allodynia in diabetic rats. *Eur. J. Pharmacol.*, **577**: 203-210, 2007.
- Ayodagan S., Yerer M.B., Goktas A. Melatonin and nitric oxide. *J. Endocrinol. Invest.*, **29**: 281-287, 2006.
- Axelrod J. and Wurtman R.J. Photic and neural control of indoleamine metabolism in the rat pineal gland. *Adv. Pharmacol.*, **6**: 157-166, 1968.
- Barrett T., Kent S., Voudouris N. Does melatonin modulate beta-endorphin, corticosterone and pain threshold? *Life Sci.*, **66**: 467-476, 2000.
- Becker-Andre M., Wiesenberg L., Schaeren-Wiemers N., Andre E., Missbach M., Saurat J.H., Carlberg C. Pineal gland hormone melatonin binds and activates an orphan of the nuclear receptor superfamily. *J. Biol. Chem.*, **269**: 28531-28534, 1994.
- Benitez-King G. and Anton-Tay F. Calmodulin mediates melatonin cytoskeletal effects. *Experientia*, **49**: 635-641, 1993.
- Berson D.M., Dunn F.A., Takao M. Phototransduction by retinal ganglion cells that sets the circadian clock. *Science*, **295**: 1070-1073, 2002.
- Billici D., Akpiner E., Kiziltunic A. Protective effect of melatonin in carrageenan-induced acute local inflammation. *Pharmacol. Res.*, **46**: 133-139, 2002.
- Boutin J.A., Audinot V., Ferry G., Delagrangre P. Molecular tools to study melatonin pathways and actions. *Trends Pharmacol. Sci.*, **26**: 412-419, 2005.
- Brainard G.C., Hanifin J.P., Greeson J.M., Byrne B., Glickman G., Gerner E., Rollag M.D. Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. *J. Neurosci.*, **21**: 6405-6412, 2001.
- Brydon L., Roka F., Petit L., de Cooper P., Tissot M., Barrett P., Morgan P.J., Nanoff C., Strosberg A.D., Jockers R. Dual signalling of Mel1a, melatonin receptor 1A, in the rat pineal gland. *J. Neuroendocrinol.*, **21**: 105-112, 2000.



- tonin receptors via G12, G13, Gq/11 proteins. *Mol. Endocrinol.*, **13**: 2025-2038, 1999.
- Bubenik G.A. Gastrointestinal melatonin: localization, function and clinical relevance. *Dig. Dis. Sci.*, **47**: 2336-2348, 2002.
- Carrillo-Vico A., Calvo J.R., Abreu P.J., Lardone S., Garcia-Maurino S., Reiter R.J., Guerrero J.M. Evidence of melatonin synthesis by human lymphocytes and its physiological significance: possible role as intracrine, autocrine and/or paracrine substance. *FASEB J.*, **18**: 537-539, 2004.
- Cardinali D.P. and Rosner J.M. Metabolism of serotonin by the rat retina "in vitro". *J. Neurochem.*, **18**: 1769-1770, 1971.
- Caumo W., Torres F., Moreira jr N.L., Auzani J.A., Monteiro C.A., Londero G., Ribeiro D.F., Hidalgo M.P. The clinical impact of pre-operative melatonin on post-operative outcomes in patients undergoing abdominal hysterectomy. *Anesth. Analg.*, **105**: 1263-1271, 2007.
- Coto-Montes A., Masson-Pevet M., Pevet P., Moller M. The presence of opiodergic pinealocytes in the pineal gland of the European hamster (*Cricetus cricetus*): an immunocytochemical study. *Cell Tissue Res.*, **278**: 483-491, 1994.
- Cuzzocrea S., Zingarelli B., Gilad E., Hake P., Salzman A.L., Szabo C. Protective effect of melatonin in carrageenan-induced models of local inflammation: relationship to its inhibitory effect on nitric oxide production and its peroxynitrite scavenging activity. *J. Pineal Res.*, **23**: 106-116, 1997.
- Dolphin P.C. G protein modulation of voltage-gated channels calcium channels. *Pharmacol. Rev.*, **55**: 607-627, 2003.
- Dubocovich M.I. and Markowska M. Functional MT<sub>1</sub> and MT<sub>2</sub> melatonin receptors in mammals. *Endocrine*, **27**: 101-110, 2005.
- Elmegeed G.A., Baluomy A.R., Abdel-Salem O.M. Evaluation of the anti-inflammatory and anti-nociceptive activities of novel synthesized melatonin analogues. *Eur. J. Med. Chem.*, **42**: 1285-1292, 2007.
- El-Shenaway S.M., Abdel-Salem O.M., Baluomy A.R., El batran S., Arbid M.S. Studies on the anti-inflammatory and anti-nociceptive effects of melatonin in the rat. *Pharmacol. Res.*, **46**: 235-243, 2002.
- Esposito D., Esposito G., Lissoni P., Paravicini L., Franchini F. Action of morphine on melatonin release in the rat. *J. Pineal Res.*, **5**: 35-39, 1988.
- Esposito E., Paterniti I., Mazzon E., Bramanti P., Cuzzocrea S. Melatonin reduces hyperalgesia associated with inflammation. *J. Pineal Res.*, **49**: 321-331, 2010.
- Gilad E., Cuzzocrea B., Zingarelli B., Salzman A.L., Szabo C. Melatonin is a scavenger of peroxynitrite. *Life Sci.*, **60**: L169-L174, 1997.
- Golombek D.A., Escolar E., Burin L.J., Brito Sanchez M.G., Cardinali D.P. Time-dependent melatonin analgesia in mice: inhibition by opiate or benzodiazepine antagonism. *Eur. J. Pharmacol.*, **194**: 25-30, 1991.
- Gomar M.D., Castillo J.L., Del Aguila C.M., Fernández B., Acuña-Castroviejo D. Intracerebroventricular injection of naloxone blocks melatonin-dependent brain [<sup>3</sup>H] flunitrazepam binding. *NeuroReport*, **4**: 987-990, 1993.
- Gomar M.D., Fernández B., Del Aguila C.M., Castillo J.L., de Dios-Luna J., Acuña-Castroviejo D. Influence of the behaviorally active peptides ACTH<sub>1-10</sub> and ACTH<sub>4-10</sub> on the melatonin modulation of <sup>3</sup>H-flunitrazepam receptor binding in the rat cerebral cortex. *Neuroendocrinology*, **60**: 252-260, 1994.
- Govitrapong P., Sawlom S., Chetsawang B., Sangchot P., Ebadi M. The bovine pineal gland contains delta and mu but not kappa or ORL 1 opiod receptor subtypes. *Proc. West Pharmacol. Soc.*, **45**: 32-35, 2002.
- Hernandez-Pacheco A., Araiza-Saldana C.I., Granados-Soto V., Mixcoatlzecuati T. Possible participation of the nitric oxide-cyclic GMP protein kinase G-K<sup>+</sup> channels pathway in the peripheral antinociception of melatonin. *Eur. J. Pharmacol.*, **596**: 70-76, 2008.
- Hill S.M. and Blask D.E. Effects of the pineal hormone melatonin on the proliferation and morphological characteristics of human breast cancer cells (MCF-7) in culture. *Cancer Res.*, **48**: 6121-6126, 1988.
- Hirata F., Hayaishi O., Tokuyama T., Seno S. In vitro and in vivo formation of two new metabolites of melatonin. *J. Biol. Chem.*, **249**: 1311-1313, 1974.
- Ismail S.A. and Mowafi H.A. Melatonin provides anxiolysis, enhances analgesia, decreases intraocular pressure, and promotes better operating conditions during cataract surgery under topical anesthesia. *Anesth. Analg.*, **108**: 1146-1151, 2009.
- Jarvis M.F. and Boyce-Rustay J.M. Neuropathic pain: models and mechanisms. *Curr. Pharm. Des.*, **15**: 1711-1716, 2009.
- Jeong J.H., Choi K.B., Yi B.C., Chun C.H., Sung K.Y., Gimm Y.M., Huh I.H., Sohn U.D. Effects of

- extremely low frequency magnetic fields on pain thresholds in mice: roles of melatonin and opioids. *J. Autonomic Pharmacol.*, **20**: 259-264, 2000.
- Kanaan S.A., Saade N.E., Haddad J.J., Abdelnoor A.M., Atweh S.F., Jabbur S.J., Salfah-Garabedian B. Endotoxin-induced local inflammation and hyperalgesia in rats and mice: a new model for inflammatory type of pain. *Pain*, **66**: 373-379, 1996.
- Klein D.C., Weller J.I., Moore R.Y. Melatonin metabolism: neural regulation of pineal serotonin, acetyl coenzyme A N-acetyltransferase activity. *Proc. Natl. Acad. Sci. U.S.A.*, **68**: 3107-3110, 1971.
- Klein D.C., Schaad N.L., Namboodiri M.A., et al. Regulation of pineal serotonin-N-acetyl transferase activity. *Biochem. Soc. Trans.*, **20**: 299-304, 1992.
- Kokki H., Vaatainen U., Pentilla I. Metabolic effects of low pressure tourniquet system compared with a high pressure tourniquet system in arthroscopic anterior crucial ligament reconstruction. *Acta Anesthesiol. Scand.*, **42**: 418-424, 1998.
- Lakin M.I., Miller C.H., Stott M.L., Winters W.D. Involvement of the pineal gland and melatonin in murine analgesia. *Life Sci.*, **29**: 2543-2551, 1981.
- Laurido C., Pelissie T., Soto-Mayano R., Valladares L., Flores F., Hernandez A. Effect of melatonin on rat spinal cord nociceptive transmission. *Neuroreport*, **13**: 89-91, 2002.
- Lerner A.B., Case J.D., Takahashi Y., et al. Isolation of melatonin, a pineal factor that lightens melanocytes. [letter] *J. Amer. Chem. Soc.*, **80**: 2587, 1958.
- Li S.R., Wang T., Wang R., Dai X., Chen Q., Li R.D. Melatonin enhances anti-nociceptive effects of delta but not mu opioid agonist in mice. *Brain Res.*, **1043**: 132-138, 2005.
- Lowenstein P., Pereya E.N., Gonzalez-Solveyra C., Cardinali D.P. Effect of naloxone on the nocturnal rise of rat pineal melatonin content. *Eur. J. Pharmacol.*, **98**: 261-264, 1984.
- Lowrie A., Jones M.J., Eastley R.J. Effect of eutectic mixture of local anaesthetic agents (EMLA) on tourniquet pain in volunteers. *Br. J. Anaesth.*, **63**: 751-753, 1989.
- Lutsch E.F. and Morris R.W. Light reversal of morphine-induced analgesia susceptibility rhythm in mice. *Experientia*, **27**: 420-421, 1971.
- Macías M., Escames G., León J., Coto-Montes A., Sbihi Y., Osuna A., Acuña-Castroviejo D. Calreticulin-melatonin. An unexpected relationship. *Eur. J. Biochem.*, **270** (5): 832-840, 2003.
- Maestroni G.J., Conti A., Pierpaoli W. Pineal melatonin, its fundamental immunoregulatory role in ageing and cancer. *Ann. N. Y. Acad. Sci.*, **521**: 140-148, 1988.
- Mantovani M., Kaster M.P., Pertile R., Calixto J.B., Rodriguez A.L., Santos A.R. Mechanisms involved in the antinociception caused by melatonin in the mice. *J. Pineal Res.*, **41**: 382-389, 2006.
- Marker C.L., Stoffel M., Wickman K. Spinal G-protein-gated K<sup>+</sup> channels formed by GIRK and GIRK2 subunits modulate thermal nociception and contribute to morphine analgesia. *J. Neuroscience*, **24**: 2806-2812, 2004.
- Marker C.L., Lujan R., Loh H.H., Wickman K. Spinal G protein potassium channels contribute in a dose dependent manner to the analgesic effect of the mu and delta but not kappa opioids. *J. Neuroscience*, **25**: 3551-3559, 2005.
- Mense S. Nociception from skeletal muscle in relation to clinical muscle pain. *Pain*, **54**: 241-289, 1993.
- Mickle A., Sood P., Zhang Z., Shahmohammadi G., Sengupta J.N., Miranda A. Antinociceptive effects of melatonin in a rat model of post-inflammatory visual hyperalgesia: a centrally mediated process. *Pain*, **149**: 555-564, 2010.
- Moore R.Y. Neural control of the pineal gland. *Behav. Brain Res.*, **73**: 125-130, 1996.
- Morris R.W. and Lutsch E.F. Daily susceptibility rhythm to morphine analgesia. *J. Pharmaceutical. Sci.*, **58**: 374-376, 1969.
- Mowafi H.A. and Ismail S.A. Melatonin improves tourniquet tolerance, and enhances postoperative analgesia receiving intravenous regional anaesthesia. *Anesth. Analg.*, **107**: 1422-1426, 2008.
- Naguib M., Hammond D.L., Schmid III P.G., Baker M.T., Cutkomp J., Queral L., Smith T. Pharmacological effects of intravenous melatonin: comparative studies with thiopental and propofol. *Br. J. Anesthesiol.*, **90**: 504-507, 2003a.
- Naguib M., Baker M.T., Spadoni G., Gregerson M. The hypnotic and analgesic effects of 2-bromomelatonin. *Anesth. Analg.*, **97**: 763-768, 2003b.
- Naranjo M.C., Guerrero J.M., Rubio A., Lardone P.J., Carrillo-Vico A., carrascosa-Salmoral M.P., Jimenez-Jorge S., Arellano M.V., Leal-Novel S.R., Leal M., Lissen E., Molinero P. Melatonin biosynthesis in the thymus of humans and rats. *Cell Mol. Life Sci.*, **64**: 781-790, 2007.
- Nosedá R., Hemández A., Valladares L., Mondaca M., Laurido C., Soto-Mayano R. Melatonin-

- induced inhibition of spinal cord synaptic potentiation in is MT2 receptor dependent. *Neuroscience Letters*, **360**: 41-44, 2004.
- Nosjean O., Ferro M., Coge F., Beauverger P., Henlin J.M., Lefoulon F., Fauchere J.L., Delagrance P., Canet E., Boutin J.A. Identification of the melatonin binding site MT3 as quinine reductase2. *J. Biol. Chem.*, **275**: 31311-31317, 2000.
- Ohkubo T., Shibata M., Takahashi H., Inoki R. Roles of substance P and somato-statin on transmission of nociceptive information induced by formalin in spinal cord. *J. Pharmacol. Exptl. Ther.*, **252**: 1261-1268, 1990.
- Onal S.A., Inalkac S., Kutlu S., Kelestimur H. Intrathecal melatonin increases the mechanical nociceptive threshold in the rat. *Agriculture*, **16**: 35-40, 2004.
- Perkarkova I., Parara S., Holecek V., Stopka P., Trefil L., Racek J., Rokyta R. Does exogenous melatonin influence the free radicals metabolism and pain sensation in rat? *Physiol. Res.*, **50**: 595-602, 2001.
- Pickard C.E. Circadian rhythm of nociception in the golden hamster. *Brain Res.*, **425**: 395-400, 1987.
- Pilcher C.W., Jones S.M., Browne J.M. Rhythmic nature of naloxane-induced aversions and nociception in rats. *Life Sci.*, **31**: 1249-1252, 1982.
- Raghavendra V., Agrewala J.N., Kulkarni S.K. Melatonin reversal of lipopoly-sacharrides-induced thermal and behavioural hyperalgesia in mice. *Eur. J. Pharmacol.*, **395**: 15-21, 2000.
- Reiter R.J. Melatonin: The chemical expression of darkness. *Mol. Cell Endocrinol.*, **79**: C153-C158, 1991.
- Reppert S.M., Weaver D.R., Ebisawa T. Cloning and characterization of a mammalian receptor that mediates reproductive and circadian responses. *Neuron*, **13**: 1177-1185, 1994.
- Reppert S.M., Godson C., Mahle C.D., Weaver D.R., Slaugenhaupt S.A., Gusella J.F. Molecular characterization of a second melatonin receptor expressed in human retina and brain. The Mel1b melatonin receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **92**: 8734-8738, 1995.
- Rosenfield J.P. and Rice P.E. Diurnal rhythms in nociceptive thresholds of rats. *Physiol. Behav.*, **23**: 419-420, 1979.
- Sandkuhler J. Models and mechanisms of hyperalgesia and allodynia. *Physiol. Rev.*, **89**: 707-758, 2009.
- Shavali B., Ho B., Govitrapong P., Sawlom S., Ajjimaporn A., Klongpanichapak S., Ebadi M. Melatonin exerts its analgesic actions not by binding to opioid receptor subtypes but by increasing the release of beta-endorphin an endogenous opioid. *Brain Res. Bull.*, **64**: 471-479, 2005.
- Shibata M., Ohkubo T., Takahashi H., Inoki R. Modified formalin test: Characteristic biphasic pain response. *Pain*, **38**: 347-352, 1989.
- Schmidtko A., Tegeder I., Geisslinger G. No NO, no pain? The role of nitric-oxide and cGMP in spinal pain processing. *Trends Neurosci.*, **32**: 339-346, 2009.
- Slominski A., Fischer T.W., Zmijewski M.A., Wortsman J., Semak I., Zbytek R.M., Slominski R.M., Tobin D.J. On the role of melatonin in skin physiology and pathology. *Endocrine*, **27**, 137-148, 2005.
- Srinivasan V., Spence D.W., Trakt I., Pandi-Perumal S.R., Cardinali D.P., Maestroni G.J. Immunomodulation by melatonin: Its significance for seasonally occurring diseases. *Immunomodulation*, **15**: 93-101, 2008.
- Srinivasan V., Pandi-Perumal S.R., Spence D.W., Moscovitch A., Trakht I., Brown G.M., Cardinali D.P. Potential use of melatonergic drugs in analgesia: mechanisms of action. *Brain Res. Bull.*, **81**: 362-371, 2010.
- Sugden D. Psychopharmacological effects of melatonin in mouse and rat. *J. Pharmacol. Exp. Ther.*, **227**: 587-591, 1983.
- Sung C.S. and Wong C.S. Cellular mechanisms of neuroinflammatory pain: the role of interleukin-1 $\beta$ . *Acta Anesthesiol.*, Taiwan, **45**: 103-109, 2007.
- Tal M. A novel antioxidant alleviates heat hyperalgesia in rats with an experimental painful peripheral neuropathy. *Neuroreport*, **7**: 1382-84, 1996.
- Tan D.X., Manchester L.C., Terron M.P., Flores L.J., Reiter R.J. One molecule, many derivatives: never ending interactions of melatonin with reactive oxygen and nitrogen species? *J. Pineal Res.*, **42**: 28-42, 2007.
- Toda N., Kishioka S., Hatano Y., Toda H. Modulation of opioid actions by nitric oxide signalling. *Anesthesiology*, **110**: 166-181, 2009.
- Tu Y., Sun R.Q., Willis W.D. Effects of intrathecal injections of melatonin analogs on capsaicin-induced secondary mechanical allodynia and hyperalgesia in rats. *Pain*, **109**: 340-350, 2004.
- Tzischinsky O. and Lavie P. Melatonin possesses time-dependent hypnotic effects. *Sleep*, **17**: 638-645, 1994.
- Ulugol A., Dokmeci D., Guray G., Sapolyo N., Ozyigit F., Tamer M. Antihyperalgesic but not

- antiallodynic effect of melatonin in nerve injured neuropathic mice: possible involvements of the L-arginine-NO-pathway and opiod system. *Life Sci.*, **78**: 1592-1597, 2006.
- Vanotti A., Osio M., Mailland E., Nascimbene C., Capiluppi E., Mariani C. Overview on the pathophysiology and newer approaches to treatment of peripheral neuropathies. *CNS Drugs*, **21**: 2-12, 2007.
- Wan Q. and Pang S.F. Segmental, coronal and subcellular distribution of 2[125]iodo melatonin binding sites in the chicken spinal cord. *Neurosci. Letters*, **180**: 253-256, 1994.
- Wan Q., Liao M., Brown G.M., Pang S.F. Localization and characterization of melatonin receptors in the rabbit spinal cord. *Neurosci. Letters*, **204**: 77-80, 1996.
- Wang T., Li S.R., Dai X., Peng Y.L., Chen Q., Wang R. Effects of melatonin on orphan FQ/nociceptin-induced hyperalgesia in mice. *Brain Res.*, **1085**: 43-48, 2006.
- Weaver D.R., Rivkees S.A., Reppert S.M. Localization and characterization of melatonin receptors in rodent brain by in vitro autoradiography. *J. Neurosciences*, **9**: 2581-2590, 1989.
- Williams L.M., Hannah L.T., Hastings M.H., Maywood E.S. Melatonin receptors in the brain and pituitary. *J. Pineal Res.*, **19**: 173-177, 1995.
- Ying S.W. and Huang Z.Q. Effects of pineal body and melatonin on sensitivity to pain in mice. *Acta Pharmacol. Sinica*, **11**: 411-414, 1990.
- Yu C.X., Wu G.C., Xu S.F., Chen C.H. Effect of melatonin on the release of endogenous opiod peptides in rat periaqueductal gray. *Sheng Li Xue Bao*, **52**: 207-210, 2000.
- Zhadnova I.V., Wurtman R.J., Lynch H.J., Ives J.R., Dollins A.B., Morabito C., et al. Sleep-inducing effects of low doses of melatonin ingested in the evening. *Clinical Pharmacol. Therap.*, **57**: 552-558, 1995.
- Zahn P.K., Lansmann T., Berger E., Speckmann E.J., Mushoff U. Gene expression and functional characterization of melatonin receptors in the spinal cord of the rat: implications for pain modulation. *J. Pineal Res.*, **35**: 24-31, 2003.