Melatonin: new applications in clinical and veterinary medicine, plant physiology and industry

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Abstract

Novel functions of melatonin continue to be uncovered. Those summarized in this report include actions at the level of the peripheral reproductive organs and include functions as an antioxidant to protect the maturing oocyte in the vesicular follicle and during ovulation, melatonin actions on the developing fetus particularly in relation to organizing the circadian system, its potential utility in combating the consequences of pre-eclampsia, reducing intrauterine growth restriction, suppressing endometriotic growths and improving the outcomes of in vitro fertilization/embryo transfer. The inhibitory effects of melatonin on many cancer types have been known for decades. Until recently, however, melatonin had not been tested as a protective agent against exocrine pancreatic tumors. This cancer type is highly aggressive and 5 year survival rate in individuals with pancreatic cancer is very low. Recent studies with melatonin indicate it may have utility in the treatment of these otherwise almost untreatable pancreatic cancers. The discovery of melatonin in plants has also opened a vast new field of research which is rapidly being exploited although the specific functions(s) of melatonin in plant organs remains enigmatic. Finally, the described application of melatonin's use as a chemical reductant in industry could well serve as a stimulus to further define the utility of this versatile molecule in new industrial applications.

REVIEW ARTICLE

INTRODUCTION

Subsequent to its discovery and characterization more than 50 years ago (Lerner *et al.* 1958; 1959), melatonin has been linked to a wide variety of functions in organisms from plants (Paredes *et al.* 2009; Iriti *et al.* 2010) to humans (Dominguez-Rodriguez *et al.* 2010; Paradies *et al.* 2010). Indeed, the functional versatility of this indoleamine has surprised even the most ardent scientists working in this dynamic field. While its actions are often characterized as being hormonal in nature, it also functions as a paracoid, an autocoid, a tissue factor and as an amine antioxidant (Tan *et al.* 2003). These multiple actions involve both receptor-mediated mechanisms (Stankov & Reiter 1990; Dubocovich & Markowska 2005) as well as processes that are receptor-independent (Reiter *et al.* 2007b; Jou *et al.* 2010). The receptor-dependent processes of melatonin involve classical membrane receptors (Pozo *et al.* 2010; Hardeland *et al.* 2011), nuclear binding sites (Acuna-Castroviejo *et al.* 1994; Carrillo-Vico

et al. 2005) as well as its ability to interact with cytosolic proteins (Hardeland 2009; Sharkey *et al.* 2010). Melatonin's multi-faceted actions make it one of the most ubiquitously acting molecules in nature (Reiter 1991). While melatonin was initially thought to be synthesized exclusively in the pineal gland of vertebrates (Axelrod 1970; Klein & Weller 1970), its production has now been documented in an uncommonly large number of vertebrate tissues (Hamm & Menaker 1980; Huether 1994) as well as in non-vertebrates which lack a pineal gland (Finnocchiaro *et al.* 1988; Hardeland & Poeggeler 2003) and also likely in plants (Murch & Saxena 2006).

This brief survey highlights some of the emerging fields of melatonin research. Of particular note is the large number of scientists from widely diverse disciplines whose investigative efforts now involve melatonin. The rapid expansion of research activities on this indoleamine has opened new vistas that will surely be exploited within the next decade.

NEW ASPECTS OF MELATONIN AND REPRODUCTIVE PHYSIOLOGY

Even before melatonin was extracted from the bovine pineal gland (Lerner *et al.* 1958, 1959), morphological changes in the appearance of cells in this gland suggested it was regulated/influenced by the light:dark environment (Quay 1956). Thus, after its discovery, the metabolic activity of the pineal gland throughout the light:dark cycle was examined and it quickly became obvious that the gland, judging from the level of precursors and the activities of the enzymes involved in tryptophan metabolism, was more active at night than during the day (Quay 1963; Axelrod & Wurtman 1968). Moreover, since it had long been suspected that some factor of pineal origin was capable of altering reproductive physiology (Kitay & Altschule 1954), it was surmised very early that annual changes in photoperiod likely impelled seasonal changes in reproductive capability (Reiter & Fraschini 1969). This was also supported by the observations that either surgical removal of the pineal gland (Hoffman & Reiter 1965, 1966) or its sympathetic denervation (Reiter & Hester 1966) prevented perturbations of the light:dark cycle from influencing the annual reproductive fluctuations in the photoperiodic rodent, the Syrian hamster (Reiter 1973).

While the activity of the pineal gland was clearly linked to fluctuations in sexual physiology induced by changes in the photoperiod, tests to document the role of melatonin in these responses initially failed (Reiter *et al.* 1974). Using a more appropriate experimental paradigm, however, it eventually became apparent that melatonin was the pineal factor responsible for the regulatory actions of the pineal on reproductive atrophy and recrudescence (Reiter *et al.* 1976; Tamarkin *et al.* 1976; Carter & Goldman 1983).

It is currently well accepted that seasonal reproductive changes in photoperiod-sensitive mammals are inextricably linked to the changing duration of nocturnal melatonin levels (Reiter 1974; Malpaux *et al.* 2002; Focada *et al.* 2006). This is true for both long day and short day breeding mammals with the molecular mechanisms of these interactions having been at least partially clarified (Vidal *et al.* 2009; Scherbarth & Steinlechner 2010).

The ability of melatonin to synchronize the annual cycle of reproduction in photoperiodic species generally involves its ability to modulate the activities of hypothalamic neurons and pars tuberalis cells which subsequently control gonadotropin secretion from the cells of the pars distalis (Malpaux *et al.* 2002; Focada *et al.* 2006; Vidal *et al.* 2009; Clarke *et al.* 2009; Schenbarth & Steinlechner 2010). At the level of the peripheral reproductive organs, however, melatonin also has important actions that are essential for optimal sexual physiology (Reiter *et al.* 2009c; Tamura *et al.* 2009). In the follicular fluid of the human vesicular ovarian follicle, the measured melatonin concentrations exceed those of simultaneously collected blood samples (Brzezinski *et al.* 1987; Nakamura *et al.* 2003). These differential levels of melatonin in follicular fluid and blood indicate an important point, namely, that melatonin concentrations in the body are not in equilibrium but vary from one fluid (or cell?) to another. This is also consistent with observations that other fluids, e.g., bile (Tan *et al.* 1999; Koppisetti *et al.* 2008) and cerebrospinal fluid (Skinner & Malpaux 1999; Tan *et al.* 2010), also have melatonin levels that exceed those measured in the peripheral circulation. Thus, as a general rule, it seems that the concentrations of melatonin are lower in the blood than they are in other bodily fluids. One seemingly important implication of this is that at least the cell membrane receptors, which exist in many cells throughout the organism, may be exposed to vastly different melatonin concentrations. Whether this has relevance to an influence on the signal transduction processes of these receptors or whether different melatonin membrane receptors respond to different concentrations of melatonin remains a subject for later research. The possibility also exists that elevated melatonin levels in some bodily fluids may be unrelated to the receptor actions of this indoleamine but rather may be essential for its functions as a direct free radical scavenger (Reiter *et al.* 2002c, 2008b; Tan *et al.* 2008; Romero *et al.* 2010).

Melatonin and ovarian function

In the ovarian follicular fluid, melatonin has ready access to the granulosa and luteal cells. In the human and in the rat ovary these cells have been found to possess the classic membrane melatonin receptors, i.e., the MT1 and MT2 receptor (Niles *et al.* 1999; Woo *et al.* 2001). After ovulation, the developing corpus luteum also contains these receptors (Soares *et al.* 2003). Melatonin has been shown to influence steroidogenesis by the ovarian granulosa cells (Yie *et al.* 1995). Receptor-independent functions of melatonin in the follicle may

be to protect especially the maturing oocyte from oxidative damage particularly at the time of ovulation, a process that is believed to involve an inflammatory response (Espey *et al.* 2003) and, as a consequence, the generation of large numbers of free radicals. The mature oocyte, which when fertilized will develop into the next generation, is a precious commodity that must be protected from damage of any type including mutilation by free radicals. Follicular fluid melatonin levels seem to reach their maximal concentrations just prior to ovulation (Tamura *et al.* 2009). Exogenously-applied melatonin may be helpful in reducing free radical-mediated damage to the maturing oocyte since the concentrations of melatonin in the follicular fluid of infertile women given 3 mg melatonin daily increased 3–4 fold (Tamura *et al.* 2008b). Whereas these findings are consistent with melatonin being taken into the ovarian follicular fluid from the blood, whether this is the case in women not supplemented with melatonin, where the concentration of the indoleamine also is greater than the level in the blood, remains to be proven. Melatonin produced in the ovaries themselves may contribute to the elevated melatonin concentrations in the follicular fluid. Recall, the activities of the two melatonin synthesizing enzymes, i.e., alkylamine N-acetyltransferase (AANAT) and acetylserotonin N-methyltransferase (ASMT) (formerly called hydroxyindole-O-methyltransferase or HIOMT) are detectable in human ovarian homogenates (Itoh *et al.* 1999).

Melatonin and the fetus

Pregnancy is a hazardous time for both the mother and the fetus. For optimal fetal development and normal fetal delivery, a healthy placenta is required. The developing fetus produces no melatonin but melatonin in the maternal blood is quickly transferred through the placenta to the fetus (Okatani *et al.* 1998). Thus, the fetal circulation exhibits a melatonin rhythm like that of the mother. Also, anything that perturbs the maternal melatonin rhythm, e.g., light at night, likewise alters the concentrations of melatonin in the fetal blood. Moreover, a faulty association between the maternal and fetal placental components could also jeopardize the amount of melatonin entering the fetal circulation. The importance of the fetal melatonin rhythm, derived from the maternal pineal gland, relates to the role of this indoleamine in synchronizing the fetal biological clock, i.e., the suprachiasmatic nuclei (SCN). Also, in the event that the fetus is rendered hypoxic (a condition that generates excessive free radicals) as a result of some abnormality, Wakatsuki and colleagues (1999) have shown that supplementing pregnant rats with melatonin results in reduced oxidative damage in the fetus. Thus, physiological levels of melatonin in the fetal circulation can be supplemented by administering pharmacological amounts of this antioxidant to the mother (Tamura *et al.* 2009). These findings could have implications for the prevention of periventricular leukomalacia and the subsequent cerebral palsy (Lee & Davis 2011).

In addition to protecting the fetus from free radical damage, the melatonin rhythm due to its actions on the fetal SCN impacts its circadian maturation. This in turn, has affects on postnatal behavior (Kennaway 2002; Torres-Farfan *et al.* 2006). In view of this, it would seem judicious that pregnant women, particularly during the third trimester of pregnancy, avoid light at night so as to preserve a normal circadian melatonin signal thereby allowing it to influence the normal maturation and development of the fetal circadian system.

Melatonin and pre-eclampsia

The placenta is a highly complex organ that, under conditions of abnormal development, can jeopardize the health of both the fetus and the mother. Interestingly, the placenta, i.e., the cytotrophoblasts as well as the syncytiotrophoblasts, have the enzymatic machinery to produce melatonin (Lanoix *et al.* 2008). The production of melatonin in this organ could be highly advantageous considering that a major disease of pregnancy, i.e., pre-eclampsia, is a high free radical-related condition (Siddiqui *et al.* 2010; Benedetto *et al.* 2011). Typically women with pre-eclampsia possess many features which are indicative of high oxidative stress, e.g., elevated placental superoxide anion radicals, reduced placental superoxide dismutase and glutathione peroxidase activities, reduced placental and whole blood glutathione and depressed levels of circulating vitamins C and E and melatonin. Additionally, there is often an elevation in maternal blood pressure and pre-eclampsia can proceed to eclampsia in which seizures occur (Saftlas *et al.* 1990). The use of melatonin has been suggested as a potential treatment for pre-eclampsia (Tamura *et al.* 2008a). Such studies seem justified considering the potent free radical scavenging activities of melatonin and its metabolites (Tan *et al.* 1993, 2008; Reiter *et al.* 2008a; Das *et al.* 2010; Milczarek *et al.* 2010; Stasiak *et al.* 2010), melatonin's ability to stimulate antioxidative enzymes (Rodriguez *et al.* 2004), and its beneficial synergistic actions with other antioxidants (Gitto *et al.* 2001; Milczarek *et al.* 2010). Additionally, melatonin has antihypertensive actions (Scheer 2005; Simko & Pechanova 2009; Reiter *et al.* 2010b) which may reduce the mean blood pressure of pre-eclamptic women and it has anti-seizure actions (Molina-Carballo *et al.* 1997) which could aid in preventing the progression of pre-eclampsia to eclampsia. Finally, melatonin has not been shown to exhibit toxicity either in the fetus or in the mother when given to pregnant rats (Jahnke *et al.* 1999).

Melatonin and intrauterine growth restriction

Intrauterine growth restriction (IUGR) of the fetus is also not an uncommon feature of abnormal placentation and other factors which reduce the blood supply and/or the availability of oxygen to the fetus (Salafia *et al.* 1992). To simulate compromised blood flow to the

placenta and fetus with the intent of limiting intrauterine growth, it is common to transiently occlude the utero-ovarian arteries in late pregnancy in rats (Tanaka *et al.* 1994). Nagai and colleagues (2008) used this method and gave supplemental melatonin to determine whether the damage inflicted in the placenta and fetus as a result of the transitory period of hypoxia and reoxygenation could be prevented. In this study, the utero-ovarian arteries of pregnant rats were occluded for 30 minutes on the 16th day of pregnancy (gestation length is 20 or 21 days). At day 20, the non-melatonin treated rats exhibited reduced placental and fetal weights and a lower respiratory control index, a marker of mitochondrial respiratory chain activity, as well as elevated levels of a damaged DNA product (8-hydroxy-2-deoxyguanosine) and redox factor-1 (which promotes the repair of damaged DNA) in the placenta. In addition to preventing the molecular damage at the level of the placenta, melatonin limited the reduction in fetal weight and fetal death resulting from the ischemia/reperfusion episode. Clearly, melatonin had limited the IUGR that resulted from a compromised blood supply. That melatonin also restored the mitochondrial respiratory control index is in line with the actions of melatonin at the level of the mitochondria (Acuna-Castroviejo *et al.* 2001, 2011; Rodriguez *et al.* 2007; Garcia-Macia *et al.* 2011) including in the human placental mitochondria (Milczarek *et al.* 2010).

A diminished blood supply to the fetus also reduces fetal nutrient delivery which negatively impacts the placento-fetal unit (Fowden *et al.* 2006) and leads to elevated oxidative/nitrosative stress in these tissues (Franco *et al.* 2007). Richter and co-workers (2009) examined the ability of melatonin to counteract the effects of malnourishment or placental efficiency, fetal weight and markers of oxidative stress in rats. Nutrient restriction was achieved by a 35% reduction in food intake during the last 5 days of pregnancy in rats while melatonin was given via the drinking fluid. Maternal undernutrition limited placental function and caused fetal growth retardation, changes prevented when melatonin was available to the undernourished rats. The protective effects of melatonin in this study were believed to relate to the direct free radical scavenging actions of melatonin and its metabolites (Hardeland *et al.* 2009; Bonnefont-Rousselot *et al.* 2011) and to its indirect antioxidant actions via stimulation of the enzymes, superoxide dismutase and catalase, which metabolize potentially toxic oxygen derivatives to innocuous products (Fowden *et al.* 2006).

Melatonin and endometriosis

Endometriosis is a severe chronic inflammatory condition in which implantation and growth of endometrial tissue occurs outside the uterine cavity (Garai *et al.* 2006). Although this condition is not life threatening, it poses a major risk factor for infertility and predisposes to ovarian cancer. Since it is an inflammatory condition, massive free radical generation occurs during endometriosis (Zeller *et al.* 1987). Data suggesting a beneficial action of melatonin in endometriosis is still fragmentary and limited but the preliminary findings suggest the indoleamine may have therapeutic value in this condition. Findings published by Güney et al (2008) confirmed, in a rat model of endometriosis, that melatonin induced regression and atrophy of endometriotic lesions and reduced the number of cyclooxyenase-2 (COX-2) positive cells and the levels of lipid hydroperoxides in the diseased tissues. In mice as well, melatonin caused the regression of an endometriosis model and arrested lipid peroxidation and protein damage in the lesions (Paul *et al.* 2008). This group also reported on a new diagnostic marker for judging the progression and severity of the disease, i.e., the expression ratio of matrix metalloproteinases (MMP-9)/tissue inhibitors of metalloproteinases (TIMP). Melatonin down regulated the activity and expression of pro-MMP-9 and elevated TIMP expression further supporting a role for melatonin in suppressing endometriosis. The regulation of MMP and TIMP by melatonin has far reaching implications considering these enzymes are involved in a number of pathophysiological conditions (Swarnakar *et al.* 2011).

Melatonin and IVF-ET

A highly important application of melatonin was recently reported when it was found that the indoleamine improved the pregnancy outcome of in vitro fertilization/embryo transfer (IVT-ET) (Tamura *et al.* 2008b). Poor oocyte quality is a major factor that reduces successful implantation in assisted reproductive technologies. The less than optimal oocyte quality is often considered to be a result of damage by free radicals, which have a major impact on reproductive physiology generally (Sugino 2005, 2007). Given that melatonin and its metabolites are versatile antioxidants (Rodriguez *et al.* 2004; Tan *et al.* 2008; Korkmaz *et al.* 2008; Reiter *et al.* 2009a; Wiktorska *et al.* 2010), Tamura and co-workers (2008b) tested whether melatonin would protect the oocyte from free radical damage and thereby improve the outcome of IVF-ET. Human ovarian follicular fluid was sampled at the time of oocyte retrieval and the level of 8-hydroxy-2-deoxyguanosine (8-OHdG), a damaged DNA product, was estimated in the fluid. The quantity of damaged DNA was found to be inversely related to degenerate state of the oocytes, i.e., the follicular fluid associated with the most deteriorated oocytes had the highest levels of 8-OHdG and there was also a negative correlation between the melatonin concentration of the fluid and intrafollicular 8-OHdG levels. When women who were undergoing IVF-ET were treated with melatonin prior to the procedure, the fertilization and pregnancy rates were improved and were correlated with a reduction in 8-OHdG and a damaged lipid product, hexanoyl-lysine adduct, in the follicular fluid. Considering the improve-

ment of both the fertilization and pregnancy rates in the women treated with melatonin, the indoleamine, presumably because of its antioxidant activities, may prove to be highly beneficial in reducing the necessity for repeated attempts at IVF-ET.

On the basis of the studies summarized here as well as those surveyed in other reviews (Malpaux *et al.* 2002; Tamura *et al.* 2008a, 2009; Revel *et al.* 2009; Casao *et al.* 2010), it is obvious that melatonin has multiple actions on the hypothalamo-hypophyseal axis as well as at the level of the peripheral reproductive organs. Whereas the bulk of the studies summarized above were performed in females, similar protective effects have been shown for the reproductive system of males, in both humans and domestic animals (Casao *et al.* 2010; Ortiz *et al.* 2011; Succu *et al.* 2011). These diverse roles of melatonin probably involve actions via membrane receptors, i.e., MTI and MT2 (Dubocovich & Markowska 2005; Hardeland 2009), and also via receptor-independent effects when melatonin and its metabolites function in the scavenging of free radicals and related derivatives (Reiter *et al.* 2009a; Paradies *et al.* 2010; Galano *et al.* 2011).

NEW ASPECTS OF MELATONIN AND CANCER

As with melatonin and reproductive physiology, the established association between melatonin and cancer has a rather long investigative history with the earliest studies claiming that surgical removal of the pineal gland, and therefore removal of a major source of melatonin, enhanced tumor growth (Rodin 1963; Das Gupta & Terez 1967). That the loss of melatonin was likely the cause of accelerated cancer cell proliferation after pineal removal became apparent about a decade later when it was shown that daily melatonin administration slowed tumor growth in rats (Lapin & Ebels 1976); in addition to melatonin, this group also argued that low molecular weight peptides from the ovine pineal gland contribute to the oncostatic actions (Lapin & Ebels 1976). Prior to melatonin being established as the major secretory product of the pineal gland, it was commonly asserted that in fact peptides, rather than the indoleamine melatonin, accounted for the endocrine effects of the pineal (Benson 1980; Pevet *et al.* 1980). This notion has been dispelled in recent decades by the failure of these investigators to identify any peptide that is synthesized and released from the pineal.

That melatonin has oncostatic actions is no longer debated (Mediavilla *et al.* 2010) and interest in the use of this endogenous non-toxic molecule as a cancer treatment is high. Despite this interest, clinical trials have been agonizingly slow to be executed. The reason for this is that melatonin per se has garnered little support from the pharmaceutical industry because it is a non-patentable molecule. Thus, drug companies are unwilling to support extensive clinical trials of an anti-cancer drug if they will not be the exclusive purveyor of the agent once its efficacy is established. Similarly, the granting agencies, whose budgets are being strained by numerous grant applications, have yet to support an expensive human trial related to the oncostatic properties of melatonin. From the pharmaceutical company perspective, however, it would seem that they should be interested in combining their toxic cancer chemotherapies with melatonin since it has repeatedly been shown to reduce the toxicity of many of the chemotherapies in common use (Reiter *et al.* 2002a, 2002b). By reducing their side effects, the dose of the chemotherapy could possibly also be increased thereby elevating its tumor-killing potential. Finally, melatonin, which itself has obvious anti-cancer actions, could further elevate the oncostatic effects of the combined therapies.

Within the last two decades, there has been a major emphasis on the ability of melatonin to inhibit especially breast (Blask *et al.* 1992, 2005; Coleman & Reiter 1992; Cos & Sanchez-Barcelo 1994; Stevens & Davis 1996; Leon-Blanco *et al.* 2003) and prostate cancer (Philo & Berkowitz 1988; Lupowitz & Zisapel 1999; Marelli *et al.* 2000; Sainz *et al.* 2005; Shiu *et al.* 2003; Shiu 2007), a trend that continues to the current time (Korkmaz *et al.* 2009; Jung-Hynes *et al.* 2010a, 2010b; Shiu *et al.* 2010). Many of these investigations have been elegant and have unequivocally established a role for melatonin as an effective experimental oncostatic agent. What is perplexing, however, is the very wide range of mechanisms proposed to explain the processes by which melatonin suppresses the growth of breast and prostate cancer cells (Cos & Sanchez-Barcelo 1994; Yuan *et al.* 2002; Leon-Blanco *et al.* 2003; Blask *et al.* 2005; Sainz *et al.* 2005; Korkmaz *et al.* 2009; Jung-Hynes *et al.* 2010a, 2010b; Shiu 2007; Shiu *et al.* 2010; Park *et al.* 2010; Proietti *et al.* 2011). How these multiple potential mechanisms by which melatonin modulates tumor cell proliferation will be reconciled awaits further investigations.

Breast and prostate cancers are by no means the only tumor types that are reportedly inhibited by melatonin. This indoleamine has been effective in restraining the growth of virtually every tumor against which it has been tested. Recently, melatonin was examined for its efficacy in diminishing the growth of pancreatic cancer (Leja-Szpak *et al.* 2010; Padillo *et al.* 2010; Gonzalez *et al.* 2011). These findings are particularly noteworthy since cancer of the pancreas is especially difficult to treat and even when all available measures are used, the 5-year survival is still less than 5% (Han *et al.* 2006).

Melatonin is well known to be a pro-apoptotic stimulus for a large number of cancer cell types (Sainz *et al.* 2003). This proved also to be the case for human pancreatic cancer cells (PANC-1) in culture. After 24 or 48 hours of incubation with melatonin (10^{-8}–10^{-12}), PANC-1 cells exhibited elevated Bcl-2/Bax and caspase 9 levels with the strongest signal of these pro-apoptotic factors being achieved with melatonin at a concentration of 10^{-12} M (Leja-Szpak *et al.* 2010). When pan-

creatic cancer cells were incubated with a combination of melatonin and luzindole (10^{-12}), a MT1 and MT2 melatonin receptor blocker, the pro-apoptotic actions of the indoleamine were completely abolished. While these findings are consistent with melatonin promoting apoptosis of pancreatic carcinoma cells via a membrane receptor-mediated pathway, considering the wide variety of mechanisms by which the indoleamine inhibits cancer cells of other types (Reiter *et al.* 2009b; Mediavilla *et al.* 2010), the processes described in this report may not be the only mechanisms by which melatonin suppressed pancreatic cancer cell growth.

Pancreatic cancer was induced in Syrian hamsters by the administration of *N*-nitrosobis (2-oxopropyl) amine (BOP). This agent was used to establish pancreatic lesions since it causes a similar pancreatic tumor pattern to that type seen in humans. Following the administration of BOP, melatonin or celecoxib, a COX-2 inhibitor, were given alone or in combination during the initiation phase, the post-initiation phase or during both phases of tumor development (Padillo *et al.* 2010). Melatonin proved more effective than celecoxib in reducing the development of pancreatic tumor nodules and improving survival of the hamsters. There was also some evidence that the combined treatment, especially during the post-initiation phase, reduced pancreatic tumor incidence but, in general, celecoxib had a very minor effect in improving the beneficial effects of melatonin (Padillo *et al.* 2010).

In the third report of this series, AR42J pancreatic tumor cells (derived from rat exocrine pancreas) were used; AR42J is the only cell line that maintains many of the characteristics of normal pancreatic acinar cells including the synthesis and secretion of digestive enzymes. When incubated in the presence of melatonin, the indoleamine caused transitory changes in cytosolic-free Ca^{2+} levels [Ca^{2+}] and mitochondrial free Ca^{2+} concentrations $[Ca2+]_m$ and induced mitochondrial membrane depolarization which led to a reduction in oxidized flavin adenine dinucleotide (FAD). Also, melatonin reduced AR42J cell viability and activated Ca^{2+}-dependent caspase-3. In view of the cellular changes observed, Gonzalez et al (2011) theorized that melatonin curtailed pancreatic cell viability via mechanisms that involved mitochondrial function impairment.

These three reports, all of which appeared within the last year, convincingly show that, at least under experimental conditions, melatonin inhibits the growth and reduces the viability of pancreatic exocrine tumors. These findings could prove to be of major importance since there are currently few adequate treatments for these very aggressive tumors in humans.

A number of the scientific publications have noted that the nighttime physiological melatonin concentration is capable of inhibiting tumor growth and that even partial suppression of nocturnal melatonin levels may promote excessive tumor metabolic activity and growth (Blask *et al.* 2005). Indeed, the nighttime inhibition of melatonin by artificial light has been frequently invoked as an explanation for the elevated incidence of breast cancer in women who work at night (Schernhammer *et al.* 2006; Erren *et al.* 2010; Kloog *et al.* 2010; Reed 2011). It is also noteworthy that since as animals (Reiter *et al.* 1980; 1981) and humans (Sack *et al.* 1986; Pang *et al.* 1998) age, their ability to produce melatonin (judging from the drop in pineal and serum melatonin concentrations) wanes and a natural consequence may be an elevated risk of developing cancer. Of course, many cancer types are, in fact, age-related; however, whether the rise in cancer incidence in the elderly has anything to do with the attenuated melatonin levels remains unproven at this point but would be worth examining. If an association is shown to exist it may be possible to reverse the trend of elevated cancer risk in an aging population by encouraging the regular use of melatonin.

The amplitude of the nocturnal rise in endogenous melatonin in humans is genetically determined. As a result, the amplitude of the nighttime increase varies widely, i.e., some individuals have a robust melatonin increase nightly while in other individuals the rise is significantly attenuated. Thus, it appears some individuals are relatively melatonin deficient even during early and middle age. If such attenuated nocturnal levels of melatonin promote tumor growth as suggested by at least one study (Blask *et al.* 2005), then people with a relative deficiency of melatonin may be prone to developing cancer at an earlier age than normal. If so, measuring the nighttime serum melatonin rise early in life may have prognostic value as a predictor of the likelihood of an individual to develop cancer.

NEW ASPECTS OF MELATONIN IN PLANTS

In reality, everything that is known about melatonin in plants must be considered as new. In the early 1990s it had been established that melatonin was very widespread in the animal kingdom, existing in organisms as diverse of humans (Vaughan *et al.* 1976) and unicellular algae (Poeggeler *et al.* 1991). This prompted scientists to search for melatonin in plants and in 1995 two publications appeared that measured melatonin in various plant organs (Dubbels *et al.* 1995; Hattori *et al.* 1995). Although initially viewed with skepticism, these findings have been repeatedly confirmed using all currently available techniques for the measurement of this indoleamine. This field of research is currently in an exponential growth phase and the findings have attracted the attention of botanists, plant physiologists, nutritionists, etc. (Paredes *et al.* 2009; Iriti *et al.* 2010; Huang *et al.* 2011; Sharman *et al.* 2011).

The concentration of melatonin in different plants and different plant organs varies widely (Reiter *et al.* 2007a). Interestingly, the highest melatonin levels measured in any plants have been in Chinese herbal medi-

cines (Chen *et al.* 2003). It is also common that plant seeds contain high melatonin concentrations, although again the levels vary widely among different seeds (Manchester *et al.* 2000). It is speculated that the high melatonin concentrations, because of the antioxidant activity of the indoleamine, aids in the germination of the seed. Seeds are rich in easily oxidized fats and high concentrations of a potent antioxidant such as melatonin would be highly beneficial in preventing molecular damage and maintaining the ability of a seed to successfully germinate.

In at least one plant, the water hyacinth, melatonin levels as well as its metabolite N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) varies throughout the light:dark cycle (Tan *et al.* 2007a). The peak melatonin levels, however, are not linked to darkness as in most vertebrates, but rather occur near the end of the light phase. Tan and colleagues (2007a) surmised that melatonin increases during the day to scavenge free radicals produced as a consequence of the process of photosynthesis. That this may be the function of melatonin in this situation is consistent with peak AFMK concentrations, which followed shortly after the melatonin peak in the hyacinth. AFMK is known to be formed when melatonin scavenges free radicals in animal tissues (Tan *et al.* 2002). The free radical scavenging activity of melatonin may also explain how plants, enriched with melatonin, resist damage when exposed to heavy metals (Tan *et al.* 2007b). The distribution of melatonin in plants includes its presence in flowers and fruits (Burkhardt *et al.* 2001; Iriti 2009; Murch *et al.* 2009, 2010). Consumption of plant products that contain melatonin cause a rise in blood melatonin concentrations (Hattori *et al.* 1995; Reiter *et al.* 2005) which correlate with the total antioxidant status of the blood (Benot *et al.* 1999; Reiter *et al.* 2005).

Okazaki and co-workers (2009) have cloned and characterized the cDNA for arylalkylamine N-acetyltransferase (AA-NAT) of *Chlamydomonas reinhardtii* (green alga). The cDNA was used to produce transgenic Micro-Tom tomato plants which then synthesized elevated amounts of melatonin. This showed that it is possible to genetically engineer plants to generate more than normal amounts of melatonin. Kang et al (2010) also overexpressed human AA-NAT in rice (*Oryza sativa* cv Dongjin) calli. The rice seedlings that grew from the calli expressed high levels of AA-NAT and melatonin. The transgenic rice plants also exhibited elevated chlorophyll synthesis during cold stress. The findings published in these two reports suggest AA-NAT, as in animals, is an essential enzyme for melatonin production in plants; moreover, the findings of these two studies have implications for nutrition, phytoremediation, resistance from harsh environmental conditions, etc. More recently, this latter group also cloned plant N-acetylserotonin methyltransferase (formerly known as hydroxyindole-O-methyltransferase) from rice (Kang *et al.* 2011). Considering the importance of rice as a dietary commodity throughout the world, engineering rice to produce increased amounts of the antioxidant melatonin could have enormous benefits.

With the idea of clarifying the functional role of endogenously produced melatonin, Okazaki et al (2010) genetically engineered tomato plants to overexpress the melatonin metabolizing enzyme indoleamine 2, 3-dioxygenase (IDO). The transgenic Micro-Tom plants expressing IDO at high levels exhibited depressed endogenous melatonin levels. Compared to leaf development in wild type plants, the T1 progeny of tomato plants with high IDO activity (and low melatonin) formed odd-pinnately compound leaves and exhibited a general leaf maldevelopment implying a metabolic role for plant melatonin in leaf maturation. Clearly, plants genetically engineered to produce reduced or exaggerated levels of melatonin could be valuable tools in defining the functional relevance of this indoleamine in plants organs.

There are a number of studies documenting a role for melatonin in enhancing the germination of seeds and functioning as a growth promoter in plants (Paredes *et al.* 2009). One of the initial studies that led to the assumption that melatonin promoted growth in plants was published by Murch et al (2001) who found that manipulating melatonin levels inhibited auxin-induced root and cytokinin-induced root organogenesis in explants of St. John's wort. Hernandez and Arnao (2005) and Arnao and Hernandez (2007) followed this with observations that melatonin in equimolar concentrations to the auxin, indole-3 acetic acid, promoted root growth in the lupin, *Lupinus albus*. Similarly, Afreen et al (2006) reported that melatonin dose-dependently promoted vegetative growth and development of the medicinal plant *Glycyrrhiza uralensis*. They also provided evidence that as the plant grew, the melatonin levels increased. Melatonin also improves the survival of the calli of *Rhodiola crenulata* after their cryopreservation (Zhao *et al.* 2011).

The most extensive studies related to the impact of melatonin and seed germination come from the research of Posmyk and co-workers (Janas *et al.* 2009; Posmyk & Janas 2009; Posmyk *et al.* 2009). They incubated either cucumber or corn seeds in a melatonin-containing solution before planting them. Melatonin generally improved seed germination in both plants, especially when the studies were carried out at reduced environmental temperature, i.e., 15 °C or 10 °C. This group also reported that plants grown from melatonin-treated seeds grew larger and produced more edible product.

Clarification of the function of melatonin in plants is a major area of research. In some cases, melatonin, an indole similar to indole-3-acetic acid, has actions like the auxin. It would seem likely that melatonin also functions as an antioxidant in plants and scavenges free radicals generated during photosynthesis.

MELATONIN: AN INDUSTRIAL APPLICATION

A publication that appeared in mid-2011 stands to revolutionize the production of graphene. Graphene, identified as the next "big thing", is a monolayer of sp^2-bonded carbon atoms that form a hexagonal two-dimensional lattice. This lattice of carbon atoms is one of the strongest metals discovered to date. Moreover, graphene is foldable, crushable and stretchable and is very light weight. The potential applications of this material are predicted to be extremely far reaching.

A common method for the preparation of graphene is the chemical exfoliation of graphite which is accomplished using powerful oxidizing agents (Stankovich *et al.* 2007; Kudin *et al.* 2008). The product produced by this process is graphene oxide nanosheets. These are subsequently chemically reduced to generate graphene nanosheets, which exhibit high electrical conductivity.

A number of methods have been used to reduce graphene oxide nanosheets to graphene including the use of strong chemical reductants such as hydrazine (Kim *et al.* 2009; Akharen & Ghaderi 2010). The use of chemical reductants, especially hydrazine, results in serious environmental contamination with negative health consequences. Thus, in addition to being corrosive and highly explosive (Schmidt 2001), hydrazine is extremely toxic to DNA, to neural tissue and to blood cells (Mo *et al.* 2001; Prabakar & Narayanan 2008) in addition to being carcinogenic and inducing hepatic and renal toxicity (Reilly & Aust 1997).

In view of the short comings of the chemical reductant, hydrazine, Esfandiar et al (in press) considered the use of other powerful reductants that are more environmentally friendly for the reduction of graphene oxide to graphene. For this purpose, they selected melatonin as a powerful bio-antioxidant. The use of melatonin as a substitute for hydrazine proved an excellent choice. Melatonin as a reductant resulted in an increased amount of absorbed nitrogen on the reduced graphene oxide nanosheets. The oxidized melatonin absorbed onto the surface of the reduced sheets acted as a stabilizer which prevented aggregation of the reduced sheets in suspension for three months. In comparison, hydrazine-reduced graphene oxide sheets are stable for only a few days. Raman spectroscopy confirmed the role of oxidized melatonin as a capping stabilizer of the reduced sheets. The authors of this seminal report predict that the use of melatonin, in lieu of hydrazine, will be a major step forward with the promise of high efficiency of deoxygenation of graphene oxide suspensions, which will be especially important in the large-scale production of graphene.

CONCLUDING REMARKS

At the time of its discovery, surely no one predicted that melatonin would turn out to be one of nature's most biologically-diverse molecules. It is indeed a "regulator of regulators" (Reiter 1980) and a multitasking agent (Reiter *et al.* 2010a). It has been described as a molecule that improves cellular physiology. This may in fact define what melatonin does on a day-to-day basis. After arriving at the cellular level, melatonin seems to function as a "molecular handyman", thereby doing what is necessary to improve the function of cells and thereby organs. These actions can be accomplished via its receptor-mediated functions or a result of the non-receptor actions of the indoleamine and its metabolites.

It may be that the precise function of melatonin remains unknown. What we are observing as the actions of melatonin are possibly the epiphenomena of more basic functions that have yet to be de-coded. There are certainly many questions related to the very complex and diverse functions of melatonin.

The discovery of melatonin throughout the animal kingdom and more recently in plants has already attracted many investigators to research melatonin. Thus, in the foreseeable future, there is every reason to believe that the general field of melatonin research will continue to mushroom. This is also emphasized by the most recent paper that illustrates the use of melatonin in industry. Once confirmed, this could lead to the use of melatonin for other industrial applications.

By necessity, this brief review had to be selective in terms of the subjects covered. There are many other burgeoning fields of melatonin research that deserved to be mentioned (Pacini & Borzani 2009; Srinivasan *et al.* 2010; Casao *et al.* 2010; Huang *et al.* 2010; Rodella *et al.* 2010; Calvo-Guirado *et al.* 2010; Yoo & Jeung 2010; Rosenstein *et al.* 2010; Hong *et al.* 2010) but were not because of space constraints. In reference to endogenous melatonin production in vertebrates, the continued misuse of artificial light, which lowers endogenous melatonin synthesis and perturbs circadian rhythms (Erren & Reiter 2008; Claustrat *et al.* 2010; Figueiro and Rea 2010), must be taken seriously as a potential causative factor of some disease states that are related to a relative melatonin deficiency or an abnormal melatonin rhythm.

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