JAMES OLCESE

Melatonin Receptors in the Human Reproductive Tract

The history of melatonin (MEL) research has long been intertwined with reproductive physiology. Soon before and especially after its characterization in the late 1950s as a tryptophan-derived molecule of the pineal gland (Lerner et al., 1960), researchers had begun finding an intriguing association between pineal activity, light, and gonadal growth (Kitay and Altschule, 1954; Fiske et al., 1960; Wurtman et al., 1961). These observations motivated basic and ultimately clinical research on MEL's involvement in reproductive biology for decades. After years of controversy resulting from often contradictory clinical findings, the dust is finally beginning to settle, so that a review of the current status of MEL in the context of human fertility is in order. Without attempting to survey the entire MEL research discipline, it is nonetheless valuable to initially cover some of the basic aspects of MEL as a hormone of the reproductive system. Thereafter, an update of the newer findings in the field of MEL research as it relates to the human reproductive tract will be provided, with the hope of stimulating new approaches and experimental data in this intriguing branch of endocrinology.

Brief background to melatonin in the context of mammalian reproduction

The hormone, melatonin (N-acetyl-5-methoxytryptamine), is a small lipophilic indoleamine generated primarily in neural tissues whose ontogeny reflects a phototransductive phylogeny, i. e. the retina and the epithalamic pineal gland. This pattern of tissue localization offers a key to understanding MEL's primary role during the course of mammalian evolution as a chemical signal encoding environmental lighting conditions ("photoperiods"). In all vertebrates, but not necessarily in other organisms (non-vertebrates, non-metazoans, fungi, plants and bacteria) where MEL synthesis has been described (Arendt, 1995), the generation and release of MEL occurs almost entirely during the dark phase of the 24-hour day/night cycle. The mechanisms driving nocturnal MEL synthesis have been studied in considerable detail in numerous vertebrate species. A key feature of the secretory pattern of MEL is its plasticity under differing photoperiods. Thus, long-duration profiles of elevated MEL are associated with short daylengths and vice versa (Goldman, 2001).

The investigation of the molecular pathways through which MEL operates to physiologically code for night length remains an active area of neuroendocrine research (see below for details). What has been convincingly demonstrated is that MEL plays a critical role in the seasonal timing of reproductive activities in a number of mammalian species, including the sheep, mink, ferrets, skunks, equines, hamsters and feral mice (Arendt, 1995). Thus, for example, hamsters lacking their pineal gland will not enter reproductive quiescence normally associated with the short days of winter. Conversely, certain species of sheep can be induced to premature seasonal reproductive activity by the administration of MEL. These species-specific differences in the response to MEL created considerable doubt and confusion initially and only in time were researchers able to recognize that MEL is neither "anti-gonadotropic" nor "pro-gonadotropic", but instead it simply encodes night-length. This information is then used in a species-specific adaptive manner to time seasonal reproduction appropriately.

The key word in the previous sentence is *time*. MEL is now seen as something different than a classical endocrine signal driving its target tissues to respond in a concentration-dependent manner and responding to negative feedbacks from downstream sources. Indeed, very few of the factors generated from its peripheral target glands (e. g. ovaries, adrenals, testes) appear capable of significantly regulating pineal MEL synthesis under normal conditions. This is not to say that gonadal or adrenal steroids are without effect on pineal function. However, their effects are small and do not appear to play a major role in the determination of either MEL secretory phase or amplitude.

The primary regulator of MEL's rhythmic secretion in mammals is the central circadian oscillator in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus, whose efferents direct sympathetic neural activity to the pineal gland and thereby stimulate MEL synthesis. Loss of sympathetic input to the pineal (or loss of the SCN) results in the abolition of measurable nocturnal MEL in the bloodstream (cf. Arendt, 1995). Interestingly, light can acutely inhibit nocturnal MEL secretion, albeit indirectly *via* the retinohypothalamic input to the SCN. Furthermore, under otherwise light-free conditions light can cause phase shifts in the MEL secretory profile whose direction depends on the phase at which it is given (Lewy et al., 1998). All of this points to the powerful role of

light on the SCN and the pattern of its output signals, such as MEL rhythms.

The re-orientation of MEL research into the domain of chronobiology has brought numerous advances in our understanding of the hormone's physiological attributes and has even spawned some important new applications for MEL—for example in the treatment of jet lag and sleep disorders (see Arendt, 2003 for review). In a small irony of history it is worth mentioning here that the Nobel laureate Julius Axelrod (whose prize was admittedly not for pineal research) published a now-forgotten but remarkably prescient paper together with Richard Wurtman in 1966, entitled "The pineal gland: a biological clock" (Axelrod and Wurtman, 1966). Up to now, however, the impact of chronobiology on human reproductive science has been minimal, hence, MEL research is confronted with a challenge that may take some years to sort out. A potentially promising direction has recently been charted by the discovery of circadian gene elements ("clock genes") in the mammalian neuroendocrine axis (Sun et al., 1997; Morgan et al., 1998; von Gall et al., 2002a; Olcese et al., 2003; Gillespie et al., 2003; Chappell et al., 2003) and in the gonads (Zylka et al., 1998; Morse et al., 2003; Bittman et al., 2003). As effects of MEL on the expression of clock genes have also been reported (Morgan et al., 1998; Roy and Belsham, 2002), it seems likely that the MEL/reproduction model may undergo another paradigm shift in the near future.

Melatonin and human reproduction: A very brief historical account

A potential role for the pineal gland in certain aspects of human reproductive function was first suggested early in the 20th century, following reports that precocious puberty was associated with pineal tumors, and that the gland decreases in size after puberty (see Kappers 1979 for historical review). Although a large body of literature has documented a decline of MEL during puberty, as first reported by Waldhauser and colleagues (1984), the current consensus tends to argue that this may merely be consequence of increasing body size (Luboshitzky and Lavie, 1999). Similarly, no clear consensus has emerged as to the potential role for MEL in the normal menstrual cycle, as some groups have reported changes in MEL during the menstrual cycle (Wetterberg et al., 1976; Webley and Leidenberger, 1986) while others find no significant alterations in MEL levels (Brzezinski et al., 1988).

A number of studies have addressed the issue of MEL and amenorrhea. Typically, a significantly elevated plasma MEL level is seen in women with hypothalamic amenorrhea (Berga et al., 1988; Brzezinski et al., 1988), although the circadian phase of MEL secretion remains normal. This could be interpreted as evidence for a negative role of MEL in the neuroendocrine processes that underlie menstrual cyclicity. Subsequent research has confirmed that MEL is elevated during both primary and secondary hypothalamic amenorrhea (Walker et al., 1996; Okatani and Sagara, 1995) as well as in athletics-induced amenorrhea (Laughlin et al., 1991). Interestingly, women with Kallmann's syndrome—a congenital hypogonadotropic defect due to improper migration and maturation of the GnRH neurons in the hypothalamus—also have significantly elevated MEL levels, suggestive of an effect of low plasma GnRH concentrations on pineal MEL secretion. Similarly, GnRH-deficient men have been shown to have an increased nocturnal MEL secretion (Luboshitzky et al., 1996, 2000a). Whether this indicates a direct inhibitory effect of GnRH on pineal MEL secretion or an indirect effect elsewhere in the brain remains unclear, although GnRH has been reported to elevate MEL secretion from the rat pineal gland *in vitro* (Itoh et al., 2003).

It is clear that humans have lost most of their inherent photoperiodism (Wehr, 2001), and yet there are still some human populations—especially those living at high latitudes—that undergo seasonal alterations in fecundity (Roenneberg and Aschoff, 1990; Rojansky et al., 1992; Lam and Miron, 1994). Both male and female reproductive parameters are subject to some degree of seasonality (e. g. Saint Pol et al., 1989; Kauppila et al., 1987). On the basis of the inverse correlation between seasonally high MEL (Stokkan and Reiter, 1994) and seasonally low ovarian activity in these high latitude populations (Kauppila et al., 1987), it is often argued that MEL may be the cause of such seasonal infertility (Partonen, 1999). Nonetheless, clear evidence for this hypothesis is still lacking. Despite suggestions that MEL could be employed as a human contraceptive (Voordouw et al., 1992; Silman, 1993) or in the treatment of pubertal disorders (Aleandri et al., 1996)—the main emphasis of MEL research today is no longer explicitly in this direction. Indeed, the consensus has emerged that truly compelling evidence for an essential role of MEL in human fertility is still outstanding (Rohr and Herold, 2002).

Melatonin receptors

The first evidence for MEL binding sites was presented some 25 years ago by Cohen et al. (1978) who reported cytosolic binding sites, and Cardinali and colleagues (1979) who showed that radiolabeled MEL specifically binds to membranous fractions of the bovine brain. Within a few years MEL was shown to be a potent modulator of dopamine release in the mammalian retina (Dubocovich, 1983). MEL's effect on pigment aggregation in amphibian dermal melanophores was shown to be blocked by pertussis toxin (White et al., 1987), providing strong support for the notion that a membrane-bound G-protein-coupled MEL receptor might exist. On the basis of these,

and many other corroborative studies, Reppert and colleagues set out to clone the MEL receptor, and succeeded to identify the first MEL receptor in amphibian melanophores and subsequently its homologues in mammals, including the human (Reppert et al., 1994, 1995). Soon thereafter a "melatonin-related receptor" with no MEL-binding capacity was also cloned and characterized (Reppert et al., 1996), to be followed by the purification and identification of a low-affinity MEL-binding protein ("MT3"), which in fact is recognized now to be the enzyme quinone reductase 2 (Nosjean et al., 2000). The physiological significance of the latter two proteins is uncertain.

Although the data are primarily from non-primate mammals and the level of receptor expression in many tissues is often rather low, especially for the MT2 subtype, the distribution of the two MEL receptors is rarely non-overlapping. In the brain, the MT1 receptor is usually found in the SCN, hippocampus, cerebellum and (with the exception of humans; cf. Barrett et al., 2003) especially in the pars tuberalis of the pituitary. The MT2 receptor is most strongly expressed in the retina, and in considerably lower levels in the SCN, hippocampus and cerebellum (Dubocovich et al., 2003). MEL receptor expression in peripheral human tissues has also been well documented (cf. von Gall et al., 2002b).

Table 1: Human reproductive tissues where the mRNA and/or protein expression of melatonin receptors (MT1/MT2) has been identified

Tissue	Receptor isoform	Molecular level (mRNA; protein*)	Citation**
(MALE)			
Prostate epithelium	MT1 ?	protein	Laudon et al. 1996; Shiu et al. 2003
Spermatozoa	??	protein	Van Vuuren et al. 1992
(FEMALE)			
Breast epithelium	MT1, MT2	mRNA and protein	cf. Sanchez-Barcelo et al. 2003 (review)
Ovarian granulosa/lutein	MT1, MT2	mRNA and protein	Yie et al. 1995b; Woo et al. 2001
Uterine myometrium	MT1, MT2	mRNA and protein	Schlabritz-Loutsevitch et al. 2003

* including radioreceptor binding data
** representative, not exhaustive

The binding of MEL to both receptor subtypes (in native or transfected cells expressing only MT1 or MT2 receptors) is in the picomolar range (cf. Barrett et al., 2003), with MT1 having a somewhat higher affinity for MEL and I-MEL. Specific MEL analogues with a higher selectivity for MT2 receptors have been synthesized, e. g. 6-chloro-MEL, 5-methoxyluzindole, and 4P-PDOT (cf. Dubocovich et al., 2003), which have enabled researchers to identify MT2 receptor-mediated effects of MEL in a number of native tissues.

Both receptor subtypes couple *via* inhibitory G-proteins to adenylyl cyclases to inhibit cyclic AMP signaling and *via* Gq-proteins to stimulate phosphatidylinositol turnover. The MT2 receptor also inhibits cyclic GMP signaling (Dubocovich et al., 2003). Other signaling pathways for MEL receptors include release of arachidonic acid (Godson and Reppert, 1997), activation of protein kinase C (McArthur et al., 1997; Hunt et al., 2001) and other kinases (cf. Dubocovich et al., 2003), and modulation of potassium channels (Nelson et al., 1996). Although non-receptor effects of MEL have also been demonstrated (cf. Tan et al., 2003) these are beyond the scope of this review, and will not be addressed here.

Melatonin receptors in the female reproductive tract: Current status

MEL has been shown to have actions on several cells of the female reproductive tract *in vitro*. The most investigated tissue in this regard is clearly breast tissue. Nonetheless, in view of the excellent recent reviews on this single subject (e. g. Sanchez-Barcelo et al., 2003) no discussion of this topic will be attempted here. There are, however, other female reproductive tract cells that respond to MEL.

Ovarian receptors

Earlier studies reported substantially higher levels of MEL in human ovarian follicles as compared to serum MEL levels (Brzezinski et al., 1987; Ronnberg et al., 1990). Subsequent findings identified a positive correlation between follicular MEL and progesterone concentrations (Yie et al., 1995a), especially for large, preovulatory follicles (Nakamura et al., 2003). These data are consistent with earlier studies that reported positive effects of MEL on progesterone secretion by human granulosa cells (Webley and Luck, 1986; Webley et al., 1988; Brzezinski et al., 1992). A modest effect of pharmacologically high doses of MEL was also reported on the release of oxyto-

cin from human granulosa cells *in vitro* (Schaeffer and Sirotkin, 1997).

Subsequent to reports of ^{125}I-MEL binding to human granulosa cell membranes (Yie et al., 1995b), the transcripts for both MT1- and MT2-receptors were identified in rat ovaries (antral follicles and CL) by Soares and colleagues (2003). Specific binding of I-MEL was shown to be estrogen-dependent since receptor density was significantly higher during proestrus than in metestrus. Pharmacological characterization also suggested constitutively active MT1R. In granulosa cells MEL inhibited FSK-stimulated cAMP *via* PTX-sensitive G-protein, which could be blocked by the antagonist 4P-PDOT.

Both receptor isoforms were also identified by RT-PCR in human granulosa/luteal cells by Woo et al. (2001). Remarkably, treatment of these cultured cells with MEL increased mRNA expression of the LH- (but not FSH-)receptor, while inhibiting expression of GnRH and the GnRH-receptor. Although without effect on its own, MEL did enhance hCG-stimulated progesterone secretion from these cells, possibly *via* the increased expression of the LH receptor. MEL also appeared to activate MAP kinase activity in cultured granulosa/luteal cells.

MEL has been shown to have antiproliferative effects on human ovarian cancer cells (Bartsch et al., 2000) and on the human ovarian carcinoma cell line BG-1 (Petranka et al., 1999). However, in a more recent study with HTOA and OVCAR-3 ovarian cancer cells that were cultured in the presence of MEL the chemotherapeutic agent *cis*-diaminedichloroplatinum (CDDP), no effects of MEL alone were seen (Futagami et al., 2001), even though MEL enhanced CDDP-sensitivity in both cell lines. Although the mechanism for this enhancement is unknown, these data point to the interesting possibility of using MEL in the future as an adjunct in human ovarian cancer chemotherapy.

Uterine receptors

Effects of photoperiod on timing of parturition in rats have been known for quite some time (e. g. Lincoln and Porter, 1976; Bosc and Nicolle, 1980). Given its role as mediator of daylength information it is thus not surprising to also find an influence of MEL on this physiological event in the rat (Bosc, 1987). Indeed, Takayama et al. (2003) showed recently in a very interesting paper that pinealectomized female rats, while showing no disturbances in estrous cyclicity nor in their ability to become pregnant, nonetheless failed to deliver their young exclusively during the daytime (the normal birthing phase), but instead gave birth across the 24 h light-dark cycle. MEL replacement was effective in restoring the daytime birth pattern when administered in the evening, but was ineffectual when given in the morning or continuously. This clearly demonstrates that the timing of birth in the rat is under circadian control, and that MEL may serve as a key circadian signal for this event. Very early reports showed direct inhibitory effects of pharmacological doses of MEL on uterine contractility (Hertz-Eshel and Rahamimoff, 1965; Burns, 1972) and the presence of MEL binding sites in the uterus (Cohen et al., 1978). Later studies have also reported inhibitory effects of MEL on uterine contractility *in vitro* following stimulation by oxytocin (Gimeno et al., 1980; Abd-Allah et al., 2003).

The precise mode of action of MEL in the uterus is however not clear. Inhibitory effects of MEL *in vitro* on prostaglandin E2 as well as F2α generation in rat tissues have been reported (Cardinali et al., 1980; Gimeno et al., 1980; Franchi et al., 1987). In view of recent work demonstrating that MEL's inhibitory effect on the rat myometrium *in vitro* could be overriden by addition of PGF2a (Ayar et al., 2001), the cyclooxygenase pathway may represent an intrauterine target for MEL. Additionally, MEL administration to nonpregnant rats has been reported to significantly inhibit the expression of uterine estrogen and progesterone receptors (Abd-Allah et al., 2003). Recently, the calcium-activated large conductance potassium channel (BK_{Ca}) has been identified as a target for MEL action in rat myometrial cells by Steffens et al. (2003). Importantly, the effects of MEL on this myometrial-specific channel were shown to vary depending on whether the animal was cycling or pregnant. Thus, MEL appeared to influence the BK_{Ca} channel *via* the pertussis toxin (PTX)-sensitive Gi-protein pathway (which inhibits cyclic AMP/protein kinase A signaling) differently in nonpregnant as compared to pregnant myometrium. In contrast, MEL consistently activated the PTX-insensitive Gq pathway in myometrial cells (thereby stimulating phospholipase C activity, which leads to mobilization of calcium from intracellular stores) as assessed by BK_{Ca} channel activity. These data may help to explain the mechanisms through which MEL can differentially modulate myometrial contractility in both nonpregnant and pregnant uteri.

In contrast to the myometrial actions of MEL described above, Zhao and co-workers (2000) reported by means of RT-PCR that endometrial stromal cells of the rat uterus express MT1 receptors. As MEL also inhibits the growth of rat uterine stromal cells *in vitro* (Zhao et al., 2002a) this laboratory then suggested the MT1 receptor may mediate MEL's antiproliferative effects on these cells. In another study these authors documented estrous cycle-dependent variations in ^{125}I-MEL binding in the endometrial cells of the rat uterus (lowest binding at metestrus) as well as inhibitory effects of ovariectomy, which could be partially counteracted by estrogen and progesterone (Zhao et al., 2002b). As MEL has been reported to be lowest at estrus in the rat (Johnson et al., 1982) the changes in MT-R expression may be in response to the ligand as well as regulation by steroids. These data need to be juxtaposed with earlier findings (e. g. Danforth et al., 1983) that showed a direct stimulatory effect of MEL on estrogen receptor binding in the rodent uterus.

In view of the accumulating data on the binding and intracellular effects of MEL on uterine tissues from animals, the report of Martensson and colleagues (1996) demonstrating MEL effects on the human myometrium was not unexpected. These authors utilized pregnant myometrial biopsies to record *in vitro* spontaneous contractility following noradrenaline (NA) treatment. Although MEL alone had no apparent action in these preparations, the hormone did significantly potentiate NA-induced myometrial contractions. Given that human labor and delivery are statistically more common during the night phase (in contrast to that of rodents—see above), it seems reasonable to propose that the potentiating effect of MEL on tocophilic stimuli may be a contributing element to this rhythm.

Table 2: Effects of melatonin in vitro on non-breast tissue or cells of the human female reproductive tract

Tissue/cell source	Effect	Citation
Granulosa cells	stimulation of progesterone secretion	Brzezinski et al. 1992
Granulosa cells	stimulation of oxytocin release	Schaeffer and Sirotkin 1997
Granulosa/luteal cells	↑ LH-receptor transcripts; ↓ GnRH, GnRH-R transcripts; activation of MAP kinase activity	Woo et al. 2001
Primary ovarian cancer cells	↑ ↓ cell growth	Bartsch et al. 2000
Ovarian carcinoma line BG-1	antiproliferative	Petranka et al. 1999
Ovarian carcinoma lines HTOA and OVCAR-3	enhancement of chemotherapeutic actions	Futagami et al. 2001
Myometrium	potentiation of contractility	Martensson et al. 1996
Myometrium	inhibition of cAMP signaling	Schlabritz-Loutsevitch et al. 2003
Endometrial carcinoma line Ishikawa	antiproliferative	Kanishi et al. 2000
Choriocarcinoma lines Jar and JEG-3	antiproliferative	Shiu et al. 2000

* representative, not exhaustive

Recently, my laboratory has extended these earlier findings by demonstrating MEL receptor expression in the human myometrium during pregnancy and in nonpregnant women (Schlabritz-Loutsevitch et al., 2003). At both the mRNA level and as assessed by radioreceptor assay, both receptor isoforms were detected. A substantial decline in receptor expression (as compared to nonpregnant tissues) was seen in the myometria of women who underwent cesarean delivery in late pregnancy prior to the onset of labor. Additionally, the inhibitory effects of MEL on cAMP signaling that were seen in cultured myometrial cells of nonpregnant women were absent in the cells of pregnant women, possibly as a result of the low receptor expression in the latter case. It will be important to determine however whether our findings of low myometrial MEL receptor expression in late pregnancy represent a normal physiological mechanism (i.e. to brake contractility until the proper gestational phase) or instead a pathological situation that might be associated with suboptimal uterine contractility and delayed onset of labor. In either instance, the ramifications of these findings for the practice of obstetrics could be very exciting.

Kanishi and colleagues (2000) demonstrated significant inhibitory effects of physiological (1 nM)—but not higher—doses of MEL on the growth of the estrogen receptor-positive human endometrial cancer cell line Ishikawa. By contrast, MEL was without effect on the growth on the estrogen receptor-negative endometrial cell line SNG-II. The inhibitory effect of MEL on Ishikawa cells could be blocked by nanomolar concentrations of 17β-estradiol and by the MEL antagonist luzindole. Subsequent work by the same laboratory (Kobayashi et al., 2003) showed saturable and specific MEL binding to Ishikawa cells, with affinity constants indicative of possible MT2 receptor expression (although confirmation of this with RT-PCR was not provided).

Antiproliferative effects of MEL have also been reported for human Jar and JEG-3 malignant trophoblastic choriocarcinoma cell lines (Shiu et al., 1999; Shiu et al., 2000). In both cell lines, MT2 melatonin receptors, but not MT1 receptors, were identified by RT-PCR, suggestive of an antiproliferative effect that may be specifically mediated *via* the MT2 receptor. MEL also inhibited the *in vivo* growth of both Jar and JEG-3 xenograft tumors in nude mice and even extended lifespan in those animals that developed choriocarcinomas (Shiu et al., 2000).

Melatonin receptors in the male reproductive tract: Current status

The potential effect of MEL on the secretion of reproductive hormones in men has been investigated for decades with no consistent conclusions emerging. Recent studies confirm that long-term MEL did not alter mean gonadotropin, testosterone, inhibin-beta secretion, prolactin nor growth hormone levels in men (Luboshitzky et al., 2000b; Rajaratnam et al., 2003).

Testicular receptors

MEL binding sites have been identified in human spermatozoa (Van Vuuren et al., 1992) and MEL-R have been shown to be present in the epididymis and vas deferens of rodents (Pang et al., 1998). Pharmacological doses of MEL (3 mg daily) reportedly lowers the sperm concentration and sperm motility of some men (Luboshitzky et al., 2002) in agreement with similar effects of MEL *in vitro* (Irez et al., 1992). However, the actual mechanisms through which MEL might influence sperm function remain unknown.

Nothing is known about the possibility of MEL having direct actions on the human testes. MEL-binding sites have been reported in rat Leydig cells (Valenti and Giusti, 2002) but no comparative data have yet been published in the case of humans.

Receptors in the prostate gland

In contrast to the paucity of data on direct effects of MEL on the human testes, there is considerable evidence for MEL having effects on the prostate gland in men. Previous autoradiographical investigations identified specific G-protein coupled MEL binding sites in the glandular epithelium of the human prostate gland (Laudon et al., 1996). Subsequent studies demonstrated an anti-proliferative action of MEL on these prostate epithelium cells *in vitro* (Gilad et al., 1996).

Since that time several groups have also shown inhibitory effects of MEL *in vitro* on the proliferation of the androgen-sensitive LNCaP prostate cancer cell line (Lupowitz and Zisapel, 1999; Xi et al., 2000; Moretti et al., 2000) as well as of the androgen-insensitive PC3 and DU145 prostate cancer cell lines (Gilad et al., 1999; Marelli et al., 2000). MEL was also able to inhibit the *in vivo* growth of LNCaP xenograft tumors—but not PC3 xenografts—in nude mice, an effect which correlated with the presence of the MT1 MEL receptor protein in the LNCaP cells and its absence in the PC3 line (Xi et al., 2001). These antiproliferative actions of MEL appear to involve G_o cell cycle arrest (Marelli et al., 2000) and correlate with significant decreases in the expression of prostate-specific antigen and cyclin A (Xi et al., 2001). Recently, remarkable evidence has been provided that MEL causes significant exclusion of the androgen receptor from the cell nucleus of LNCaP cells (Rimler et al., 2001) as well from the nuclei of PC3 cells that were stably transfected to express the androgen receptor (Rimler et al., 2002).

Table 3: Effects of melatonin on cells derived from the human male reproductive tract

Cells	Effect	Citation*
Spermatozoa	↓ sperm motility	Irez et al. 1992
Prostate epithelial cells	antiproliferative	Gilad et al. 1996
Prostate cancer line LNCaP	antiproliferative	Lupowitz and Zisapel 1999; Xi et al. 2000
LNCaP prostate cancer cells	nuclear exclusion of androgen receptor	Rimler et al. 2001
Prostate cancer lines PC3 and DU145	antiproliferative	Marelli et al. 2000

* representative, not exhaustive

Perhaps the most impressive findings in this context of MEL and human prostate cancer are the *in vivo* clinical data reported by Shiu and colleagues (2003). In this study, specific high affinity MEL binding sites pharmacologically suggestive of the MT1 receptor were identified in excised prostate tumor tissue of an elderly man. More importantly, following castration the patient developed a biochemical relapse, which could be contained for several weeks by administration of 5 mg MEL daily (thereafter, his prostate-specific antigen titer increased much more slowly than expected). These results provide valuable new clinical evidence for an oncostatic action of MEL on human prostate cancer.

MEL and MEL-receptors: Therapeutic potential in reproductive medicine

After nearly 40 years of clinical research on the physiological role of MEL, there is still a sense that even more work has to be done to understand this hormone. Much like the tyrosine-derived thyroid hormones, the tryptophan-derived pineal hormone MEL appears to have actions on a tremendous number of biochemical and physiological processes, especially when blood levels are high, as in the rare MEL-secreting tumor or following exogenous administration (Arendt, 2003). This could be interpreted to mean that the healthy adult human body may either not have an absolute requirement for MEL, or that the effects of MEL are very subtle, perhaps indicative of primarily a synergist mode of action.

Obviously, negative changes in the expression of MEL receptors over the course of pre- and post-natal development, as has been described in animal models (cf. Johnston et al., 2003), are suggestive of functions that may be important at the early stages of life. In this context, if one can assume that MEL is somehow involved normally in the regulation of certain aspects of human development, then the numerous findings of MEL having antiproliferative effects on human tumors begins to make

some sense. Quite likely, many of the same MEL-sensitive pathways that are employed by developing tissues may turn out to the pathways recruited by many tumor cells. In as much as evidence is beginning to suggest some similarity between cell cycle and circadian processes (Fu and Lee, 2003), this may also help to explain how a hormone like MEL can act both as a feedback signal on SCN activities and at the same time have oncostatic properties.

With the development of novel, selective ligands for the MT1 and MT2 receptors (Audinot et al., 2003) as well as pharmacological approaches to selectively inhibit MEL synthesis in the pineal gland (Ferry et al., 2004), the field of MEL research is now approaching maturity. It has been a long, exciting and sometimes aggravating "adolescence", but it seems reasonable to believe that the coming years will spawn yet more fascinating insights into how MEL receptors can modulate the human reproductive tract, both physiologically and pharmacologically.

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