The pineal gland and reproduction

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Introduction

The importance of the pineal gland was perceived long ago: in the 16th century the gland was thought to be the seat of the soul! However, it is only in the past three decades that remarkable advances in the knowledge of the functional significance of the epiphysis have been made. Through its endocrine activity, via mechanisms and physiological correlations that are not yet entirely understood, the pineal regulates or takes part in the control of numerous functions of an organism. Investigations in both animals and humans have provided evidence that the gland plays an important role in the regulation of both the circadian and seasonal rhythms in a variety of species (Morgan and Williams, 1989; Namboodiri et al., 1991; Bartness et al., 1993; Reiter, 1993; Weaver et al., 1993; Masson Pevet and Gauer, 1994) and in the endocrine control of reproductive physiology (Kinson and Peat, 1971; Hoffman, 1973; Brzezinski and Wurtman, 1988; Matthews et al., 1993). Furthermore, epiphysis activity seems to be important in modulating the activity of the immune system (Maestroni, 1993). In addition, several studies have investigated a possible role of the gland in other physiological processes, such as the regulation of body temperature (Saarela and Reiter, 1994) and weight (Lerchl and Schlatt, 1993), the inhibition of tumoral growth (Bartsch et al., 1991; Maestroni, 1993) and even an influence on life span (Oaknin Bendahan et al., 1995).

Nevertheless, many of the observed physiological correlations are still incompletely understood, and there remains much to investigate about the activities and the physiological role of this endocrine organ, especially in humans. For this reason, the epiphysis is attracting the interest of researchers. As regards reproduction, the gland has been demonstrated to exert an important regulation on seasonal reproductive changes in seasonally breeding animals. In humans, who are not seasonal breeders, the pineal appears to be involved in the neuroendocrine regulation of puberty and reproductive physiology; however, many aspects of the role of the pineal in human reproduction

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remain obscure. The aim of this study is to update current knowledge about the role of the pineal in reproductive physiology.

**Physiology of the pineal body**

The pineal body is the only endocrine gland directly influenced by the external environment via the retina; in fact, the gland converts environmental signals into neuroendocrine messages (Reiter, 1983; Binkley, 1993; Pevet, 1993). The information is transmitted from the retinal photoreceptors to the suprachiasmatic nuclei, then to the paraventricular nuclei and, through the intermediolateral cell column, to the superior cervical ganglia. Noradrenergic fibres originating from superior cervical ganglia have their terminals in the pineal body (Reiter, 1983; Klein, 1985; Binkley, 1993) (Figure 1).

These fibres stimulate either β- or α-adrenergic receptors of the pinealocyte (Arendt et al., 1985; Klein, 1985; Vanecek et al., 1985). The activation of these receptors synergistically increases intracellular cAMP and cGMP: in fact, α1-adrenoceptor activation has proved to significantly potentiate β-adrenergic stimulation of both cAMP and cGMP. The increase in intracellular cAMP enhances N-acetyltransferase (NAT) activity (Klein, 1985; Sugden et al., 1985; Vanecek et al., 1985). Thus serotonin, which is produced in two steps (hydroxylation and decarboxylation) from tryptophan within the pinealocyte (Axelrod, 1974), is converted to N-acetylserotonin by NAT (Klein, 1979). N-Acetylserotonin is finally converted into the hormone melatonin (5-methoxy-N-acetyltryptamine) by the pineal-specific enzyme hydroxyindole-O-methyltransferase. Thus, NAT activity is under the control of the retino-pineal pathway and represents the rate-limiting factor in the synthesis of melatonin (Klein, 1979; Namboodiri et al., 1991) (Figure 2). Interestingly, a large nocturnal increase in NAT activity has been demonstrated (Sugden et al., 1985). Such an increase is mediated by noradrenaline released from sympathetic nerve terminals in the pineal, and it causes a notable increase in melatonin synthesis during the night.

Melatonin is the major, or at least the most studied, secretion product with which pineal physiological properties appear to correlate, but recent studies have also ascribed importance for pineal function to other indoles (Pevet, 1983). Melatonin is secreted into the blood with an endogenous and individual rhythm synchronized by the dark–light cycle: the plasma concentrations of the hormone reach a peak during the night-time, at least in the absence of light, and the persistence of high concentrations of melatonin is proportional to the duration of darkness (Goldman, 1991; Reiter, 1991, 1993; Pevet, 1993). Exposure to light rapidly inhibits melatonin synthesis by the epiphysis and its secretion into the blood. Thus, because of changes in the duration of night and day, the rhythm of melatonin release is also influenced by the cycle of the seasons. Indeed, the pineal gland, through its hormonal secretion, informs the whole organism about the current phase both of the day and of the year (Reiter, 1991).

Melatonin secretion may, however, be influenced by additional factors. Animal studies suggest that temperature (Zatz et al., 1994) and social cues may affect melatonin
secretion and seasonal rhythms (Wayne et al., 1989). Moreover, since the precursor tryptophan is supplied to the gland by the circulating blood, dietary intake of tryptophan may influence melatonin concentrations (Heuther et al., 1992; Kennedy, 1994). Dopaminergic, serotonergic, γ-aminobutyrate and benzodiazepine receptors have also been detected in the pineal (Ebadi and Govitrapong, 1986), suggesting a possible role for these neurotransmitters in the control of melatonin secretion. Experimental evidence suggests that melatonin synthesis is in part under serotonergic control (Den Boer and Westenberg, 1990) and may be suppressed by benzodiazepines (McIntyre et al., 1993). In addition, it has been suggested that in the rat melatonin controls its own rhythmic production by directly entraining a circadian pacemaker modulating the NAT activity rhythm (Humlova and Illnerova, 1990).

Role of the pineal in reproduction

The pineal in seasonal and non-seasonal breeders

The reproductive function shows both qualitative and quantitative differences, related to the seasonal cycle (especially winter and summer), in both females and males. This feature is especially evident in animals that are seasonal breeders, in which variations in the reproductive cycle reveal they are timed by changes in the photoperiod (Tamarkin et al., 1985). The pineal, through its endocrine activity synchronized by the dark–light cycle, regulates seasonal changes in the reproductive function of these animal species (Reiter, 1991, 1993; Weaver et al., 1993). In fact, changes in the duration of night and day that are associated with the cycle of the seasons result in a different secretory profile of melatonin: the nocturnal release of the hormone is longer in duration in winter than in summer.

Long-day breeders, such as the hamster, are reproducively active during the summer, when the nights are shorter; in these species the reproductive function decreases to a minimum in winter months, when the nights are longer (Silman, 1993). By exposing hamsters to short photoperiods, inhibition of the reproductive system is obtained until there is testicular involution in males and anoestrus in females (Hoffman, 1973; Lerchl and Nieschlag, 1992). The gonadal regression is followed by a return to normal function, suggesting the development of insensitivity of the reproductive axis to regressive photoperiods (Lerchl and Nieschlag, 1992; Lerchl and Schlatt, 1993). In addition, gonadal development in pubertal hamsters is arrested by exposure to short photoperiods (Buchanan and Yellon, 1991). Pinealectomy, however, prevents gonadal regression in hamsters exposed to a short photoperiod (Hoffman, 1979). With melatonin it is possible to mimic all of these effects of photoperiod on the reproductive function (Duncan et al., 1990; Buchanan and Yellon, 1991; Goldman, 1991; Badura and Goldman, 1992; Pevet, 1993). Thus, in the hamster, melatonin plays an inhibitory role in reproduction. There is evidence to suggest that this antigonadal action is exerted by inhibition of gonadotrophin-releasing hormone (GnRH) (Buchanan and Yellon, 1991) (Table I).

In contrast, in short-day breeders such as the sheep reproductive activity is associated with a decrease in the length of the day, the breeding season being autumn and winter. Melatonin exerts a stimulatory effect on the reproductive axis in this species (Karsch et al., 1984). Studies in pinealectomized ewes treated with long-term melatonin infusion have reported that short-day melatonin patterns produce an increase in the pulse frequency of luteinizing hormone (LH) secretion (Bittman et al., 1985). Recently, contemporaneous stimulation of GnRH and LH pulsatile secretion by short-day-like melatonin administration has been demonstrated (Table I), whereas LH release and reproductive activity are inhibited by a long-day pattern of melatonin (Vigie et al., 1995). It appears that melatonin mediates the influence of photoperiod on LH pulsatile secretion by driving the responsiveness to oestadiol negative feedback (Bittman et al., 1985). After pinealectomy, ewes are unable to synchronize their reproductive responses to seasonal changes in the duration of night and day (Wayne et al., 1990; Woodfill et al., 1991, 1994).

The rat is a non-seasonal breeder, but in this species too, reproductive function is influenced by photoperiod, melatonin administration or pinealectomy. Light exposure and pinealectomy are associated with an enhancement in gonadal function (Kinson and Peat, 1971), while melatonin administration is capable of delaying the onset of puberty, blocking the pubertal increase in gonadotrophins and inhibiting nalofoxine-induced LH pulsatility (Aubert et al., 1988). The onset of puberty is also sensitive to variations in photoperiod (Ramaley and Bunn, 1972). Furthermore, both the oestrous cycle and ovulation are reported to be influenced by the pineal. Melatonin administration during the pro-oestrous prevents the LH surge and ovulation (Ying and Greep, 1973). There is also evidence that the pineal has a role in the timing of the pro-oestrous LH surge in the rat (Chiba et al., 1994). In-vitro studies suggest that melatonin is capable of influencing hypothalamic GnRH secretion (Rasmussen, 1993) and luteinizing hormone-releasing hormone (LHRH)-induced pituitary LH release in this species (Vanecek and Klein, 1992; Hattori et al., 1995) (Table I).
Table I. Melatonin and reproduction of seasonal breeders, non-seasonal breeders and humans

<table>
<thead>
<tr>
<th>Species</th>
<th>Breeding season</th>
<th>Influence of melatonin on HPO axis</th>
<th>Effects of long-term melatonin administration</th>
<th>Mechanism of action of melatonin</th>
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<tbody>
<tr>
<td>Hamster (long day breeder)</td>
<td>spring–summer</td>
<td>inhibitory</td>
<td>delayed puberty, gonadal involution</td>
<td>GnRH inhibition</td>
</tr>
<tr>
<td>Sheep (short day breeder)</td>
<td>autumn–winter</td>
<td>stimulatory</td>
<td>enhanced reproductive activity, increased pulsatile LHRH and LH</td>
<td>GnRH stimulation</td>
</tr>
<tr>
<td>Rat (non-seasonal breeder)</td>
<td>—</td>
<td>inhibitory</td>
<td>delayed puberty, absence of the pro-oestrous LH surge</td>
<td>GnRH inhibition</td>
</tr>
<tr>
<td>Human</td>
<td>—</td>
<td>inhibitory</td>
<td>decreased plasma LH (with absence of the mid-cycle surge), oestradiol &amp; progesterone, anovulation</td>
<td>GnRH inhibition, GnRH-induced LH release inhibition, direct action on gonads</td>
</tr>
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GnRH = gonadotrophin-releasing hormone; LHRH = luteinizing hormone-releasing hormone; LH = luteinizing hormone; HPO = hypothalamic–pituitary–ovarian.

Central binding sites for melatonin-mediated control of reproduction

Experiments utilizing microimplants of melatonin and techniques involving destruction of specific brain areas show that the target sites, which subserve the melatonin control of seasonal responses, are the suprachiasmatic nucleus in the Siberian hamster, the anterior hypothalamus in the Syrian hamster, and the medio-basal hypothalamus in the sheep (Bartness et al., 1993; Malpaux et al., 1993). Autoradiographic techniques have localized the receptors that mediate this role of melatonin to the pars tuberalis in the rat and the Rhesus monkey, which are not seasonal breeders (Nakazawa et al., 1991; Weaver et al., 1993), and in many mammals (Gauer et al., 1994). Such receptors are characterized by high affinity and highly species-specific distribution (Reiter, 1993; Weaver et al., 1993). Furthermore, receptor density in the pars tuberalis of different species of animals exhibits seasonal variations (Masson Pevet and Gauer, 1994). Autoradiographic techniques (using \(^{125}\)I-labelled melatonin) have also detected the presence of these receptors in the pars distalis in humans, but with an inconsistent distribution, and their relative absence in the pars tuberalis; such data suggest that there are different neuroendocrine mechanisms of response to melatonin in humans. Conversely, autoradiography has also localized the receptors which mediate melatonin influence on circadian rhythms to the suprachiasmatic nuclei (Weaver et al., 1993). Data regarding central melatonin binding sites are summarized in Table II.

The pineal in humans

The seasonality of reproduction represents the mechanism by which nature ensures the occurrence of births at a time of the year suitable for offspring survival, in relation to appropriate environmental conditions and food supply. Humans are not seasonal breeders: sexual activity is not related to season and not necessarily connected with reproduction. Importance is ascribed to food supply, artificial light, heating and social life in reducing seasonal and circadian influence on human reproduction. Furthermore, especially in western countries, various confounding factors must be taken into account: contraception, family planning, diet, stress (Rojansky et al., 1992). In seasonally breeding species there is a relative uniformity of the melatonin release pattern among individuals. In the adult human, plasma concentrations of the hormone are on average higher during the night than in the day; yet considerable inter-individual differences have been reported not only in nocturnal serum melatonin concentrations, but in some cases also in the secretion rhythm (Lerchl and Partsch, 1994). This may represent the expression of evolutionary changes, or be due to exposure to artificial light (Silman, 1993) or to the influence of the other above-mentioned factors.

Table II. Central binding sites for melatonin-mediated control of reproduction

<table>
<thead>
<tr>
<th>Species</th>
<th>Central target sites</th>
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<tbody>
<tr>
<td>Siberian hamster</td>
<td>suprachiasmatic nucleus(^a)</td>
</tr>
<tr>
<td>Syrian hamster</td>
<td>anterior hypothalamus(^a)</td>
</tr>
<tr>
<td>Sheep</td>
<td>medio-basal hypothalamus(^b)</td>
</tr>
<tr>
<td>Rat</td>
<td>pars tuberis(^c)</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>pars tuberis(^c)</td>
</tr>
<tr>
<td>Human</td>
<td>suprachiasmatic nucleus(^c)</td>
</tr>
<tr>
<td>Human</td>
<td>pars distalis(^c)</td>
</tr>
</tbody>
</table>

\(^a\)Assessed by brain lesion techniques.
\(^b\)Assessed by intracranial application of microimplants of melatonin.
\(^c\)Assessed by autoradiographic techniques (\(^{125}\)I-labelled melatonin).
**Seasonal trends in human reproduction**

Although humans are not seasonal breeders, seasonal trends in their reproductive function have been described. In a recent study, seasonal variations in human conception and birth rates in different geographical areas were analysed (Rojansky et al., 1992). In particular, in northern countries, the conception rate has been reported to be higher in summer than in winter and, as a consequence, the birth rate reaches a maximum in the spring season (Timonen et al., 1965; Puolakka et al., 1985). In contrast, in hot climate areas of the world, a peak in the conception rate during winter (Mathers and Harris, 1983) and a decrease in the birth rate during spring (Levine et al., 1990) have been documented. Moreover, the season in which there are peaks in the birth rate has been found to be related to latitude (Batschelet et al., 1973).

In hot climates, where there is a minimal seasonal variation in photoperiod, temperature has been especially implicated in producing seasonal changes in conception rates. In northern countries, where the seasonal contrast in luminosity is considerable, the photoperiod is more likely to affect human reproductive axis activity (Rojansky et al., 1992). During the dark season, reduced activity of the anterior pituitary–ovarian axis (Kauppila et al., 1987b) and an increase in serum melatonin have been documented by population studies in such countries (Kauppila et al., 1987a). In addition, the season is reported to affect plasma concentrations of melatonin and LH significantly. Nocturnal plasma melatonin concentrations on day 10 of the menstrual cycle have been found to be higher in winter than in summer. Conversely, nocturnal plasma LH levels are higher in summer than in winter (Kivelä et al., 1988). All of these data are consistent with the above findings regarding conception and birth rates in northern countries. With the exclusion of a correlation with temperature in these countries, it has been suggested that seasonal changes in daylight, through melatonin secretion, may affect human reproductive function (Kauppila et al., 1987a).

The conception rate may also be critically influenced by nutritional status, diet, stress and weight loss. Various factors have been implicated in seasonal fluctuations in the conception rate: frequency of intercourse, ovulation rate, quality of ovum and zygote, endometrial receptivity and sperm quality (Rojanski et al., 1992).

The importance of some of these factors is also suggested by in-vitro fertilization results, which have also been found to exhibit seasonality. In particular, seasonal changes have been documented not only in the fertilization and pregnancy rates, but also in the quality of the embryo and the number of oocytes retrieved. Furthermore, such seasonal differences have still been observed after adjusting for number of oocytes retrieved. This may be related to the quality of the oocyte or the spermatozoon, or to endometrial receptivity (Stolwijk et al., 1994).

Seasonal effects on oocyte quality (Jongbloet, 1975) and endometrial receptivity (Timonen et al., 1964) have been described. As regards sperm quality, an investigation carried out in Texas on 131 men reported that sperm concentration, count and motility were significantly lower in summer. Furthermore, in the offspring of these men, a significantly low birth rate was found to occur in spring (Levine et al., 1990). Since temperature critically affects spermatogenesis, changes in scrotal temperature have been proposed as the major factor related to seasonal sperm quality: the higher the temperature the lower the sperm quality (Levine et al., 1990; Levine, 1991). It must be remembered that melatonin reportedly plays an important role in the thermoregulatory processes of an organism (Saarela and Reiter, 1994), although no correlation with the above data has been found so far. As regards the contribution of ovulation rate to seasonal conception rate, light appears to have an influence. The sensitivity of ovulation to light is suggested by the documented decrease in anterior pituitary–ovarian function and increase in serum melatonin during the dark season in northern countries (Kauppila et al., 1987b), where conception and multiple pregnancy rates are higher in summer than in winter (Puolakka et al., 1985).

Thus, available data seem to indicate that melatonin partially mediates seasonal fluctuations in the human reproductive function. This is also suggested by evidence from some pathologies influenced by the season, such as depression and seasonal affective disorders, which are often associated with menstrual and ovulation irregularities. Winter depression is reported to be linked to abnormally delayed circadian rhythms; the onset of melatonin secretion has been observed to occur later in these patients. Phototherapy has proven to be effective for the treatment of winter depression, by inducing the earlier onset of melatonin production with a phase advance of circadian rhythms (Sack et al., 1990). Sensitivity to phototherapy has also been described in seasonal affective disorders, which must be regarded as a clinical category characterized by the triad hypersomnia, hyperoxia and weight gain and, usually, association with symptoms of depression. Interestingly, these disorders appear to be influenced by latitude (Attar Levy et al., 1990). Circadian abnormalities may also be involved in the pathogenesis of pre-menstrual depression. Phototherapy benefits patients suffering from this syndrome, in which an altered secretory profile of melatonin has been observed (Parry et al., 1990).
The pineal and puberty

Experimental evidence indicates that the pineal plays an important role in the neuroendocrine control of puberty in animals (Ramaley and Bunn, 1972; Aubert et al., 1988; Buchanan and Yellon, 1991). Melatonin also appears to be involved in the endocrine modulation of human sexual maturation. In humans, serum melatonin concentrations are reported to decrease progressively with advancing age, and reach values $<450$ pmol/l ($≈100$ pg/ml) at 10 years of age (Attanasio et al., 1985; Waldhauser et al., 1988; Cavallo, 1992). The hypothalamic–pituitary–gonadal axis, which is very active during fetal life and the first year of life, remains quiescent until approximately the age of 10 years. After this, there is a reactivation of the hypothalamic–pituitary axis. A progressive increase occurs in the amplitude and frequency of GnRH pulses; consequently, the pulsatile secretion of LH and follicle stimulating hormone (FSH) increases similarly, with the subsequent onset of pubertal phenomena (Sizonenko, 1989).

It has been reported in animals that, through its nocturnal secretion of melatonin, the pineal exerts an inhibitory role on the hypothalamus and on pubertal maturation (Sizonenko et al., 1985; Buchanan and Yellon, 1991). Thus, it is likely that melatonin also exerts a similar inhibitory effect on GnRH hypothalamic secretion in humans. It has been postulated that, before puberty, even if they progressively decrease, melatonin concentrations are too elevated to allow hypothalamic activation; however, at $≈9$ or 10 years of age the decline of serum melatonin below a threshold value ($≈500$ pmol/l $≈115$ pg/ml) represents the activating signal for the hypothalamic pulsatile secretion of GnRH and thereafter the onset of pubertal changes (Silman, 1991).

Since the production rate of melatonin does not change with age, it has been suggested that the decreasing concentrations of melatonin seen with advancing age are due to the increase in body mass (Waldhauser et al., 1988; Young et al., 1988). However, according to another hypothesis, the decrease with age of nocturnal serum melatonin is not fully explained by an increase in body mass, since even independently of weight the onset of sexual maturation is significantly related to melatonin concentrations (Waldhauser et al., 1991). Neither can the decrease in melatonin be explained by increased concentrations of gonadotrophin and sex steroids at puberty, since it has been proved that GnRH-agonist therapy does not alter plasma melatonin (Berga et al., 1989; Waldhauser et al., 1991). These data suggest that the reduction in nocturnal serum melatonin is not connected simply with an increase in body mass, but is also temporally related to sexual maturation (Waldhauser et al., 1991).

Conversely, some researchers, on the basis of experiments using female Rhesus monkeys, postulate that elevated nocturnal melatonin concentrations do not play a role in the control of either the onset of puberty or ovulatory function in adults (Wilson et al., 1992). In contrast to this hypothesis, evidence from human pathological conditions also suggests the suppressive role of high serum melatonin on pubertal development, pulsatile secretion of GnRH and ovarian function (Cavallo, 1993). Children with precocious puberty have been found to have lower nocturnal serum concentrations of melatonin than age-matched prepubertal children (Waldhauser et al., 1991), whereas children with delayed puberty present higher nocturnal melatonin concentrations than age-matched normal children (Cohen et al., 1982). In pathologies characterized by inactivity of the GnRH pulse generator, such as hypothalamic amenorrhoea or anorexia nervosa, the amplitude of nocturnal melatonin has been reported to be significantly higher than in normal controls, with values similar to the prepubertal ones (Berga et al., 1988; Brzezinski et al., 1988; Tortosa et al., 1989).

Interestingly, research on rats suggests a possible influence of the maternal pineal during gestation on the gonadal and genital development and function of offspring (Jarrige and Boucher, 1992). However, investigations are needed to evaluate this hypothesis in humans.

Melatonin and the hypothalamic–pituitary–ovarian axis

Data from human puberty lend support to the hypothesis that the pineal exerts an inhibitory function on the reproductive axis. The pineal suppressive role is also indicated by melatonin administration tests. It has been reported that long-term daily administration of melatonin, alone (300 mg) or associated with norethisterone (0.75 mg), induces in the fourth month of medication a significant decrease in LH (with absence of the mid-cycle peak), oestradiol and progesterone plasma concentrations. Furthermore, a combination of 300 mg of melatonin with 0.15 mg of norethisterone or 75 mg of melatonin with 0.3 mg of norethisterone, administered for 21 days, has also been reported to inhibit ovulation, suggesting an additive or synergic effect of the two hormones and the possibility of a new contraceptive (Voordouw et al., 1992).

However, only scant data are available in humans, especially with regard to where and how the pineal suppressive role is exerted. There is evidence that the pineal is involved in the control of the pulsatile secretion of LH (Brzezinski et al., 1987). A negative relationship between nocturnal serum melatonin and LH concentrations has been documented at different pubertal stages (Waldhauser et al., 1984). Interestingly, the influence of the season on
the ovulatory LH surge has been described (Testart et al., 1982) and a role for melatonin in the timing of the LH surge has also been suggested (Brzezinski et al., 1987). As yet, however, it has not been clearly demonstrated in humans whether melatonin exerts these effects by acting at the hypothalamic level or directly at the pituitary level.

There is evidence to suggest that the inhibitory role of melatonin is exerted at the hypothalamic level, influencing the pulsatile secretion of GnRH. In patients with functional hypothalamic amenorrhea, whose main hormonal feature is decreased GnRH/LH pulsatility, a significant increase in the nocturnal peak amplitude and duration of melatonin has been documented (Berga et al., 1988; Brzezinski et al., 1988). In amenorrheic athletes, who have alterations of the hypothalamic–pituitary–ovarian axis similar to those observed in hypothalamic functional amenorrhea, melatonin secretion presents a higher nocturnal peak and a 2 hour delay of peak offset (Laughlin et al., 1991). In patients with anorexia nervosa, also characterized by suppressed hypothalamic pulsatile release of GnRH, enhanced diurnal and nocturnal mean plasma melatonin values with anticipation of nocturnal melatonin increase have been reported (Tortosa et al., 1989). Since long-term administration of melatonin is also capable of inhibiting ovulation (Voordouw et al., 1992), the nocturnal amplified melatonin concentration in these conditions does not appear as an epiphenomenon, although the mechanism of pathological increase in melatonin remains to be elucidated.

Studies have been carried out to investigate the mechanism by which melatonin can inhibit the hypothalamic secretion of GnRH. Melatonin has been proposed to act by directly suppressing the hypothalamic pulsatile secretion of GnRH (Bittman et al., 1985). Conversely, it has been suggested that this inhibition is mediated by a change in dopaminergic and opioidergic activity (Rasmussen, 1993). However, the mechanism by which melatonin inhibits GnRH pulsatile release is not yet clear.

In rats, melatonin has been observed to inhibit the pituitary response to LHRH (Martin et al., 1980; Vaneeck and Klein, 1992; Hattori et al., 1995). This has not been confirmed in humans (Weinberg et al., 1980; Brzezinski and Wurtman, 1988). In addition, an inhibin-like anti-gonadotrophic activity associated with the protein–peptide extracts of ovine pineal has been identified (Bhagat and Duraiswami, 1992).

The influence of the pineal on reproductive function, however, is not limited to the hypothalamic–pituitary level. There is evidence for the existence of melatonin receptors also in the testis and ovary of certain animals and of humans (Cohen et al., 1978; Ayre and Pang, 1994). In addition, the presence of melatonin has been demonstrated in human pre-ovulatory follicular fluid at a concentration significantly higher than in peripheral serum and with both circadian and circannual variations (Ronnberg et al., 1990). All of these data indicate the possibility of a direct action of melatonin on the gonads. Furthermore, melatonin has been reported to stimulate progesterone synthesis in vitro by human corpus luteum cells (MacPhee et al., 1974) and by granulosa cells luteinized in vitro (Webley and Luck, 1985). Melatonin also enhances the stimulatory effect of human chorionic gonadotrophin on progesterone production by human granulosa lutein cells (Brzezinsky et al., 1992). These studies therefore suggest that melatonin may play a role in the modulation of luteal function and that an abnormal melatonin concentration might result in altered ovarian function. Data regarding melatonin influence on the human hypothalamic–pituitary–ovarian axis are summarized in Table I.

In rats, melatonin has been found to increase serum prolactin concentrations (Moreno et al., 1992). In women of reproductive age a facilitatory role of melatonin in thyrotrophin-releasing hormone-induced prolactin secretion has been reported: this should be a sex-specific effect (Terzolo et al., 1991). Conversely, no facilitatory function has been observed in the response of FSH, LH or thyroid-stimulating hormone to the respective releasing hormones or of cortisol to adrenocorticotropic hormone (Terzolo et al., 1991).

**Melatonin and the menstrual cycle**

Although considerable inter-individual differences in plasma melatonin concentrations in humans have been reported (Lerchl and Partsch, 1994), intra-individual variations seem to be small (Arendt, 1979). A daily rhythm in serum melatonin concentrations can be observed in normal women: diurnal melatonin concentrations are low (with reported levels ranging from undetectable values to 25 pg/ml = 108 pmol/l); a nocturnal rise starts at ∼9 p.m. and reaches a peak at ∼3 a.m. (reported mean levels: 83 pg/ml = 358 pmol/l); a rapid decrease takes place between 5 a.m. and 7 a.m. (Brzezinski et al., 1988).

With regard to an association between melatonin concentrations and the menstrual cycle, conflicting results emerge from the literature. A few studies have reported an increase in serum melatonin during the luteal phase, preceded by a mid-cycle hormonal decrease, and thus a role for melatonin in the modulation of the menstrual cycle has been suggested (Hariharasubramanian et al., 1984, 1986). To the contrary, recent investigations have excluded a correlation between serum melatonin concentrations and different phases of the menstrual cycle (Brzezinsky et al., 1988; Berga and Yen, 1990). In particular, plasma melatonin concentrations measured in four different phases of
the menstrual cycle (assessed by obtaining daily samples for measurement of serum FSH, LH, oestradiol and progesterone) exhibited no consistent variation in circadian pattern related to a specific menstrual phase. Therefore, serum melatonin does not appear to be affected by fluctuations in sex steroids during the menstrual cycle, and melatonin secretion would not seem to be involved in the modulation of menstrual cyclicity (Berga and Yen, 1990). This hypothesis would appear to be confirmed by the finding that the plasma melatonin concentrations do not seem to be influenced by changes in sex hormone concentrations related to pregnancy, ovulation induction with gonadotrophins and contraceptive use (Delfs et al., 1994). However, according to another hypothesis, the lack of chronological correlation between plasma ovarian hormones and melatonin concentrations does not mean that melatonin has no role in modulation of the menstrual cycle (Brzezinski et al., 1988). In fact, a chronological correlation between the onset of the LH pre-ovulatory surge and the early morning decrease in serum melatonin concentration has been reported, suggesting a role for melatonin in the timing of the mid-cycle LH surge (Brzezinski et al., 1987).

Evidence supporting melatonin regulation of the menstrual function also emerges from pathology. Interestingly, in women with amenorrhoea of hypothalamic origin, not only a considerable increase in the nocturnal peak amplitude but also prolonged melatonin secretion during the daytime have been reported (Berga et al., 1988; Brzezinski et al., 1988; Tortosa et al., 1989; Laughlin et al., 1991). This suggests an alteration in the rhythm of melatonin secretion. Importantly, the duration of melatonin secretion has been found to represent a critical component of the melatonin signal which elicits cyclic reproductive responses in the hamster and sheep (Bartness et al., 1993). In addition, menstrual cycle disorders are often associated with depression, which appears to be related to chronobiological abnormalities and to be sensitive to phototherapy (Sack et al., 1990). All of these data suggest that melatonin may control menstrual cyclicity, and that this control is exerted not only by its concentration but also by its secretion rhythm. Moreover, these findings lend support to the hypothesis that alterations in the circadian system may be involved in the pathogenesis of menstrual irregularities. This is also consistent with the finding of melatonin receptors in the suprachiasmatic nuclei (Weaver et al., 1993). Significantly, in women with a history of menstrual disorders and anovulation, regularization of the cycle length has been obtained by exposure to continuous low nocturnal light for more than one cycle (Dewan et al., 1978). Hence, due to the ability to phase-shift the circadian system (Humlova and Illnerova, 1990; Deacon et al., 1994; Dawson et al., 1995), the use of phototherapy or melatonin in the therapeutic approach to non-organic menstrual irregularities deserves to be more extensively tested.

**Conclusion**

The pineal gland appears to play an important role in the neuroendocrine control of reproductive physiology. This is well documented in seasonally breeding animals, in which reproductive activity is clearly influenced by the seasonal changes in the duration of day and night. Humans are not seasonal breeders and, especially in western societies deeply conditioned by progress, environmental impact is certainly less important than in animals. Nevertheless, seasonal trends have been demonstrated in human reproduction. In humans the pineal gland also appears to play an important role in the neuroendocrine control of sexual maturation and of reproductive function, although many aspects remain to be elucidated. The pineal seems to exert its hormonal effect at different levels of the reproductive axis: at the hypothalamic–pituitary level and directly at the gonadal level. Furthermore, melatonin appears to increase prolactin secretion. Although not all researchers agree, it has been suggested that melatonin may be involved in the modulation of menstrual cyclicity.

**References**


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