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Chronobiology in endocrinology

Sergio SENSI, Valeria PACE PALITTI and Maria Teresa GUAGNANO

Istituto di Medicina Interna, Cattedra di Clinica Medica, Università degli Studi "G. D'Annunzio", Chieti, Italy

Summary. - *Temporal endocrine structure (TES).* It can be defined as a combination of predictable hormonal changes that are time-related. Regarding their frequency, endocrine rhythms may be *circadian, ultradian* and *infradian.* In this context, the *endocrine circadian time structure* (ECTS), that is closely dependant of some areas of the hypothalamus, is of particular interest. *Long* and *short* loop feedback link together the various components: central nervous system (CNS), hypothalamus and anterior pituitary with target glands and tissues. The hypothalamic neuropeptides (releasing hormones or factors - RH or RF - or inhibiting hormones or factors IH or IF) presently known are: thyrotropin releasing hormone (TRH); luteinizing releasing hormone (LH RH); prolactin releasing factor (PRF); Prolactin Inhibiting Factor (PIF); Corticotropin Releasing Factor (CRF); Growth Releasing hormone (GH RH). Some general remarks on endocrine rhythms should be noted: the circadian changes in hormones may depend on each other; even an apparently subordinate rhythm should be considered a *true independent rhythm*; accurate studies have shown that hormonal secretion occurs in all cases according to a rhythmic organization at many levels; these rhythms may not be evident at a first analysis. The *hormone secretion* is basically pulsating which makes it difficult to draw standard reference values. Although an ECTS is present at the cell level, in organs etc., it is evident that a rhythm hierarchy exists. *Hormonal secretion and sleep-wake cycle.* Although several reports state that no rhythm is totally dependent on the sleep-wake cycle, from a general point of view the hormone secretion rhythms can be divided in: sleep-dependent rhythms and sleep-independent rhythms. *Meal-timing and hormonal secretion.* In animals, meal-timing is a powerful synchronizer; however, there are no definitive and conclusive data to prove that meal-timing is a true synchronizer also in humans, although there have been some reports suggesting it. *Endocrine rhythms.* Data regarding the endocrine rhythms (circadian-ultradian-infradian) of the numerous hormones as GH; prolactin; aspects of temporal pattern of CRF-ACTH-corticosteroid and of hypothalamic - pituitary - thyroids axis; hypothalamic - pituitary - ovaric steroid and testosterone axis are reported. The study of a possible rhythmic pattern of insulin has been approached from many points of view as the basal rhythmicity of insulin; the diurnal variation of efficacy of injected insulin and of insulin responsiveness to insulinogenic stimuli.

Key words: biological rhythms, chronobiology, chronoendocrinology, hormone rhythms.

Riassunto *(La cronobiologia in endocrinologia).* - *Struttura temporale endocrina (STE).* Con il termine di STE si definisce la combinazione delle oscillazioni temporali ormonali ritmiche e quindi prevedibili. I ritmi possono essere, riguardo alla loro frequenza, circadiani, ultradiani, infradiani. In questo contesto si è data particolare importanza alla struttura temporale endocrina circadiana (STEC) che è strettamente collegata ad alcune aree dell'ipotalamo. Feed-back a lunga e breve catena collegano insieme i vari componenti: sistema nervoso centrale; ipotalamo; ipofisi con le ghiandole bersaglio e i tessuti. I neuropeptidi ipotalamici (releasing hormones o factors - RH, RF - or inhibiting hormones o Factors - IH, IF) attualmente conosciuti sono: thyrotropin releasing hormone (TRH); luteinizing releasing hormone (LH-RH); prolactin releasing factor (PRF); prolactin inhibiting factor (PIF); corticotropin releasing factor (CRF); growth releasing hormone (GH-RH). Vengono sottolineate alcune caratteristiche dei ritmi endocrini: i ritmi endocrini possono dipendere l'uno dall'altro; tuttavia anche ritmi apparentemente subordinati hanno una propria ritmicità autonoma; anche se la secrezione ormonale avviene secondo un'organizzazione ritmica a vari livelli: circadiani, ultradiani, infradiani, fondamentalmente è pulsatile; quest'ultimo carattere rende difficoltosa l'elaborazione dei valori standard di riferimento; la STEC è presente a qualsiasi livello (cellulare, organi, tessuti, etc.), tuttavia è evidente l'esistenza di una gerarchia di ritmi. *Secrezione ormonale e ritmo sonno-veglia.* Nessun ritmo circadiano ormonale è totalmente dipendente dal ritmo sonno-veglia; da un punto di vista generale i ritmi circadiani possono essere divisi in sonno-dipendenti e sonno-indipendenti. *Meal-timing (MT) e secrezione ormonale.* Nell'animale il meal timing è un potente sincronizzatore; non vi sono prove sicure che l'MT sia un vero sincronizzatore anche nell'uomo sebbene alcune ricerche suggeriscano questo comportamento. *Ritmi endocrini.* Vengono riportati i dati riguardanti i ritmi endocrini (circadiani, ultradiani, infradiani) quali GH; prolattina; aspetti del comportamento ritmico del CRF-ACTH-corticosteroidi, dell'asse ipotalamico-ipofisario-tiroideo; dell'asse ipotalamico-ipofisario-gonadico (steroidi ovarici e testosterone). Per quanto riguarda il comportamento ritmico dell'insulina, questo è stato studiato da molti punti di vista quali la ritmicità basale dell'insulina; le variazioni diurne dell'efficacia dell'insulina iniettata e della responsività insulinica a stimoli insulinogenici.

Parole chiave: cronobiologia, cronoendocrinologia, ritmi biologici, ritmi ormonali.

Outlines of neuroendocrinology

Temporal endocrine structure (TES)

It can be defined as a combination of predictable hormonal changes that are time-related. The definition covers a range of rhythmic frequencies and at any level: organs and tissues, cells and subcellular structures. Regarding their frequency, endocrine rhythms may be *circadian, ultradian* and *infradian*. In this context we will pay particular attention to the endocrine circadian time structure (ECTS).

The ECTS is closely dependent on some areas of the hypothalamus. In the report "Neural Control of Pituitary Gland" in 1955, Harris stated that all the blood reaching the anterior hypophysis via the portal system had initially been in contact with the eminence of the hypothalamus by means of a primary capillary plexus [1]. Later studies defined the morphofunctional characteristics of the monoaminergic system and reported the capacity of the hypothalamus to produce releasing hormones or factors (RH) and inhibiting hormones or factors (IH) [2-5].

The basis of neuroendocrinology were established, concentrating particularly on the study of the neural control of hormonal secretion and also dealing with the interactions between the monoaminergic system and the behaviour of the subject (psychoneuroendocrinology) and, on a wider scale, the relationship between the rhythmic endocrine activity of the body and neural activity (chrononeuroendocrinology) [6-10].

The hypothalamic neuropeptides (RH, IH) known so far at the hypothalamic level are shown in Fig. 1. *Long* and *short* loop feedback link together the various components: central nervous system, hypothalamus, anterior pituitary and target glands and tissues. "Both these feedback systems are essentially closed-loop systems, which allow self-regulation and avoid overshoot in the production of any secretory product. Such systems also respond to exteroceptive and interoceptive stimuli, though they are apparently not involved in the function of the hypothetical "clock(s)" which regulates the circadian periodicity of many pituitary and releasing hormone concentrations. Such periodicity persists in the absence of target organ gland secretions and exhibits phase characteristics similar to those seen in intact subjects, but at higher hormonal levels, which reflect the absence of target organ feedback processes" [11].

Thyrotropin releasing hormone (TRH). - This was the first RH to be isolated; its activity is not completely clear but it is thought to stimulate the release of prolactin (PRL) and thyrotropin (TSH). TRH, T4 and T3 in turn act directly on the anterior pituitary gland to control the secretion of TSH.

Luteinizing releasing hormone (LH-RH). - Both natural and synthetic LH-RH provoke the release of LH and FSH. Although it is defined a *releasing factor*, it has also been shown capable of hormonal synthesis [12].

Growth releasing hormone (GH-RH). - GH secretion regulation is provided not only by GH-RH but also by *somatostatin*, which has an inhibiting action on the release of the hormone.

Prolactin releasing hormone (PRH). - Together with the TRH, which releases PRL, there is a PRH, which seems to act independently from TRH. Prolactin secretion regulation is carried out by an inhibitory hormone, *prolactin inhibiting factor* (PIF).

The release of adrenocorticotropic hormone is induced by a *corticotropin releasing hormone or factor* (CFR) [13-15].

Recently, for a further insight into the neuroendocrine regulation of anterior pituitary function, in women with *functional hypothalamic amenorrhea* (FHA), serum LH, FSH, cortisol, GH, PRL, TSH concentrations were measured simultaneously at frequent intervals for 24 h. The 24 h secretory pattern of each hormone except TSH was altered in the women with FHA. Compared to normal women, those with FHA had a 53% reduction in LH pulse frequency and an increase in the mean LH interpulse interval; LH pulse amplitude was similar. The 24 h integrated LH and FSH concentrations were reduced. Pituitary hormone increases the response to the simultaneous i.v. administration of GnRH, CRF, GHRH and TRH. The response was normal also in the group of women with FHA, except for a blunted PRL response to TRH [16].

Neurotransmitter regulation of pituitary hormone secretion

No central nervous system areas have been reported as being able to specifically regulate the

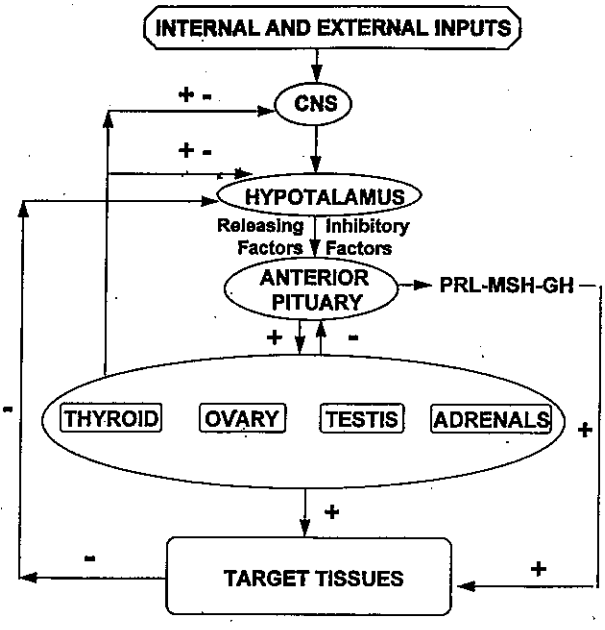

Fig. 1. – Neuroendocrine network indicating the relationship among its components: the central nervous system; hypothalamus, anterior pituitary and target glands and tissue; +: Stimulatory effect; –: Inhibitory effect.

release of a pituitary hormone. Fig. 2 shows a possible model for CRF regulation by the hypotalamus. The CRF neuron ending at a portal capillary gets in contact with a receptor for a 5-HT pathway that releases CRF after stimulation of the cholinergic neuron. One may conclude that the final common pathway to CRF release is cholinergic (Ach). One of the two cholinergic pathways is placed between the 5-HT pathway and the CRF cell, suggesting that this may be used to control the circadian rhythm. The CRF neuron could be under the inhibitory control of both a noradrenergic (NA) pathway and GABA inhibitory neuron [17]. Fig. 2 summarizes the state of knowledge and the controversies regarding the neurotransmitter regulation of pituitary hormone secretion. The neurotransmitter may act on the RH, since receptors for neurotransmitters have been found in the pituitary gland. For PRL, an inhibitory action of the DA on the pituitary gland has been reported [18, 19].

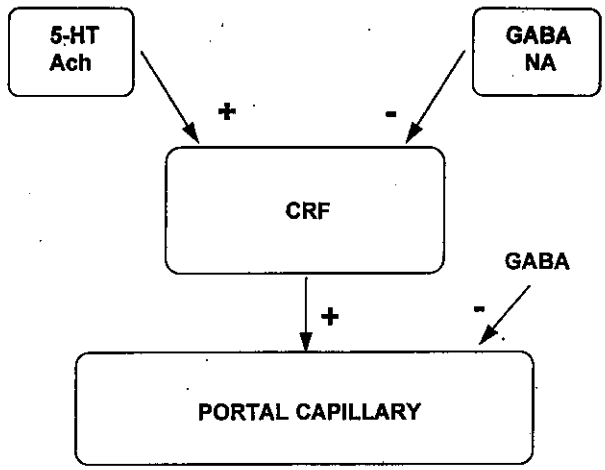

Fig. 2. – Suggested model for the control of corticotropin releasing hormone (CRH) from the hypothalamus +: Stimulatory effect; –: Inhibitory effect.

Central neural mechanisms and endocrine rhythms regulation

Three alternative models have been suggested for the mammalian circadian oscillating system as illustrated in Fig. 3. In the first and simplest model there is a driving oscillator (DO) that is an active cellular unit capable of maintaining a self sustaining oscillation with its own independent driving influence.

By light and other inputs the oscillation is transmitted to subordinate centers A and B and from these to others, C, D and E. In this system, the destruction of the driving oscillator would bring about the loss of all the subordinate circadian rhythmic functions, while the phase-shift of the driving oscillator would lead to the same consequences, that is the dephasing of the subordinate centers. The destruction of a single connection

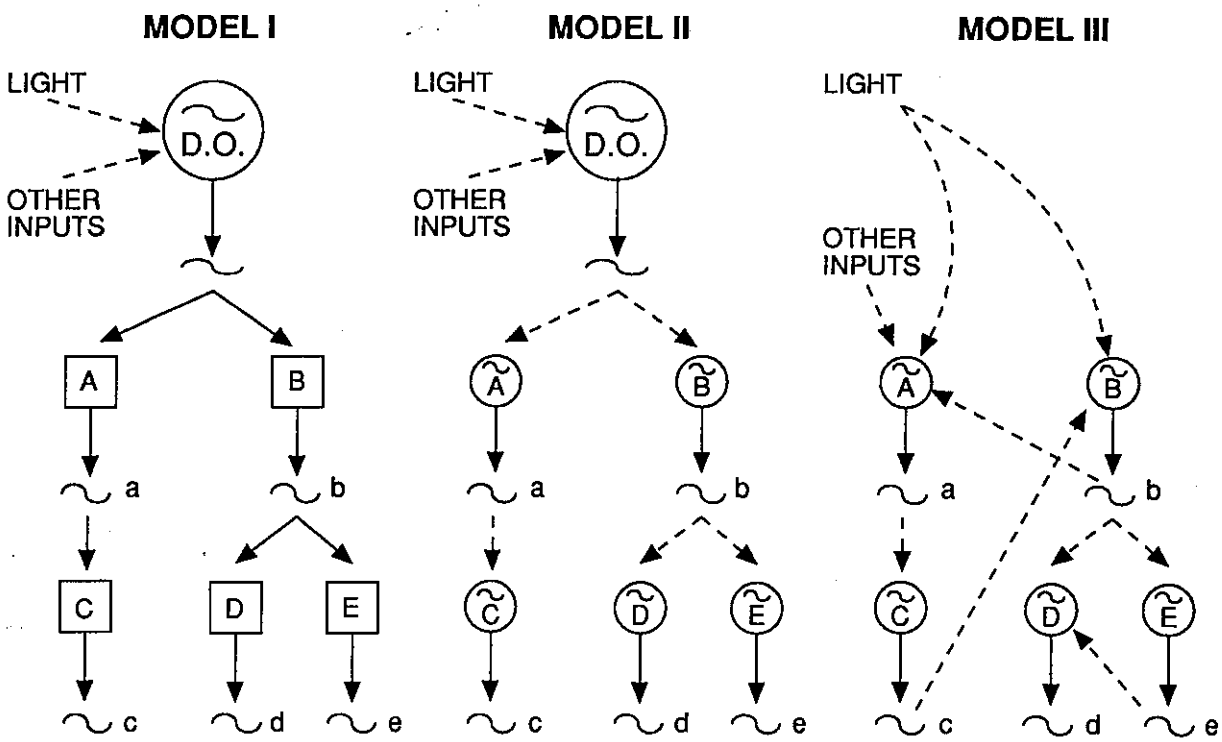

Fig. 3. – Representation of three alternative models for the mammalian circadian oscillating system. DO: driving oscillators (see text).

between the DO and any one of the subordinate centers leads to the loss of that single circadian rhythmic function.

The second model consists of a hierarchically organized set of oscillators. The DO will entrain secondary (A and B) and tertiary (C, D and E) oscillators but unlike the first model, if the DO (S) is or are eliminated, the secondary oscillating mechanisms will be able to maintain their own autonomous rhythm.

The third model is a multioscillator system organized in a non-hierarchical manner. There is no DO and the different centers can interact with each other and with external inputs [20-22].

General remarks on endocrine rhythms

The daily hormonal variation was first described in 1943 by Pincus (urinary ketosteroid secretion) [23]. In chronobiological research, the studies have been concentrated on the *endocrine circadian time structure* (ECTS), as it is the easiest to extrapolate and to apply in practice.

In this context some general points should be noted.

The circadian changes in hormones may depend on each other; even an apparently subordinate rhythm should, however, be considered a *true independent rhythm.*

Accurate studies have shown that hormonal secretion occurs in all cases according to a rhythmic organization at many levels. However, this rhythm may not be evident at a first analysis, since it has not been proved that all hormones have a circadian rhythm only and they may thus undergo periods of infradian and, particularly, of ultradian rhythm.

What should be underlined is that hormone secretion is basically pulsating; the pulsation of hormone secretion makes it difficult to draw a standard reference curve and explains the difference in hormone levels observed in various subjects examined and in the same subject over the same observation period. The statistical analysis of the rhythmic pattern of hormone secretion eliminates the minor components and clearly evidences the circadian rhythm, with similar peak and nadir values in the different subjects [24-29].

Moreover, most of the data obtained in the different research centers regarding the rhythmic oscillation of hormone levels are quite comparable from one subject to another when sampling is fairly frequent and at regular intervals, for example every hour, or fraction of an hour.

Fig. 4 summarizes the circadian pattern of 8 hormones: cortisol, GH, aldosterone, PRL, testosterone, TSH, LH and FSH when the sampling is hourly or at every fraction of an hour. The use of intensive and, at the same time, extensive sampling has allowed a more detailed and realistic picture of the range of physiological variation of hormonal secretion. In addition, the application of more analytical techniques (cluster analysis, spectral analysis, etc.) performed simultaneously has revealed multiple ultradian rhythms for several hormones [30-39].

Although an ECTS is present at the cell level, in organs etc., it is evident that a rhythm hierarchy exists. The circadian rhythmic variation in neurotransmitter and neuropeptide levels can have a circadian pattern similar to that of nerve cells but, on account of their intrinsic hierarchical role, they promote an oscillatory function which becomes the most important for the whole circadian time structure [40, 41].

Hormonal secretion and sleep-wake cycle

The relationship between circadian rhythmsand the sleep-wake cycle is particularly important in the field of endocrine rhythms. As suggested by Aschoff, "*in view of the multi-oscillatory structure of the circadian system, it is of great interest to*

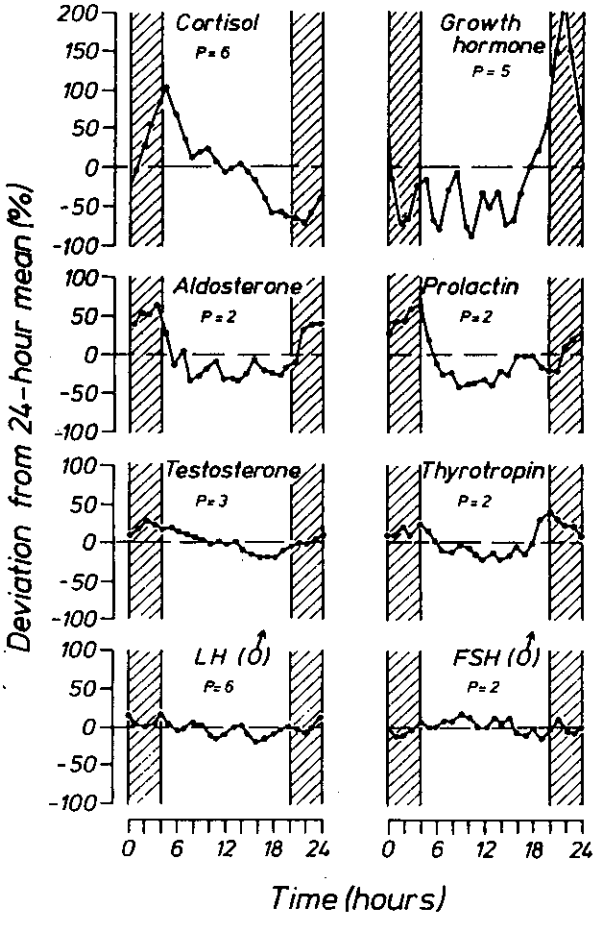

Fig. 4. – Circadian time structure of eight plasma hormones in man. Mean values from a number of studies (P) with sampling occurring at hourly intervals at least.

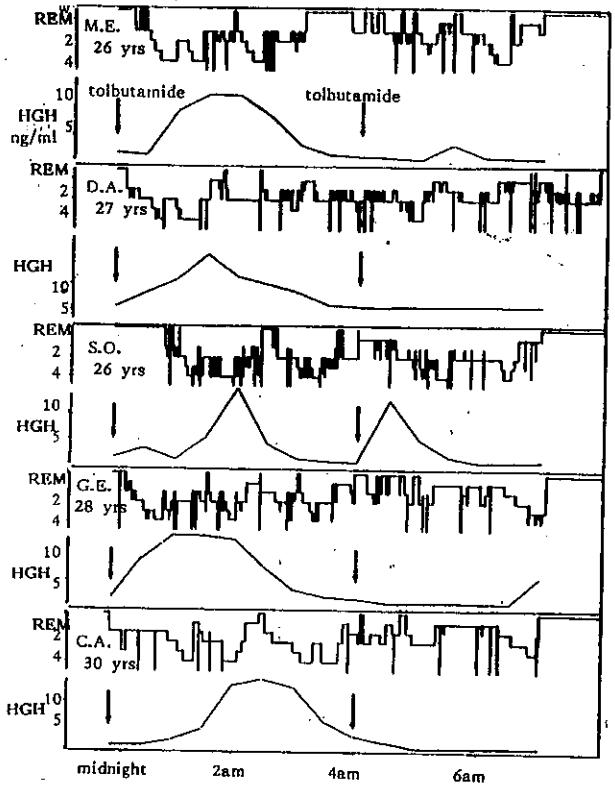

Fig. 5. – An experiment which shows the strong correlation between the stages of sleep and GH secretion in healthy males administered 250 mg Tolbutamide every 4 hours is represented. It can be noted that in the last part of the night hormone secretion peaks are not documented, even in the presence of REM phases.

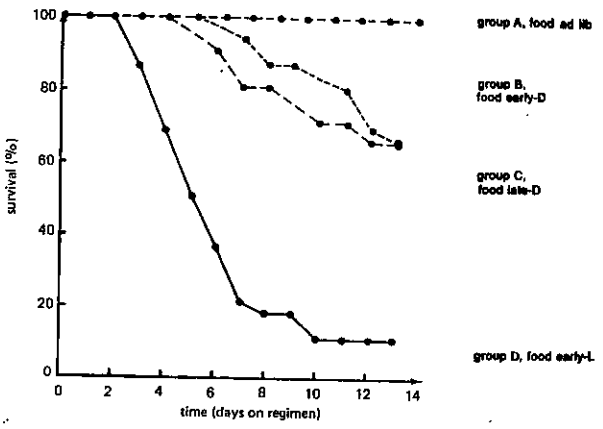

Fig. 6. – Manipulation of mealtiming modifies the toxic effect of drug (phenohorbital sodium). Group A: food allowed at libitum; Group B: food in the early hours of dark; Group C: food in late hours of dark; Group D: food in the early hours of light; Light/Dark: 12/12.

know whether a variable represents a circadian rhythm in itself or whether its rhythm is a (passive) consequence of another rhythmic variable" [42]. Although several reports state that no rhythm is totally dependent on the sleep-wake cycle, from a general point of view the hormone secretion rhythms can be divided in: 1) sleep-dependent rhythms; 2) sleep-independent rhythms.

Rhythms that seem to be strictly sleep-dependent, for example GH and prolactin secretion, actually display a sleep-independent rhythmic component showing (*a*) a basic (minimal) hormone secretion without sleep, (*b*) a sleep-independent (small amplitude) rhythm of secretory activity and (*c*) a strong circadian rhythm of responsiveness to sleep [42, 43] (Fig. 5).

Unlike GH, *cortisol* has been shown to have a strong independent periodic component [43-47].

Mealtiming and hormonal secretion

In animals meal-timing is a powerful synchronizer. If a mouse, a nocturnal rodent, is allowed to eat only in the first 4 h of daylight in otherwise unaltered environmental conditions, it shows a greater susceptibility to the toxic effects of adriamicine and this animal has a clearly decreased survival time compared to another which is offered food in the first 4 h of night-time [48] (Fig. 6).

So far there are no definite and conclusive data to prove that meal-timing is a true synchronizer also in humans, although there have been some reports suggesting this. For example, in volunteers on a free diet without calorie intake restrictions, according to the protocol of (*a*) any time of the day, (*b*) only breakfast and (*c*) only dinner, examination of the rhythms showed that some hormonal rhythms, such as GH and insulin, underwent a phase shift. It was concluded that meal-times are an important synchronizer of some circadian rhythms also in humans and that meal-timing can influence many rhythms. In studies carried out by Reinberg *et al.* on shiftworkers in oil refineries, the time structure of some variables was evaluated, including the circadian secretion of some hormones whose rhythms were normal during night, evening and morning-work, according to the different work-periods. Therefore it was concluded that the most powerful syncronizer was not the meal-timing but, in fact, the manipulation of the rest-activity and light-dark cycles. This determined a phase-shift in the circadian rhythms studies, where meal-timing was a far weaker synchronizer [48, 49].

When obese subjects were subjected to an experiment very similar to the above-mentioned model, that is with a single meal at 10.00 or 18.00, the meal-timing manipulation provoked a phase shift in the circadian oscillations, which have a strong exogenous component as carbohydrate and lipid oxidation and, among the hormones, urinary catecholamines. In this study, however, an endogenous rhythmic component emerged which was not influenced by the meal-timing, that is energy expenditure, plasma cortisol and urinary electrolytes [50-52] (Fig. 7). It has recently been shown that fasting (5

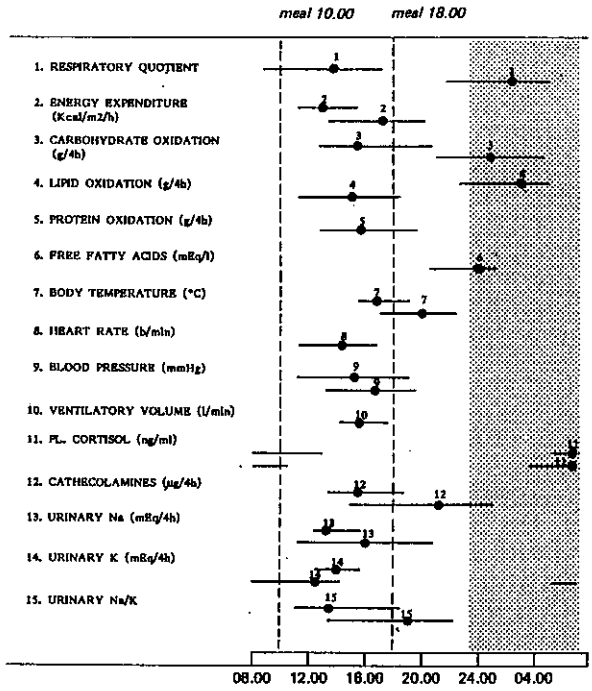

Fig. 7. – The effect of manipulation of mealtiming on circadian rhythms (peak; confidence limits).

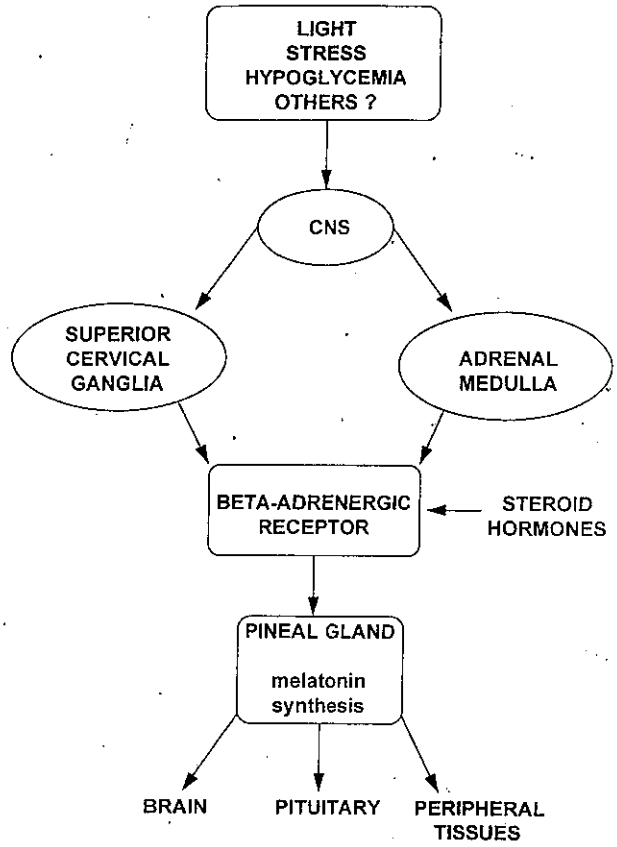

Fig. 8. – Diagram showing the factors that control pineal hormone secretion of pineal hormone (see text).

days) increases serum cortisol in normal subjects and that an increase is associated with distinct changes in the pulsatile, circadian and ultradian pattern, of cortisol [53].

Pineal gland

Although connected to the epithalamus by a peduncle, the pineal does not receive a direct nerve supply from this source. Rather, it is innervated by postganglionic nerve fibers that arise in the cervical simpathetic ganglia and travel along the great vein of Galen, the blood vessel into which pineal secretions drain. The sympathetic inflow to the pineal is in turn regulated by impulses arising in the suprachiasmatic nuclei, paired structures lying just above the optic chiasm. This nucleus is innervated by a direct nerve pathway from the retina termed the retinohypothalamic tract. Changes in external lighting influence pineal activity (and other endocrine functions) via this pathway even when pathways mediating conscious light perception have been severed.

The present interest in the study of the links between endocrine circadian rhythms and the pineal gland (PG) was triggered by several recent works.

The PG in animals is a photoreceptor organ which secretes a hormone, *melatonin*, when the animal is in the dark and stops secreting when the animal is exposed to artificial or sun-light. Fig. 8 illustrates the factors involved in the control and delivery of pineal hormones.

The major input controlling pineal melatonin synthesis is norepinephrine released from postganglionic sympathetic nerves. The rate at which norepinephrine is released is suppressed by environmental lighting (via a pathway involving the retinas and accessory optic tracts), causing a generalized sympathetic activation; the latter also affect the pineal via epinephrine released into the circulation. In the absence of cyclic changes in environmental lighting (e.g., when animals are housed under continuous darkness), melatonin synthesis and secretion continue to exhibit circadian rhythmicity, which requires the intactness of the pineal's sympathetic nerves. Once released, the norepinephrine and epinephrine act via beta-adrenergic receptors on the surface of the pineal cells. Drugs that activate noradrenergic receptors directly or indirectly (e.g., L-dopa) can also accelerate melatonin biosynthesis [54].

The rhythmic pattern of PG secretion is present also in the blind and in subjects kept continuously in the dark which suggests that this hormonal secretion is not controlled by the light-dark cycle but by an internal synchronizer.

The synthesis of melatonin may be influenced by stress. This has been observed indirectly in humans;

in patients administered antidepressant drugs, an increased synthesis of melatonin was noted. Wurtman *et al.* suggested that people subjected to continuous stress could have marked changes in melatonin synthesis [54, 55].

A great deal of interesting data has been provided by experimental animal *pinealectomy*, which induces a pituitary-gonadal stimulation in male and female animals. Removal of the pineal gland before puberty in animals causes a premature sexual development and increased LH and FSH levels. An adrenocortical activation has been reported in pinealectomized animals, demonstrated both by increased weight and secretion of aldosterone and corticosterone and by increased thyroid weight and hormone secretion.

Many studies have provided evidence of an antigonadal activity by melatonin. In hamsters, the long periods spent in the dark would seem to provoke an increase in melatonin production leading to a reduced capacity of reproduction and even to gonadal atrophy; pinealectomy prevents this effect. The same effects have been reported in animals with blindness from an increase in melatonin synthesis with antigonadal activity. The action of melatonin seems to be exerted on the hypothalamus-pituitary axis rather than directly on the pituitary gland [55-59].

Short-term fasting inhibits the nocturnal melatonin secretion in the healthy man [60].

Changes of melatonin secretion have been described in some psychiatric disorders and the mean 24 h melatonin level is significantly higher in anorectic than in obese patients [61, 62].

Endocrine rhythm

CRF, ACTH and corticosteroids

In the field of endocrine rhythms, the circadian (ultradian and infradian) time structure of CRF, ACTH and corticosteroids has undoubtably been the most studied.

The CTS of CRF, ACTH and corticosteroids represents a network of exemplary paradigmatic rhythms of the previously hypothesized models.

The circadian rhythm of cortisol is characterized habitually by a hormonal peak on awakening and a rapid decrease in the first hours of the day. The increase in hormone concentrations in humans is during the early hours of the morning while in the nocturnal rodent it is during the first hours of the night (Fig. 4). Cortisol oscillation depends on the light-dark synchronizer, since the peak is dephased if the light-dark sequence is inverted [15].

Information regarding the sleep-wake cycle can be found in the general paragraph; however, it should be remembered that, unlike many others, the cortisol rhythm is not sleep-dependent.

Meal-timing does not influence the circadian rhythmic oscillation of plasma cortisol secretion [49-52].

ACTH rhythm. - In humans, if the number of samplings is increased, a cyclic variation in ACTH plasma concentration can be observed which is identical to that reported for cortisol; the peaking of the former coincides with that of the latter. With samples every 5 min, ACTH peaks can be seen which do not coincide with those of cortisol. With samplings every 10 min for a duration of 24 h in humans, a significant secretory burst of ACTH and of beta-endorphine can be observed over 24 h. The bursts are simultaneous and both are followed after 10 min by cortisol pulses [63].

Circadian rhythm of cortisol responsiveness to different stimuli. - A circadian rhythmicity in animals and recently in humans of ACTH secretion after injection of pyrogens and CRF has been demonstrated. The circadian oscillation of hypothalamic CRF persists also after hypophysectomy, preceeding 4 h from the increase of the concentration of plasma corticosteroids [64-65]. These data suggest that NCS may play a fundamental role in producing the periodic CRF secretion, independently of any type of feedback control [65]. Of further evidence is the documentation that the plasmatic concentration of ACTH is able to maintain the circadian oscillation even after adrenalectomy.

The interruption of the circadian oscillation of ACTH and corticosteroid plasma levels is obtained in the animal after hypothalamic suprachiasmatic deafferentation and nuclear lesion.

In rats with disease involving the hypothalamic-limbic area, an alteration in the rhythmic plasma corticosteroid levels is also documented [66].

On the basis of the observation of the circadian variation of secretion from *isolated adrenal cultures*, there is disagreement concerning the capacity of a rhythmic hormonal secretion. It is unclear whether the rhythmic oscillation of ACTH and corticosteroids should be considered endogenous.

Even in the absence of sure evidence of a free-running rhythm under strictly constant conditions, adult and blind humans showed a free-running rhythm. Additional credit in sustaining a prevalent endogenous component in the circadian rhythmicity of ACTH and cortisol is given by the fact that many days are necessary (from 3 to 9) to determine a phase-shift of the hormonal rhythmicity, which may be achieved by inverting the light-dark cycle or with a desynchronization after transmeridian flight [67].

In *Cushing's disease* there is a loss of the circadian rhythm, the morning peak disappears and a continuous and regular oscillatory component is observed. In *Addison's disease* the rhythmic variations of ACTH seem not to depend upon feedback control from cortisol since they persist in Addison's disease and after adrenalectomy [68].

Growth hormone (GH)

Rhythmicity of GH. - The neurotransmitter regulation of GH is norepinephrinergic, dopaminergic and serotoninergic, but for some investigators the circadian oscillation of GH release could be mainly cholinergic [15]. Studies of GH secretion are particularly complex if we consider that the hormonal levels depend upon two distinct hormonal activities such as somatostatin, which inhibits the release of GH, and GHRH (growth hormone releasing hormone). The initial input generating the neural activation responsible for the variation of the concentration of GH plasma levels is given by environmental factors (like stress), internal factors such as hypoglicaemia and mainly sleep (Fig. 5). This type of signal may be occasional or repetitive; when repetitive it is responsible for the rhythmicity of GH secretion. Sleep is a potent influence upon GH rhythmicity; the peak in GH secretion is associated with the first part of sleep. When the onset of sleep is shifted, there is a shift in GH secretion; if the sleep-wake rhythm is reversed, there is a reversal of GH secretion.

Nevertheless, there is evidence that peaks of GH plasmatic levels occur also diurnally, therefore independently from sleep.

To conclude, it is unlikely that other synchronizers such as the light-dark cycle, meal-timing and social habits, when compared with the sleep-wake cycle, can be considered equally important in the determination of the GH secretion peaks. In normal subjects on a low-calorie diet, the highest GH secretion always occurs during the night-time, independently from the previous period of caloric deprivation; furthermore the peak of hormonal secretion is unsuppressed after glucose infusion and continues during sleep [69].

GH secretion occurs in association with a slow wave stage (SWS) only during the first part of the night; in fact even if there are SWS also between 4 am and 8 am, GH secretion is low or absent [15].

Ontogenetically the rythmic release of GH sleep-dependent develops during the first year of life; the amplitude and frequency of the oscillations fall progressively with age, particularly after puberty [70-74].

Ultradian rhythms. - Together with the circadian rhythmic component, when hormonal secretion is studied using sampling at more frequent intervals of time (20 min), ultradian peaks can be observed. GH secretion seems to be oscillatory rather than episodic [24].

Using ultrasensitive immunoradiometric assay for GH determination and performing the cluster algorhythm and spectral analysis, some Authors recognized a rhythmic ultradian secretory pattern with pulses occurring nearly every two hours. In conclusion, GH levels oscillate with a range of 3 orders of magnitude with highly diverse amplitudes [75-79].

Comparing the influence of delayed sleep onset and temporary slow-wave-stage (SWS) deprivation on nocturnal GH and cortisol release in man, a dissociation of secretory peaks from SWS is documented, since the increase of GH secretion occurs subsequently to sleep onset rather than during the main part of SWS.

Nocturnal cortisol release is delayed when sleep onset is delayed. Thus the timing of both nocturnal GH and cortisol secretion seems more dependent on sleep onset than on SWS [80].

Under some pathological conditions associated with an alteration in GH secretion like *acromegaly*, a circadian sleep-dependent rhythmicity cannot be documented, even though episodes of GH release independent of the sleep-wake cycle have been demonstrated in this disease.

Prolactin

A circadian rhythmicity of PRL secretion with a peak during the onset of sleep was documented when sampling was performed every 20 min. PRL secretion peak occurs in the same period as that of GH but PRL shows a slower pattern and generally begins 10 to 60 min from the onset of sleep and lasts for a longer period [81-83].

Differently from GH, a strict correlation between PRL secretion and sleep stages 3 and 4, responsible for a fall of the GH secreting peak, lacks any influence on PRL secretion. The hypothalamic control on PRL secretion is represented by *prolactin inhibitory factor* (PIF), released by dopaminergic nerve impulses, but also dopamine may inhibit prolactin secretion influencing directly the pituitary. *Phenothiazines*, L-dopa dopamine receptor antagonists, induce an increase in PRL plasma levels. The dopamine precursor L-dopa inhibits PRL release and its nocturnal increase associated with sleep; consequently the hormonal circadian rhythm disappears. *Serotonin and GABA* increase prolactin plasma secretion; this may suggest that they play a role in modulating the rhythmicity of PRL plasmatic levels; however this has not yet been clearly demonstrated.

Estrogens may increase PRL plasma levels; the administration of ethynil-estradiol produces an increase in PRL plasma levels with a more evident nocturnal secretion, without modifying the oscillatory rhythm of hormonal secretion. During the early phases of *pregnancy* there is an increase in PRL levels most likely caused by the increased estrogen production; during the whole gestational period the rhythmic pattern of PRL plasma levels with a nocturnal peak is maintained [84-90].

In addition to the known circadian rhythm, *multiple ultradian PRL rhythms* were documented, with periodicities ranging from 22 to 242 min. Spectral analysis performed in some studies evidenced ultradian cycles with periodicities ranging from 30-32 to 234 min. The assessment of episodic

Table 1. – Neurotransmitter regulation of pituitary hormone secretion

	Growth hormone	Prolactin	Gonadotropin	TSH	ACTH
Norepinephrine	↑	—	—	?↑	↑+↓
Dopamine	↑	↓	↓ or ↑	↓ or ↑	?↓
Serotonin	↑	↑	?↓	?↓	↑+↓
GABA		↑	?↑		?↓
Histamine			?↑		
Acetylcholine	?↑	↑ or ↓			↑

Note: ↑ = increase, ↓ = decrease, — = no effect, ↑ = predominant effect.

PRL pulsatility by Cluster analysis showed numerous peaks/h.

Veldhuis and Johnson concluded that PRL release in normal young humans is characterized by significant circadian and ultradian periodicities, by discrete episodic pulsations that occur nearly every 95 min and also by temporal coupling with LH [29].

The secretory pattern of prolactin during the *menstrual cycle* has been studied and showed the presence of a monthly rhythm with an increase in PRL in the last period of the follicular phase, during the LH peak. The menstrual cycle oscillation is superimposed on the circadian variation; this rendered difficult the identification of the PRL monthly rhythmicity [91-92].

An increase in prolactin induced by the administration of TRH has been documented; this increase is more evident when the TRH-test is performed in the late afternoon rather than in the morning. PRL secretion induced by TRH was found to be more evident during the preovulatory period and the luteal phase when compared with the early and follicular phase [93].

One of the most potent influences for the secretion of PRL is represented by *lactation* occurring through nipple stimulation; PRL plasmatic concentration is known to increase 6-10 fold a few min from the beginning of suckling and this increase occurs not only in women after delivery but also in 1/3 of women not in puerperium. Actually there are no conclusive data concerning the influence of suckling on the rhythmic oscillation of PRL plasma levels.

In *pituitary tumors* with or without galactorrhea, associated with a constant increase in PRL levels, disappearance of the hormonal nocturnal peak is documented; the same occurs in *hypothalamic tumors* associated with the increase in PRL plasma levels.

A rise in PRL plasma levels associated with an attenuation or even the disappearance of the nocturnal prolactin peak has often been found in the *amenorrhea and galactorrhea syndrome, with oral contraceptive use, in idiopathic galactorrhea with amenorrhea and in the Chiari/Frommel syndrome* [94].

Some investigators have found in Cushing's disease a marked reduction or absence of the nocturnal peak of prolactin; the same pattern was documented in *unstable diabetes* and *anorexia nervosa* [95, 96].

In women with *primary empty sella* both the responses to TRH stimulating test and metoclopramide and the circadian variation of PRL levels were evaluated. A hormonal pattern characterized by an increase of PRL response to TRH and metoclopramide and a decrease of the PRL circadian variation were documented. The disappearance of the signs of intracranial hypertension restored the normal PRL dynamics but the endocrine alteration improved only moderately [97].

To conclude, a circannual variation in the amplitude of PRL evaluated with the Cosinor test has been reported in females with *breast cancer* [98].

Temporal pattern of the hypothalamic-pituitary-thyroid axis

TRH (thyrotropin releasing hormone). - Due to the difficulty in direct sampling of the pituitary gland and in the portal system, studies of TRH in the human have not been reported.

An experimental study on the circadian TRH variations in the rat hypothalamus may be of some interest for the inverted specularity, as seen for many circadian oscillations in this animal when compared with man. TRH levels in the anterior pituitary of the rat present a circadian pattern and are directly correlated to the variations of TSH, both showing a diurnal zenith and a nocturnal nadir (the opposite is seen in the 24 h TSH pattern in the human) [99].

TSH (thyroid stimulating hormone). - TSH shows a pulsatile secretion like other pituitary hormones

with a periodism ranging from 0 to 30 to 100 min, and an amplitude from 15 to 90% when compared with the mean values [100].

When blood samples were taken from healthy males every 10 min over the course of 24 h, cluster analysis showed that the pulsatile TSH activity was evident mainly between 8.00 pm and 4.00 am.

Most consideration was given to the *circadian rhythm of TSH*. It is well known to represent a *marker* for the organization of the endocrine temporal pattern like cortisol, ACTH and prolactin. The characteristics of this rhythm with a nocturnal acrophase between 8.00 pm and 4.00 am and an amplitude ranging in percentage between 70 and 140% of the mesor values, have been well delineated.

In contrast, for some authors it is evident that the average waveform derived from the circadian rhythm of the hormonal levels in 24 h cannot be described by a single sinusoidal function.

Also evident is the presence of a central control mechanism for the circadian TSH secretion since hypothalamic deafferentation suppresses the day/night variations; this is supported by the fact that after surgery for hypothalamic tumors in human subjects, low levels of TSH without any circadian rhythm were documented.

Infradian TSH rhythms with insignificant monthly modifications have been studied in females with normal menstrual cycles but a circannual rhythm with the acrophase in August is documented [101-109].

Also the TSH response to TRH shows a circadian oscillation with a peak between 10 pm and 2 am in males as well as in females. Although there are many hypothesis regarding the mechanisms of the circadian variations of TSH, most authors assume that their pattern is parallel to the changes in TRH, even though a possible role of other hormones and/or neurotransmitters is accepted [110].

Thyroid hormones. - Data from different experimental protocols concerning total *tri-iodothyronine* (T3) and *thyroxine* (T4) show considerable disagreement.

Some authors do not report the presence of a circadian oscillation whereas others document a circadian rhythm with different characteristics, peaks in the morning, afternoon and evening, with a prevalence for the evening [111-115].

These contrasting data may be due to the complexity of thyroid hormone metabolism responsible for the decreasing rhythmic variations, especially in physiological conditions.

More concordant seem to be those regarding the circadian temporal pattern of the free fractions, *FT3* and *FT4*.

The presence of a rhythm has been documented with an afternoon acrophase for FT4 around 4.00 pm and for FT3 around 1.00 pm [116].

Recent studies on thyreoglobulin, which is to be considered a real pro-hormone, report a circadian temporal pattern with an acrophase in the afternoon, around 3.00 pm [117].

Also the *reverse T3*, the main metabolite of the thyroid hormones, the *protein binding iodine* (PBI), the *T3-resine uptake* (T3RU) and the total concentration of the *thyroxine binding globulin* (TBG), the most important protein vehicle for the thyroid hormones, have all been investigated and have shown a circadian rhythm.

Ultradian oscillations of thyroid hormones. - The total and free thyroid hormones show ultradian oscillations, with a 30 min period and a synchronism between T3 and T4. The amplitude of the oscillation is significantly higher than the variation coefficient of the sampling method and is around 10-15%.

Infradian rhythms. - Circannual rhythms of T4 (with the acrophase in September), T3 (with the acrophase in March), FT3 (with the acrophase in October) and thyroglobulin have been reported [102].

Infradian rhythms of thyroid volume. - The introduction of ultrasonographic imaging allowed a detection of the variations of thyroid volume occurring during the menstraul cycle with the acrophase in the middle of the cycle [101]. A circannual variation of the sonographic thyroid volume with a winter acrophase has also been observed [102].

Organization of the temporal pattern during thyroid diseases. - It has been hypothesized that physiological thyroid function may be the synchronizer in the organization of the temporal pattern in contrast with thyroid disease, leading to significant modifications. At present these hypothesis have not found definite evidence.

First of all we must consider the repercussions of *hypothyroidism* or *hyperthyroidism* on the temporal pattern of the hypothalamus-pituitary-thyroid hormone axis. Reports of a possible persistence of a circadian rhythm of TSH during primary hypothyroidism are controversial and no definitive evidence has been found to support these hypothesis. Some authors report a significative circadian oscillation in the presence of low TSH levels, others in the presence of high TSH levels and some others in neither case [118].

The circadian rhythm of TSH during *autoimmune hyperthyroidism* has also been evaluated, with highly sensitive methods of immunoradiometric dosage showing a loss of the rhythm in most cases.

The early studies have been performed on the possible temporal oscillations of thyroid hormones in disease conditions of the thyroid; the absence of a circadian variation in the free thyroid hormones has been shown in primary hyper - as well as

hypothyroidism. A wider study, in contrast, reports the persistence of the circadian rhythm of FT4 and FT3 in antiphase with respect to controls in *Basedowian disease, hypothyroidism* and in the *adenoma* in latent hyperfunction, in which case there is also a circadian variation in T3 and T4 with a synchronous acrophase at 3.00 pm [119].

Other alterations of functions displaying a circadian rhythmic oscillation in normal subjects, such as body temperature, heart rate and blood pressure, do not seem to undergo significant changes regarding the circadian time pattern in primary hyperhypothyroidism. The same proved true for the circadian rhythm of plasma cortisol, which is unchanged, while the circadian oscillation of serum prolactin disappears in hyperthyroidism but is still present in primary hypothyroidism [119].

Organization of the time pattern of the hypothalamus/pituitary/thyroid system during extrathyroid diseases. - A certain amount of data has been gathered, particularly concerning the circadian rhythm of TSH in various non-thyroid diseases. As already mentioned, this rhythm is now considered to be a marker for hypothalamic function and any disorders former are therefore regarded as disorders of the latter. Alterations in the circadian variation of TSH have been found in central hypothyroidism [120], non thyroid diseases due to low T3 syndrome [121], both ACTH dependent and independent Cushing's syndrome [122], prolactin-secreting adenomas, depressive illnesses and in the keto-acidosic decompensatioin of diabetes mellitus. These data suggest a possible use of the TSH rhythm in the study of the pathophysiology of the hypothalamus [123].

Influence of drugs on the organization of the time pattern of the hypothalamus/pituitary/thyroid system. - In this regard, numerous studies have been performed on the influence of the neuroreceptor agonists and antagonists on the circadian rhythm of TSH [124]. Research has been carried out on the effects of doses of hydrocortisone on the circadian rhythm of TSH and on the I^{131} release by the thyroid. The hormone provokes the disappearance of the rhythms in both cases [125].

Lastly, chronobiological experience can be drawn from the oral administration of T3 in three daily doses as the best therapeutic protocol to inhibit the response of TSH and TRH in subjects totally thyroidectomized for thyroid cancer [126], and for the abolition of the nocturnal TSH peak in subjects in long-term suppressive therapy with L-T4 [127, 128].

The hypothalamic-pituitary-gonadal axis and ovaric steroids

Reliable data concerning the neurotransmitter regulation of the hypothalamic-pituitary-gonadal axis are currently not available. Thus, a fall in LH secretion after treatment with antiserotoninergic drugs has been reported in the rat. Furthermore, there is no evidence of a circadian rhythmicity of LH plasma levels before puberty or in adulthood [117], but in the hypothalamic-pituitary-gonadal axis infradian and ultradian periodicities are found. These investigations were possible thanks to recent analytical tools such as cross-correlation and discrete peak detectional algorhythm in the young man [128].

The oscillatory patterns of hypothalamic, pituitary and ovaric hormones may differ in reference to the menstrual cycle and to the time of observation (pubertal or pre-post pubertal) [129].

Ultradian and infradian rhythm of gonadotropins and ovaric steroids during the pre-pubertal phase. - The study of ultradian and infradian rhythms in pre-pubertal children is complex due to the small amounts of hormones present. Nevertheless, an ultradian secretion of LH can be observed in pubertal and pre-pubertal girls. It has been suggested that the progression from the pre-pubertal to the pubertal phase may be linked to the amplitude of the rhythmic oscillation of LH secretion rather than to the increasing frequency of the episodic activity [130].

As previously mentioned, a circadian rhythm of LH secretion with an increase during sleep, as in males, has been reported in pubertal girls. LH secretion is sleep-dependent, occurring during non-REM sleep and ending at the onset of REM sleep. Unlike the sleep-induced LH release associated with testosterone (T) secretion observed in the male, the estradiol circadian rhythm in the female has a peak in the daytime rather than simultaneous to the LH peak; in other words, there is a paradoxal increase in secretion of the target gland when the pituitary stimulation is at its lowest level [131, 132]. The circatrigintan rhythm is the most noted; during the menstrual cycle there is a cyclic increase in FSH and LH but also many other hormones, like PRL, TSH, GH and ACTH may oscillate during the cyclic variation [133-136].

LH-FSH. Ultradian rhythm of LH and FSH after puberty. - A pulsatile episodic LH secretion with a change in LH levels within 5 min has been documented when performing sampling more frequently. During the whole cycle, except for the late luteal phase, there is an oscillation with a 90 min period [137, 138].

Gonadotropins in menopause. - The most interesting observation during the menopausal and postmenopausal period and after surgical castration is the rapid variation of daily LH and FSH levels; with the disappearance of the gonadal-pituitary feed-back, there is an increase in amplitude of the pulsatory rhythmicity without modifications in frequency [139, 140].

Polycystic ovary syndrome. - During the polycystic ovary syndrome there is a persistence of the LH pulsatile activity with an increase in the amplitude and frequency. As in normal females, the gonadotropin pulsatile secretion is accompanied by a pulsatile release of estrogens and androgens which cannot be correlated [139-141].

Hypothalamic-pituitary-testosterone axis

The study of the variations in hormonal secretions of the hypothalamic-pituitary - gonadal axis in men has produced conflicting data on the possible or definite existence of circadian, infradian and ultradian rhythms of the hormones of the LH, FSH and testosterone (T) axis due to the complexity of the interdependent relationships, to the different patterns according to the period in which subjects were investigated (pubertal or pre-post pubertal) and because of a limited population and/or sampling [142, 143].

Ultradian LH rhythms: when sampling was performed every 15 min for 12 h, a pulsatile LH secretion rhythm was reported with intervals of approximately 90 min. These investigations have not been confirmed by other researchers who believe there is no repetitive ultradian rhythm of LH release but an intermittent rather than a cyclic secretion [144].

Whether the mechanism of LH secretion is cyclic or not, it has been suggested that it may be induced by the CNS through the hypothalamic LH-RH. This may be confirmed by the evidence that alpha-blockade agents, such as phentolamine, abolish LH secretion which is *alpha*-adrenergic dependent. While no circadian rhythmic variation is reported for LH, FSH, T level, on the other hand, a circadian variation, although of very low amplitude, when blood sampling was performed every 20 min or every hour is shown. This has been confirmed by many studies [144-149].

The majority of subjects showed a night-time increase in T, but a strict temporal relationship between LH and T secretion has not been clearly established.

Some reports suggest that the increase in T secretion may be linked to the increase in PRL activity occurring in the same period. In fact, when PRL is administered together with LH in the animal, te stosterone plasma levels increase three-fold with respect to the administration of LH alone and enhances the LH- induced increase in the size of the testis. In humans, the role of PRL in increasing the responsiveness of the Leydig cells to gonadotropines has not yet been clarified; the administration of dopamine, which slows the PRL increase, determines an increase in T.

Premature puberty. - Subjects with premature puberty, most of whom show an increase in testosterone and androstenedione levels, present a pulsatile LH secretion during sleep.

Testosterone infradian rhythms. - With sampling every 4 h every other month for a year, some research groups have reported a circannual rhythm in young men with a peak in plasma testosterone during the winter months [150].

Glucose-insulin

The study of a possible rhythmic pattern of insulin, whether circadian, infradian or ultradian, has been approached from many points of view with often conflicting results.

The basal values of plasma insulin were studied in animals kept in conditions of a fixed light-dark cycle; in fasting animals glucose levels and insulin levels rose towards the end of the light period, suggesting that the changes in insulin were not secondary to changes in blood glucose [151]. In man it has not been possible to demonstrate significant changes in plasma insulin levels, throughout the day in normal or diabetic subjects [152]. In contrast, in other studies, lower plasma concentrations have been observed in the afternoon with respect to morning levels; the afternoon insulin levels were not associated with variations in blood glucose levels.

More recently, it is suggested that basically insulin is released in a pulsatile fashion which seemingly is erratic but at close analysis displays a free running cyclic rhythmicity of 8-32 min duration [153, 154].

A diurnal variation of injected insulin was reported in healthy men who were given insulin six times on different days in random order; by statistical evaluation (Cosinor test) significant circadian rhythms for the hypoglycemic action of exogenous insulin with a peak in the morning is documented [155]. Other reports showed a variation in the hypoglycemic response to endogenous insulin, demonstrating a higher blood glucose/plasma insulin ratio in the afternoon. Gibson and Jarret reported that the effect of intravenous insulin on blood glucose has a lesser hypoglycemic effect in the afternoon. Other researchers claim that diabetics respond differently to injected insulin at different times [156-158].

Diurnal variation of insulin responsiveness to insulinogenic stimuli. We found the existence in healthy subjects of a morning-afternoon variation in insulin response to glucose load which appeared increased in the morning [159, 160]; these data were confirmed by others [161, 162]. We documented subsequently, by means of microscopic analysis in healthy subjects, a circadian rhythm of tolbutamide induced insulin secretion [163, 164].

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