

NEURO-ENDOCRINOLOGIE

Development and organization of the hypophysiotropic hypothalamus driving the pituitary-gonadal axis in the rhesus monkey

Développement et organisation fonctionnelle de l'hypothalamus contrôlant l'axe hypophyso-gonadique chez le singe rhésus

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RÉSUMÉ - Le but de cette revue est de décrire, tout particulièrement chez le singe rhésus, l'ontogenèse et l'organisation fonctionnelle du générateur de pulse à GnRH au niveau de l'hypothalamus. Cette fonction est assurée chez les primates, par un groupe d'environ 1 000 neurones répartis diffusément dans l'ensemble de l'hypothalamus. Le GnRH, initialement produit sous forme d'une pré-hormone, est déversé dans la circulation porte-hypothalamo-hypophysaire et va permettre la libération de FSH et LH.

SUMMARY - The purpose of the present review is to describe, with particular emphasis on the rhesus monkey, the ontogeny and functional organization of the hypothalamic GnRH pulse generator. Control of pituitary-gonadal axis in higher primates is provided by a group of some 1,000 GnRH neurons that are diffusely distributed throughout the hypothalamus. After synthesis of a prehormone and formation of the mature decapeptide, GnRH is released in the hypophysial portal circulation and stimulates FSH and LH production.

INTRODUCTION

The drive to the pituitary-gonadal axis in higher primates is provided by a group of some 1,000 gonadotropin releasing hormone (GnRH) neurons that are diffusely distributed throughout the hypothalamus [34]. The perikarya of these neurons synthesize a prohormone, which is then processed to form the mature decapeptide. Many GnRH neurons project to the hypophysial portal circulation where they synchronously release a pulsatile discharge of the decapeptide, the principal releasing factor stimulating luteinizing hormone (LH) and follicle stimulating hormone (FSH) secretion. Each discharge of GnRH is robustly correlated with a volley in multiunit electrophysiological activity in the hypothalamus [42], and this hypophysiotropic system is often referred to as the GnRH pulse generator

[12, 29]. The purpose of the present review is to describe, with particular emphasis on the rhesus monkey, the ontogeny and functional organization of the GnRH pulse generator.

FETAL ORGANIZATION OF THE GnRH PULSE GENERATOR

Interestingly, as first demonstrated for the rat [22], GnRH neurons in higher primates such as the rhesus monkey are born early in fetal life in the olfactory placode and enter the forebrain before migrating to the hypothalamus [32]. The essential components of the GnRH pulse generating neural network appear to be organized by mid-fetal development in higher primates, as reflected at this stage of development, by a pulsatile pattern of secretion of fetal pituitary gonadotropin

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[11], by an operational pituitary-gonadal feedback loop [31], and by the ability of fetal GnRH neurons to restore ovarian cyclicity in hypothalamic lesioned monkeys [33]. The neurobiological organization of the *in situ* GnRH pulse generator in the hypothalamus of the fetal primate has received little attention. It seems reasonable to propose, however, that the mechanisms that underlie the generation of pulsatile GnRH secretion at this stage of development are comparable to those that operate postnatally. In this regard, studies of immortalized mouse GnRH neurons [15, 41] have provided evidence that has

led to the view that GnRH neurons possess properties that endow them with intrinsic pulsatile behavior. This line of thinking has been reinforced by the recent report that primary cultures of embryonic GnRH neurons from the monkey exhibit a pulsatile pattern of peptide release [38]. On the other hand, Bourguignon and his colleagues have demonstrated that rat retrochiasmatic explants, which contain GnRH axons severed from their cell bodies, continue to secrete their peptide in a pulsatile fashion [30]. This finding suggests that synchronized pulsatile release is imparted by non-GnRH elements in the mediobasal hypothalamus (MBH).

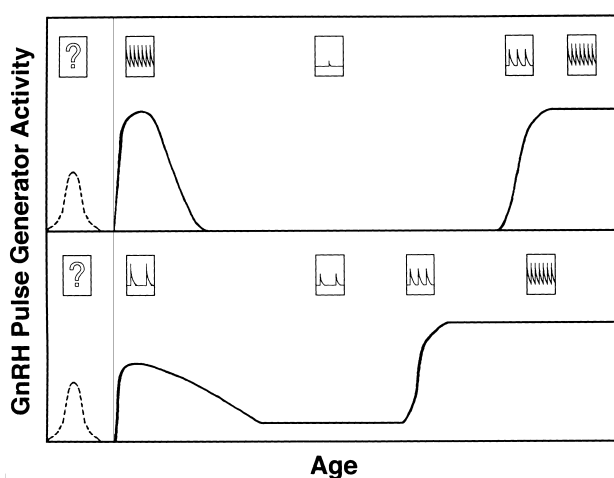


FIG. 1. — Schemata of the ontogeny of open-loop GnRH pulse generator activity in the male (top panel) and female (bottom panel) monkey. The insets indicate the frequency and amplitude of pulsatile GnRH release at critical stages of development. Reprinted with permission from [24].

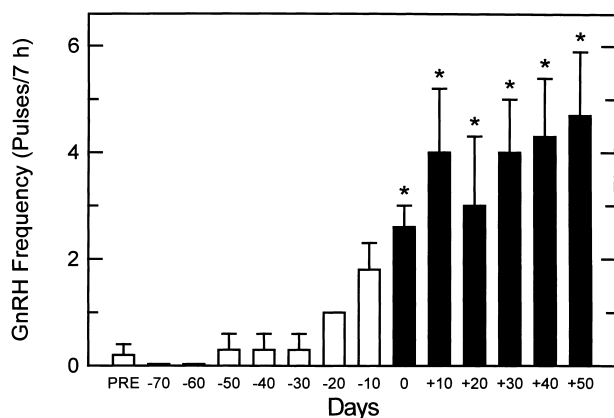


FIG. 2. — Abrupt pubertal acceleration in open-loop GnRH pulse frequency, as measured indirectly in male monkeys ($n = 5$) using LH discharges from the *in situ* pituitary sensitized to GnRH by intermittent priming with the exogenous decapeptide. The age at which enhanced pubertal GnRH release was observed was labelled Day 0 and data for individual animals were aligned to the reference point. Asterisks indicate significant difference from PRE ($p < 0.05$). Reprinted with permission from [36].

POSTNATAL ONTOGENY OF GnRH PULSE GENERATOR ACTIVITY

Higher primates exhibit a unique postnatal developmental pattern of GnRH pulse generator activity [23], and this is most graphically manifest in the open loop condition, which is shown for a representative primate in *figure 1*. By infancy, the GnRH pulse generator of the male monkey has acquired the capacity to operate at a circrchoral frequency typical of that of the postpubertal animal. At approximately 6 months of age, however, the GnRH pulse generator is brought into check leading to the hypogonadotropic state that guarantees the quiescence of the prepubertal testis. The prepubertal restraint on pulsatile GnRH release is maintained for approximately 2 years and then abruptly lifted [36], with pulse frequency in the agonadal state accelerating explosively over a period of 30 to 40 days to terminate the prepubertal phase of development (*figure 2*).

The postnatal development of open-loop GnRH pulse generator activity in the female monkey differs in several respects from that in the male (*figure 1*). In the female, the GnRH pulse generator does not operate at the adult circrchoral frequency during infancy [25] and the prepubertal restraint is applied less forcibly, and for a shorter duration, than in the male [28]. Although not established empirically, it is likely that the sexual differentiation of the ontogenic pattern of pulsatile GnRH release in the monkey results primarily from prenatal exposure of the fetal hypothalamus to testicular testosterone secretion.

In both male and female, the GnRH pulse generator is subjected to diurnal modulation during infancy, with increased activity of this neuroendocrine system being observed at

night. During prepubertal development, a similar diurnal modulation of the GnRH pulse generator is observed in the female [21] where the prepubertal brake on GnRH release is less marked than in the male. Although the lifting of the prepubertal restraint on the GnRH pulse generator is initially manifest at night in both sexes, nighttime augmentation of pulsatile GnRH release persists into adulthood only in the male.

NEUROBIOLOGY OF THE PREPUBERTAL RESTRAINT ON PULSATILE GnRH RELEASE

The finding that repetitive stimulation of the GnRH neuronal network of the prepuber-

tal monkey with a glutamate receptor agonist results in the immediate activation of an adult-like pattern of pulsatile GnRH release that leads to precocious gonadal function [9, 27], suggests that the prepubertal restraint on pulsatile GnRH release is determined by developmental changes in an upstream input to the neurons responsible for the secretion of this peptide. Recent studies of the agonadal male monkey indicate that the loss of this restraining input to the GnRH pulse generator of the prepubertal hypothalamus is associated with an upregulation of the gene encoding the releasing factor [5]. That this pubertal change in gene expression has not been previously reported [13, 40] is probably due to the earlier use of gonadally intact models where amplified steroid feedback resulting from hypothalamic puberty may be anticipated to limit developmental changes in GnRH mRNA levels. This notion is supported by the findings that castration in adult male monkeys and estradiol treatment of ovariectomized monkeys elicits an increase and a decrease, respectively, in GnRH mRNA levels in the MBH [6 and El Majdoubi, Sahu and Plant, unpublished observations].

That the prepubertal restraint on pulsatile GnRH release and GnRH gene expression is imposed by a reversible inhibitory input is supported by ultrastructural studies indicating a decline in axosomatic input to GnRH neurons at the onset of puberty (*figure 3*). Because of the studies of Terasawa and her colleagues showing that hypothalamic γ -aminobutyric acid (GABA) release declines with the onset of puberty [16], and that interruption of GABA synthesis or action by local administration of antisense oligonucleotides for the mRNA for the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD) or an antagonist to the GABA A receptor, respectively [16, 17], elicits GnRH release during prepubertal development, GABA has been considered as the most likely candidate for this inhibitory input. Recently, however, the pubertal upregulation of the GnRH gene and the reaugmentation of pulsatile GnRH release has been found to be associated with a decline, in the MBH, of the mRNA encoding neuropeptide Y (NPY) [5]. Since Pau *et al.* [19] have demonstrated that injection of this neuropeptide into the third cerebroventricle of the adult ovariectomized rhesus monkey inhibits GnRH release (*figure 4*), and since NPY neurons in the MBH are found in regions that also contain GnRH perikarya [39], it seems reasonable to propose that NPY must be considered, along with GABA, as a

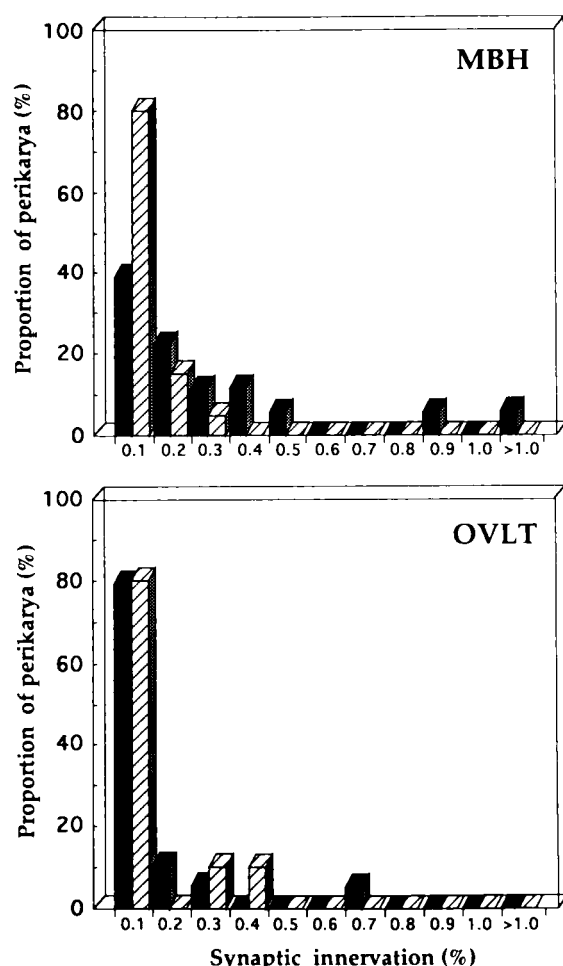


FIG. 3. — Differences in synaptic input onto GnRH perikarya in juvenile (solid bars) and adult (hatched bars) male monkeys as reflected by the frequency distribution of GnRH cell bodies in the MBH (top) and OVLT (bottom) with increasing percentages of their perikaryal membrane occupied by synaptic densities. Reprinted with permission from [20].

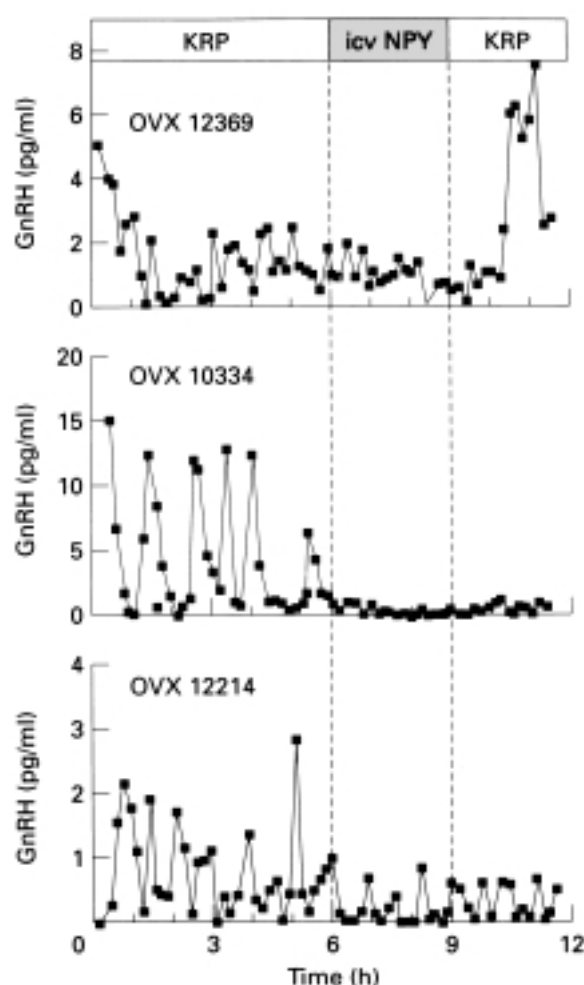


FIG. 4. — Suppression of GnRH release measured in hypothalamic perfusates in response to the continuous infusion of NPY (broken lines and stippled bars) into the third cerebro-ventricle of three ovariectomized adult monkeys. KRP-Krebs Ringer phosphate buffer. Reprinted with permission from [19].

potential component of the prepubertal brake on the GnRH pulse generator.

In addition to potential neuronal signals that might mediate the prepubertal brake to the GnRH neuron, the possibility that the hiatus in GnRH release at this stage of development also involves glial inputs has been proposed [13]. In contrast to the scant innervation of GnRH neurons, glial ensheathment of both GnRH perikarya and axons is substantial [20, 43; Durrant and Plant, unpublished observations]. Moreover, in the female rhesus monkey, the pubertal reaugmentation in pulsatile GnRH release is associated with an increased hypothalamic expression in the gene encoding transforming growth factor α (TGF α) [13]. Since this

growth factor, which is produced by glial cells, has been shown to stimulate GnRH release in the rat [18], it has been proposed by Ojeda and his colleagues [13] that the pubertal increase in TGF α may be the trigger for the reaugmentation of pulsatile GnRH release at this stage of development. The recent finding that GnRH axons make numerous *en passant* « synaptoid » contacts with astrocytes in the monkey median eminence (Durrant and Plant, unpublished observations), however, raises the alternate possibility that the TGF α response at puberty is a result of the reactivation of the GnRH neuronal network.

In summary, it should be emphasized that the foregoing observations on the potential role of neurotransmitters and neuromodulators in triggering the reaugmentation of pubertal GnRH release at the end of the juvenile phase of development have yet to be placed into a unifying hypothesis to account for the onset of primate puberty.

PERIPHERAL SIGNALS COORDINATING DEVELOPMENTAL CHANGES IN GnRH PULSE GENERATOR ACTIVITY

It is reasonable to propose that activation of the neurobiological mechanisms that trigger the reawakening of the GnRH pulse generator is coordinated, at least in part, by peripheral signals that reflect somatic growth and other aspects of development. In this regard, the attainment of a particular proportion of body fat has long been argued by Frisch and her colleagues [8] to be requisite for the onset of human puberty. Interest in this notion has recently been rekindled because of the discovery of leptin, a protein derived from adipocytes, that provides the hypothalamus with a somatic signal that relays information on fat mass to the central neural control systems regulating feeding behavior. There is also no doubt that leptin is able to exert significant effects on the hypothalamic-pituitary-gonadal axis [1, 3]. Moreover, in man, mutations of the genes coding for leptin or the leptin receptor result in disorders of pubertal development [4, 35]. These observations, together with the tantalizing finding that circulating leptin concentrations rise in association with the onset of puberty in both normal boys and girls [10, 14], may be taken to argue that leptin is the trigger for the onset of puberty. The foregoing considerations may be countered by a comparative argument. Namely, the fundamental hall-

marks of the ontogeny of the GnRH pulse generator in monkey and man appear to be identical (*figure 1*) and unique to higher primates. The most parsimonious explanation for this similarity across species of higher primate is that the lifting of the prepubertal brake on the GnRH pulse generator in man and monkey is cued by the same developmental signal. If this argument is accepted, then the hypothesis that leptin is the trigger for primate puberty must be rejected because in the male monkey, in contrast to man, a rise in circulating leptin concentrations does not precede the pubertal reaugmentation of GnRH pulse generator activity, as reflected by initiation of nocturnal testosterone secretion ([26] *figure 5*). While the foregoing argument fails to support the idea that leptin and adipose tissue comprise the pubertal clock, they do not detract from the notion derived from studies of rat that, in the context of developmental changes in GnRH pulse generator activity, leptin serves as a permissive circulating signal of nutritional status. Moreover, undernutrition in the adult monkey leads to impaired GnRH pulse generator activity [2] and therefore to a pseudoprepubertal condition. If leptin is established to be the permissive metabolic signal that allows optimal GnRH pulse generator activity in nonfasted adults [7], it would be reasonable to predict that this

role of leptin would also be operational during other stages of development, such as puberty, when GnRH pulse generator activity is being expressed. Accordingly, low levels of circulating leptin at this critical stage of development would mask the manifestation of the pubertal reawakening of the GnRH pulse generator, triggered by the true pubertal clock. What remains to be resolved is the reason for the difference in the peripubertal pattern in circulating leptin in monkeys and boys. Perhaps, this is related to differences in the proportional increase of fat to overall body weight during the pubertal growth spurt that is seen in both species [37].

THE GnRH PULSE GENERATOR IN THE ADULT

The GnRH pulse generator of the adult comprises an integral component of the feedback loops that regulate ovarian cyclicity and spermatogenesis, and forms the interface between the central nervous system and the pituitary-gonadal axis. In the latter role, the GnRH pulse generator relays to the reproductive axis the impact of stress, the consequences of metabolic imbalance arising from factors related to nutrition and exercise, and the influence of exteroceptive cues such as photoperiod. The neurobiological inputs that mediate the many factors that determine GnRH pulse generator activity in the adult are poorly understood but perhaps may be anticipated to involve novel mechanisms. This is because classical synaptic inputs to GnRH perikarya are scant [20, 34] and it is difficult to envision how such a limited signalling mode, alone, could effect such diverse control of the reproductive axis.

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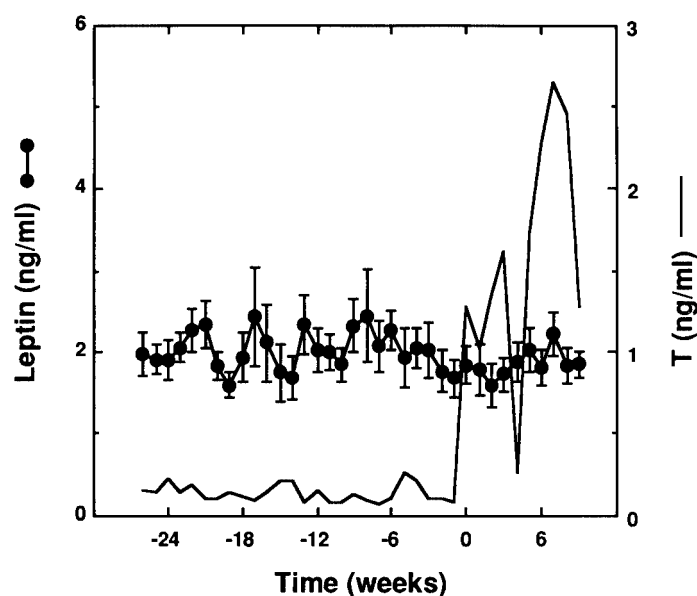


FIG. 5. — The absence of a relationship between circulating leptin concentrations (closed circles) and the onset, at week 0, of nocturnal testosterone secretion (open circles) in the male rhesus monkey. The dramatic increase in testosterone secretion indicates that the pubertal re-augmentation of pulsatile GnRH release has occurred. From [26] with permission.

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