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Electrophysiological correlates of pulsatile and surge gonadotrophin secretion

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The hypothalamic gonadotrophin-releasing hormone (GnRH) pulse generator governs intermittent discharges of GnRH into the pituitary portal circulation and, consequently, modulates the pulsatile pattern of gonadotrophin secretion. Electrophysiological correlates of pulsatile gonadotrophin secretion have been demonstrated in the mediobasal hypothalamus of monkeys, rats and goats by recording multiple unit activity. A temporal coincidence between characteristic increases in multiple unit activity and gonadotrophin pulses in the circulation is seen under a variety of physiological and experimental conditions in all three species examined, providing evidence that hypothalamic multiple unit activity originates in the GnRH pulse generator. During a preovulatory gonadotrophin surge induced by oestrogen in ovariectomized animals or occurring spontaneously in intact animals, GnRH pulse generator activity is decelerated, suggesting that it is not involved in generating the gonadotrophin surge. The gonadotrophin surge may be generated by an oestrogen-responsive neuronal complex intrinsically different from the GnRH pulse generator, the electrical operation of which remains unknown.

The secretion of gonadotrophins from the pituitary in female mammals occurs in two different forms; one is pulsatile and the other is the preovulatory surge. The pulsatile nature of gonadotrophin secretion was first noted in ovariectomized monkeys (Dierschke *et al.*, 1970) and subsequently in all male and female mammals studied. The female-specific gonadotrophin surge is evoked by oestrogen secreted from growing follicles and induces ovulation and luteinization. Pulsatile gonadotrophin secretion is elicited by intermittent release of gonadotrophin-releasing hormone (GnRH) from the hypothalamus into the pituitary portal circulation (Clarke and Cummins, 1982), while a large amount of GnRH is released during the preovulatory gonadotrophin surge (Levine and Ramirez, 1982; Moenter *et al.*, 1991; Xia *et al.*, 1992). Each bolus of GnRH release results in an episode of pulsatile gonadotrophin secretion and is the consequence of the synchronous discharge of GnRH neurones in the hypothalamus. The neuronal circuitry responsible for the rhythmic activation of GnRH neurones is known as the 'GnRH pulse generator'.

The GnRH pulse generator is a key determinant of reproductive function in mammalian species (Knobil, 1980; Lincoln *et al.*, 1985; Levine *et al.*, 1991). The pulsatile pattern and the pulse frequency of gonadotrophin secretion are essential for the normal expression of gonadal function. Frequency modulation of pulsatile GnRH release may be a principal regulatory mode underlying the central control of reproductive function. The GnRH pulse generator is thought to be the most important final common mediator of all factors influencing reproduction conveyed through the brain. Therefore, GnRH pulse generator activity provides an insight into the way the brain integrates and recognizes internal and external signals that control reproductive function. A method for measuring episodic increases in hypothalamic electrical activity associated with pulsatile gonadotrophin secretion has been developed in monkeys (Knobil, 1981), rats (Kimura *et al.*, 1991) and goats (Mori *et al.*, 1991).

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Recording of hypothalamic electrical activity

Attempts to determine the electrical events associated with pulsatile gonadotrophin secretion were first made in rhesus monkeys by Knobil and coworkers using a multiple unit activity (MUA) recording technique. They recorded characteristic increases (volleys) in MUA coincident with the initiation of each luteinizing hormone (LH) pulse in the mediobasal hypothalamus (MBH) of ovariectomized monkeys (Knobil, 1981). In these early studies, single, acutely placed electrodes were used to record hypothalamic MUA in anaesthetized animals. Subsequently, recordings of greater clarity were obtained in both anaesthetized and conscious monkeys fitted with chronically implanted multiple electrode arrays in the MBH (Wilson *et al.*, 1984).

In our laboratory, MUA volleys correlated with LH pulses in the peripheral circulation in the MBH of freely moving rats (Kimura *et al.*, 1991) and 'Shiba' goats (Mori *et al.*, 1991) were obtained by means of a similar recording technique. A multiple electrode array was used consisting of 4–6 teflon-insulated platinum-iridium wires (75 μm in diameter), encased together

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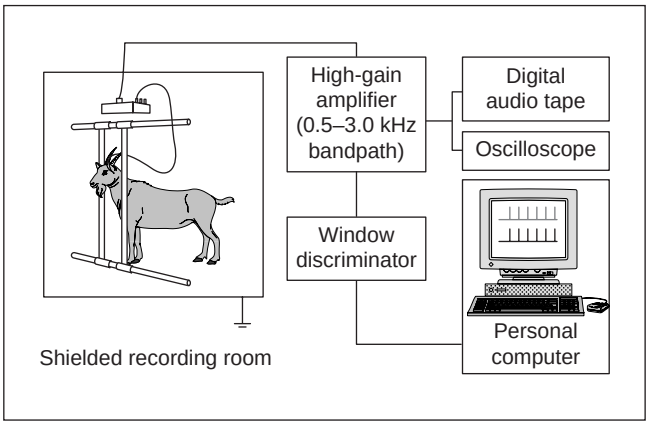

Fig. 1. Schematic illustration of the recording system for multiple unit activity (MUA) in goats. The electrode assembly is stereotaxically implanted in the mediobasal hypothalamus (MBH). The goat is tied loosely to the stanchion, and a buffer amplifier is plugged directly onto the electrode assembly. Differential input signals between two electrodes are further amplified by a high gain amplifier and fed into the amplitude discriminator. The processed activity rate is stored in a personal computer, and unprocessed signals are also recorded on a digital audiotape for off-line analysis of MUA.

in a stainless steel guide tube for chronic implantation in the brain. The tip of the electrode was targeted at the MBH, including the arcuate-median eminence. After 1–2 weeks recovery, goats were transferred to the recording room and tied loosely to stanchions where they can feed and rest during the recording period. In rats, MUA recordings were started around 5 days after brain surgery. At the time of recording, a buffer amplifier-integrated circuit is plugged directly onto the electrode assembly to reduce the noise along the signal path and to filter out artefacts from other sources of electrical activity, such as muscle contraction. Differential input signals between two electrodes are further amplified by means of a high gain amplifier and fed into the amplitude discriminator, so that processed activity rate is stored in a personal computer. Unprocessed signals are also recorded on a digital audiotape for further offline analysis of MUA (Fig. 1).

The MUA recording technique makes it possible to carry out real-time analysis of the GnRH pulse generator activity in conscious and unrestricted animals for several months. Moreover, in theory, the effects of hormones, nutrition, suckling stimuli, stressors, environmental cues and pheromones can be assessed at both the hypothalamus and the pituitary independently and simultaneously.

Electrophysiological correlates of pulsatile gonadotrophin secretion

GnRH pulse generator activity in ovariectomized animals

An abrupt increase in hypothalamic MUA associated with each LH pulse has been demonstrated in monkeys, rats and goats. An example of the correlation between hypothalamic MUA and pulsatile LH profiles in an ovariectomized goat is shown (Fig. 2). MUA volleys are characterized by an initial

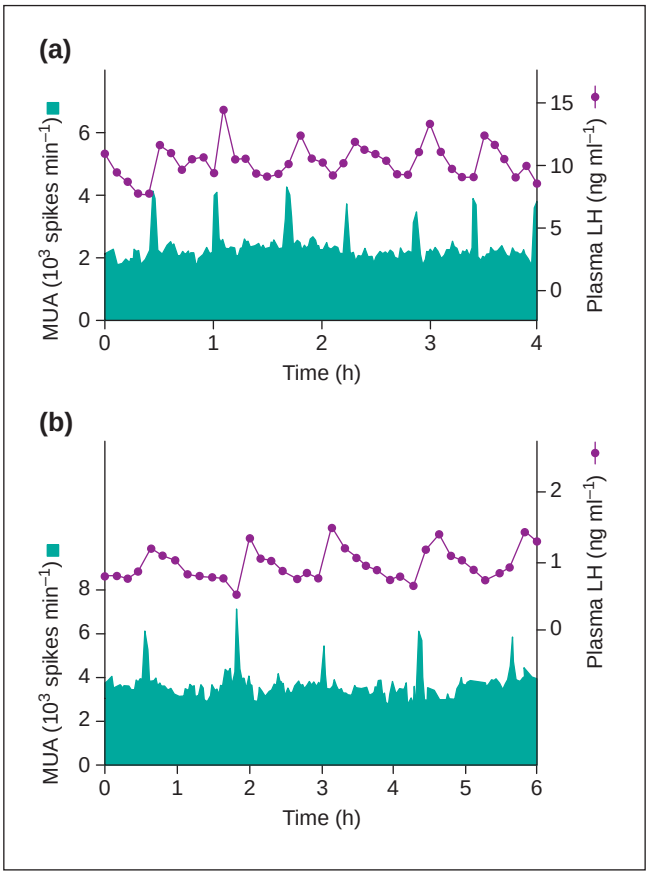

Fig. 2. Representative example of hypothalamic multiple unit activity (MUA) and plasma LH profiles in (a) ovariectomized and (b) intact female goats. MUA is expressed in terms of spikes min^{-1}. Note the temporal relationship between MUA volleys and LH pulses.

brief overshoot that reaches a peak value within 30 s and then declines gradually to the baseline value. An MUA volley invariably precedes each LH pulse, and this temporal association between the two remains consistent, even when the pulse frequency is altered by treatments with gonadal steroids (Mori *et al.*, 1991; Tanaka *et al.*, 1994).

Use of the technique for sampling pituitary portal blood originally developed for sheep (Clarke and Cummins, 1982) has detected LH pulses in the systemic circulation that are preceded by discrete abrupt increases in GnRH secretion in ovariectomized goats (Tanaka *et al.*, 1997). The interval between the GnRH pulse and following LH pulse is virtually identical to that between a MUA volley and the succeeding LH pulse.

Ovariectomized monkeys, rats and goats show species-specific MUA volley characteristics. For example, the interval between volleys is about 60, 40 and 20 min, and volley duration is about 15, 4 and 2 min in ovariectomized monkeys, goats and rats, respectively. The MUA volleys in ovariectomized monkeys have a plateau phase after the initial overshoot that is not as obvious in ovariectomized rats and goats (Wilson *et al.*, 1984). The duration and frequency of MUA volleys appear to be regulated independently. This view is supported by the observations that both barbiturates and morphine reduce

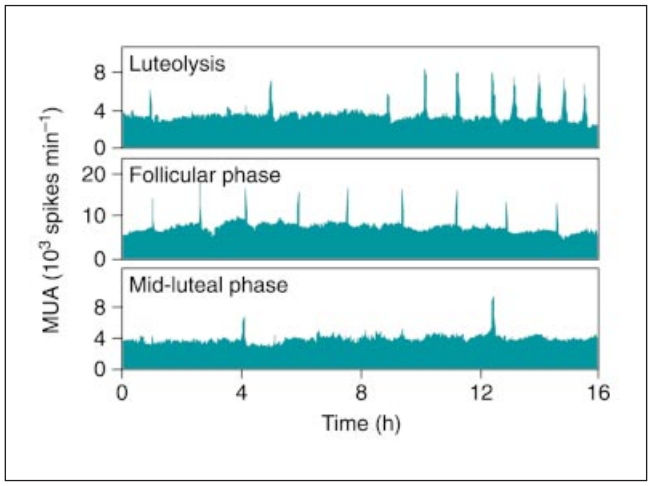

Fig. 3. Changes in the frequency of multiple unit activity (MUA) volleys during the oestrous cycle of a female goat. MUA is expressed in terms of spikes min^{-1}.

volley frequency, while barbiturates lengthen and morphine reduces volley duration (Wilson *et al.*, 1984; Williams *et al.*, 1990b). However, the mechanisms regulating the duration and frequency of MUA volleys remain to be resolved. All that can be said at present is that short-loop and ultrashort-loop feedback actions of LH and GnRH on the GnRH pulse generator, respectively, do not seem to be involved in the rhythmic activation and deactivation of the generator, because neither systemic (Kesner *et al.*, 1986) nor intraventricular (Ordog *et al.*, 1997) administration of GnRH, its agonist or antagonist affect volley duration or frequency.

The frequency of MUA volleys determines the frequency of GnRH pulses directly, and the role of changing frequency in the control of gonadotrophin secretion has been clearly demonstrated (Pohl *et al.*, 1983). Although the physiological significance of changes in the duration of the MUA volley is unclear, the relationship between volley duration and the dynamics of LH pulses has been studied extensively in ovariectomized monkeys (Williams *et al.*, 1990a). Since no significant correlation has been obtained between volley duration and the magnitude of LH pulses, all of the GnRH secreted per pulse seems to be released at the onset of each MUA volley. The remainder of the increase in electrical activity is unlikely to have further influence on GnRH secretion, although effects on other systems cannot be excluded.

Continuous monitoring of the GnRH pulse generator activity has been possible by recording the MUA, and the diurnal variation in the frequency of MUA volleys has been examined in monkeys, rats and goats. In the diurnal monkey, as anticipated from previous studies of LH pulse frequency, the frequency of MUA volleys is reduced markedly at night (O'Byrne *et al.*, 1993a), while in the nocturnal rat, the frequency of LH pulses increases during the dark phase (Hiruma *et al.*, 1992). The volley frequency in goats does not change over 24 h (T. Tanaka, T. Shimizu and Y. Mori, unpublished). These findings suggest that the circadian oscillation of the GnRH pulse generator activity varies from species to species.

The identity of the neural circuits generating MUA volleys in the MBH is uncertain. However, histological observations show that the tip of most of the active electrodes, from which the MUA volleys are recorded, are located in the arcuate nucleus and median eminence, indicating that the volleys may be recorded from the GnRH axonal tract or terminal field. In rats, MUA volleys associated with LH pulses do not occur in the preoptic area, although the majority of GnRH neurones are located in this area (Kawano and Daikoku, 1981). The reason may be that GnRH neurones are few in number and widely scattered in the preoptic area (Wray and Hoffman, 1986). Alternatively, the preoptic area may not be substantially involved in generating pulsatile LH secretion in ovariectomized rats, since LH pulses are maintained even after the complete deafferentation of the hypothalamus (Blake and Sawyer, 1974; Soper and Weick, 1980). Recent results from our laboratory support the view that MUA volleys are not discharged from GnRH neurones themselves but from a neuronal oscillator located in the MBH that activates GnRH neurones. However, it is possible that MUA volleys are a secondary consequence of GnRH neuronal activation.

It is possible to access the hypothalamic systems controlling the pulsatile secretion of GnRH directly by monitoring hypothalamic MUA. The effects of a wide range of substances regulating GnRH pulse generator activity measured as a correlate of MUA volleys have been investigated in ovariectomized animals. Substances shown to affect MUA volleys include corticotropin-releasing hormone (Williams *et al.*, 1990c), opioid peptides (Williams *et al.*, 1990b; Kimura *et al.*, 1991; Nishihara *et al.*, 1991), GnRH (Kesner *et al.*, 1986; Ordog *et al.*, 1997), noradrenaline (Kaufman *et al.*, 1985; Nishihara *et al.*, 1991), dopamine (Kaufman *et al.*, 1985), γ-amino butyric acid (Kimura *et al.*, 1993), blood glucose (Chen *et al.*, 1992), endotoxin (Takeuchi *et al.*, 1997; Yoo *et al.*, 1997a), cytokine (Yoo *et al.*, 1997a,b), prostaglandins (Yoo *et al.*, 1997b), sex steroids (Kesner *et al.*, 1987; Tanaka *et al.*, 1994; Nishihara *et al.*, 1994; Ordog and Knobil, 1995) and pheromones (Hamada *et al.*, 1996).

GnRH pulse generator activity in intact animals

Recordings of MUA volleys coincident with LH pulses have been made in intact monkeys (O'Byrne *et al.*, 1991) and goats (Tanaka *et al.*, 1995). Representative hypothalamic MUA together with plasma LH profiles in a female goat during the follicular phase of the oestrous cycle are shown (Fig. 2). The correlation between MUA volleys and LH pulses was evident. Similar correlation was obtained in intact female monkeys by using a radiotelemetoric monitoring system. However, in goats and monkeys, the frequency of MUA volleys associated with GnRH pulse generator activity was lower in intact animals than in ovariectomized animals.

Specific MUA volleys were recorded continuously in intact female goats to examine changes in GnRH pulse generator activity throughout an entire ovulatory cycle (Tanaka *et al.*, 1995). The duration of the ovulatory cycle in Shiba goats is 3 weeks, and hormonal profiles during the cycle have been described by Mori and Kano (1984) and Mori (1992). Patterns of MUA volleys at three different stages of the ovulatory cycle are shown (Fig. 3). Near the end of spontaneous luteolysis, MUA volley frequency increases abruptly and then reduces but remains relatively high during the follicular phase. The volley

frequency is low throughout the luteal phase. Thus, there is a reciprocal relationship between MUA volley frequency and plasma progesterone concentrations, suggesting a suppressive effect of progesterone on the GnRH pulse generator activity. However, when this possibility was investigated in ovariectomized goats, progesterone in combination with oestradiol suppressed MUA volley frequency, but progesterone alone was ineffective (Tanaka *et al.*, 1994).

The duration of the MUA volley varies during the oestrous cycle; it is shorter in the follicular phase and longer in the luteal phase than that in ovariectomized animals. A similar tendency for volley duration change during the oestrous cycle is also observed in monkeys, although the duration in both follicular and luteal phases is much shorter than that in ovariectomized monkeys. After ovariectomy, volley duration in monkeys increases progressively for a few months (O'Byrne *et al.*, 1993b), which may reflect the increase in the excitability of the GnRH pulse generator due to the removal of an inhibitory effect of ovarian steroids. Consistent with this view, oestradiol administered at physiological concentrations shortens volley duration in ovariectomized monkeys (Kesner *et al.*, 1987). Oestrogen affects the excitability of hypothalamic neurones directly (Nishihara and Kimura, 1989) or indirectly by changing the sensitivity to neurotransmitters (Nishihara *et al.*, 1986). Morphological changes, such as synaptic remodelling caused by oestrogen (Garcia-Segura *et al.*, 1986), may be involved in slow changes in volley duration.

Electrophysiological correlates of surge gonadotrophin secretion

Steroid-induced gonadotrophin surge in ovariectomized animals

A large amount of GnRH is released into the portal circulation in female rats (Levine and Ramirez, 1982), ewes (Moenter *et al.*, 1991) and monkeys (Xia *et al.*, 1992) during the gonadotrophin surge. Although the neuronal mechanisms involved in inducing the GnRH surge remain to be identified, the gonadotrophin surge is thought to be a consequence of an increase in the frequency or amplitude of gonadotrophin pulses. However, recent electrophysiological studies have shown decreased GnRH pulse generator activity during either an oestrogen-induced LH surge in ovariectomized animals, or a spontaneous preovulatory LH surge in intact animals.

The electrical activity of the hypothalamic GnRH pulse generator during a preovulatory-like LH surge was first recorded in ovariectomized rhesus monkeys injected with oestrogen at doses designed to achieve physiological concentrations (Kesner *et al.*, 1987). After oestrogen injection, serum LH concentrations decreased initially, before the LH surge. MUA volleys faded coincident with the disappearance of LH pulses, but remained suppressed even during the LH surge.

In ovariectomized goats, an LH surge was induced by programmed administration of oestradiol, mimicking the preovulatory increase in oestradiol, and hypothalamic MUA was recorded (Tanaka *et al.*, 1992). Representative patterns of MUA volleys and plasma LH profiles are shown (Fig. 4). Plasma LH concentrations increased abruptly about 10 h after the start of oestrogen infusion with a peak value occurring at around 14 h. MUA volleys occurred throughout the experimental period,

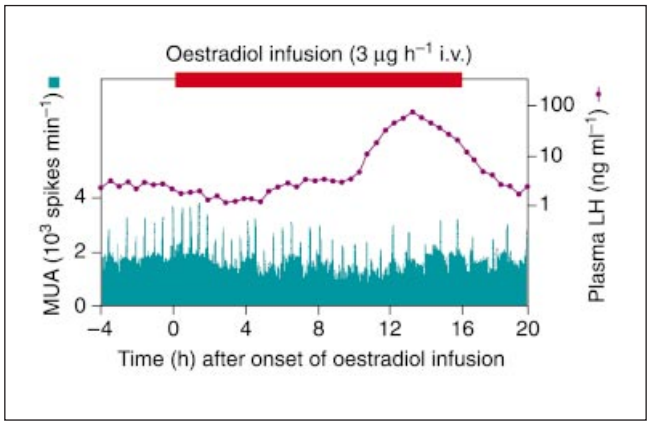

Fig. 4. Representative example of hypothalamic multiple unit activity (MUA) and plasma LH profiles during intravenous infusion of oestradiol in an ovariectomized goat. MUA is expressed in terms of spikes min^{-1}, and plasma LH is plotted at 30 min intervals. Note the decrease in MUA volley frequency during the oestradiol-induced LH surge.

although their frequency decreased with time during oestrogen infusion and became more pronounced after the onset of the LH surge.

In ovariectomized rats, after the implantation of Silastic tubing containing oestradiol, MUA volley frequency decreased gradually and then volleys stopped (Nishihara *et al.*, 1994). In the afternoon, 3 days later, a surge of LH release was observed, whereas neither MUA volleys nor characteristic changes in the baseline activity associated with the LH surge emerged. The next day, a much greater LH surge induced by the injection of progesterone was found not to be associated with either MUA volleys or changes in baseline electrical activity.

It is concluded that MUA volleys are totally suppressed in monkeys and rats, while they persist, but at low frequency, in goats during a steroid-induced LH surge. This species difference may be explained, at least partially, by differences in oestrogen concentrations in the peripheral circulation. The concentration of serum oestrogen required to induce an LH surge is much lower in goats than it is in monkeys and rats. If higher doses of oestrogen were infused into ovariectomized goats, MUA volleys might be suppressed completely.

Preovulatory gonadotrophin surge in intact animals

During the menstrual cycle in rhesus monkeys, MUA volley frequency increases as functional luteolysis progresses, reaching approximately one volley per hour in the first few days of the follicular phase, and then decreases markedly, coincident with the initiation of the preovulatory LH surge (O'Byrne *et al.*, 1991). During the LH surge, MUA volleys are very infrequent and, subsequently, resume a frequency of approximately one volley per 4 h, characteristic of the early luteal phase. These observations are in keeping with those during the oestrogen-induced LH surge in ovariectomized monkeys.

The spontaneous preovulatory LH surge in intact goats is also accompanied by a transient, but not precipitous, decrease in MUA volley frequency (Tanaka *et al.*, 1995). The relationship

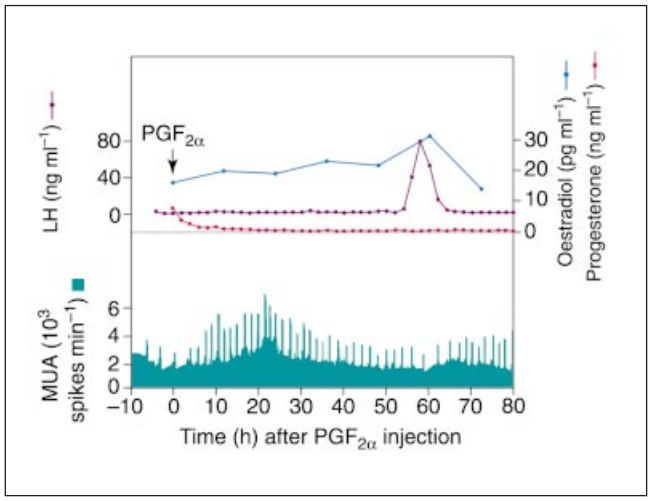

Fig. 5. Representative example of hypothalamic multiple unit activity (MUA) and plasma LH, oestradiol and progesterone profiles after $PGF_{2\alpha}$ injection into an intact female goat during the mid-luteal phase. MUA is expressed in terms of spikes min^{-1}, and plasma concentrations of LH and progesterone are plotted at 2 h intervals, while those of oestradiol are plotted at 10 h intervals. Note the decrease in MUA volley frequency during the pre-ovulatory LH surge.

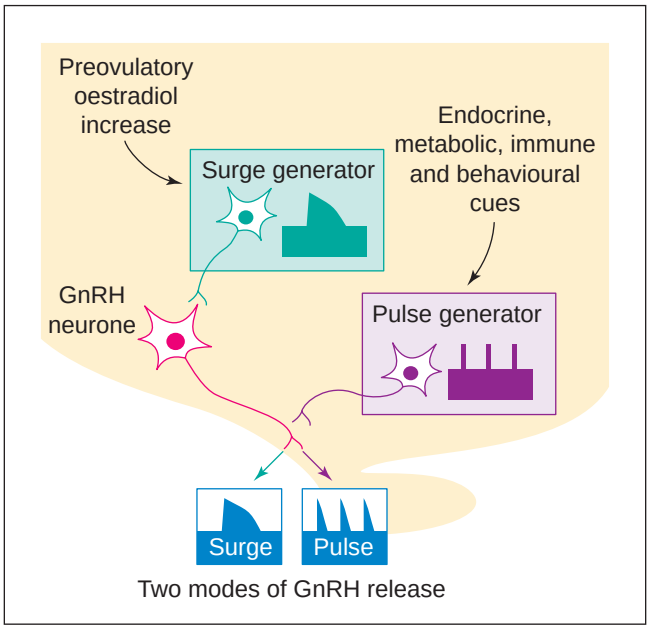

Fig. 6. Schematic illustration of the GnRH pulse- and surge-generating systems. There are two distinct neuronal circuits, the pulse and surge generators, involved in regulating the excitability of GnRH neurones in the hypothalamus. The GnRH pulse generator mediates the negative feedback action of oestrogen as well as endocrine, metabolic, immune and behavioural cues. The GnRH surge generator mediates the positive feedback action of oestrogen during the preovulatory period.

between serum LH, progesterone, oestradiol and changes in MUA volleys during the oestrous cycle has been investigated in female goats after injection of prostaglandin $F_{2\alpha}$ during the mid-luteal phase (Fig. 5). MUA volley frequency increased abruptly as progesterone concentrations decreased and continued to accelerate in the early follicular phase. The high frequency of MUA volleys was maintained throughout the follicular phase except for the period around the preovulatory LH surge, when the volley frequency was lowered temporarily. After this decline, volley frequency returned to the pre-surge value and, thus, there was a reciprocal relationship between volley frequency and oestradiol concentrations in the late follicular phase. These results are again in accordance with the observation in ovariectomized goats that the frequency of MUA volleys decreases during an oestrogen-induced LH surge.

A model for the surge-generating system

In all three species of animals examined, a decrease in GnRH pulse generator activity occurs during either a spontaneous or an induced LH surge. This finding suggests that the GnRH pulse generator is not involved in generating the LH surge and, therefore, that the LH surge is not a consequence of an increase in the frequency or amplitude of GnRH pulses. In ewes, frequent portal-blood sampling showed that GnRH pulses are superimposed on increasing GnRH baseline concentrations during the ascending limb of the GnRH surge (Evans *et al.*, 1995). Therefore, it seems that the GnRH surge is induced by neuronal circuitry that is different from the GnRH pulse generator.

Suppression of GnRH pulse generator activity during the surge coincides with increases in circulating concentrations of oestrogen, LH and GnRH. Although injection of GnRH into the third ventricle of ovariectomized ewes resulted in a significant reduction in the frequency of pulsatile LH secretion (Naylor *et al.*, 1989), the results of the studies in monkeys described above are incompatible with the presence of short-loop or ultrashort-loop feedback regulation of GnRH pulse generator activity. Similarly, there was no change in the frequency of MUA volleys after either peripheral or intracerebroventricular administration of GnRH in ovariectomized goats (A. Yamada, T. Tanaka, H. Kamomae and Y. Mori, unpublished). It is concluded that the deceleration of GnRH pulse generator activity during the gonadotrophin surge is the consequence of the increase in oestrogen concentrations in the peripheral circulation.

A large increase in GnRH release from the hypothalamus into the pituitary portal circulation is required to evoke the LH surge. Therefore, the excitability of GnRH neurones must be much increased during the surge. This view is supported by the finding in rats that expression of c-Fos, which may be a marker of increased neuronal excitability, is seen in GnRH neurones in the afternoon of pro-oestrus (Lee *et al.*, 1990). However, the electrical activity in the MBH does not show any discernible change during the LH surge, suggesting that the recorded MUA volleys are not discharged from GnRH neurones or their axon terminals.

Goats, monkeys and rats appear to share a common neuronal mechanism controlling the release of GnRH during the LH surge, which occurs in response to circulating oestrogen. Although GnRH neurones themselves do not have oestrogen receptors (Shivers *et al.*, 1983), dense collections of

cells containing oestrogen receptors occur in the hypothalamus (Pfaff and Keiner, 1973). This finding suggests that the actions of oestrogen on GnRH neurones are not direct, but are mediated via those oestrogen responsive cells. The observation that the baseline MUA recorded from the MBH does not change during the LH surge suggests that oestrogen-responsive neuronal systems located in brain regions other than the MBH participate in generating the LH surge, while the MBH, the electical activity of which is suppressed by oestrogen, is mainly involved in generating LH pulses. Therefore, it is hypothesized that there are two neuronal circuits involved in regulating the excitability of GnRH neurones (Fig. 6). One is the GnRH pulse generator that mediates the negative feedback action of oestrogen, together with endocrine, metabolic, immune and behavioural cues. The other is the GnRH surge generator that mediates the positive feedback action of oestrogen during the preovulatory period, the precise neurobiological basis and electrical operation of which remain to be elucidated.

Conclusions

The electrophysiological characteristics of hypothalamic GnRH pulse generator activity as measured by recording MUA in the medial basal hypothalamus have been investigated in primate, rodent and ruminant species. MUA volleys are tightly correlated with gonadotrophin pulses except during the gonadotrophin surge, when volley frequency decreases. At that time, GnRH and gonadotrophin secretion increase. Since the identity of the neurones from which specific MUA is recorded is unknown, care needs to be taken in interpreting the results obtained with this approach. Nevertheless, the dissociation of GnRH pulse generator activity from the mechanism controlling the gonadotrophin surge supports the view that different mechanisms underlie the GnRH pulse and surge generating systems.

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