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CENTRAL SITES CONTROLLING PITUITARY SECRETION IN THE RHESUS MONKEY

MICHEL FERIN
Institute for Reproductive Sciences, Columbia University College of Physicians and Surgeons, New York, NY 10032, U.S.A.

SUMMARY

The experimental evidence on the sites involved in the oestrogen–gonadotropin feedback loop in female rhesus monkeys is reviewed and discussed. The medial basal hypothalamic (MBH)–pituitary area is considered to contain all elements necessary for "cyclic" gonadotropin secretion control. MBH activity, however, is probably modulated by brain structures residing outside the area. Oestrogens can release gonadotropins and modulate prolactin secretion by a direct feedback effect at the anterior pituitary gland.

In the female, pituitary hormones initiate follicular development, thereby inducing ovarian steroid production and secretion. Oestrogens, in turn, feedback to the brain-pituitary axis to modulate gonadotropin output. "Tonic" secretion of gonadotropins is under the control of a negative or inhibitory feedback loop, whereby oestradiol maintains low LH and FSH circulatory levels; in rhesus monkeys, ovariectomy results in an increase in gonadotropin secretion [1]. The midcycle ovulatory "surge" of gonadotropins results from a positive or stimulatory feedback effect by oestradiol; active immunization to oestradiol suppresses cyclic LH release and renders previously cycling rhesus monkeys anovulatory [2]. Oestrogens may also influence prolactin secretion in rhesus monkeys since basal prolactin concentrations in ovariectomized animals are significantly reduced.

The site(s) at which oestradiol exerts its influence on gonadotropin and prolactin secretion, whether brain or pituitary, remain(s) controversial. This paper will briefly review the experimental evidence, obtained in female rhesus monkeys, in our and other primate laboratories, on the hypothalamic sites possibly involved in the oestrogen–gonadotropin feedback loop and discuss results suggesting that a major site for oestrogen control of gonadotropin and prolactin release may well reside within the anterior pituitary gland itself.

ROLE OF THE HYPOTHALAMUS

Surgical deafferentation experiments, in which the medial basal hypothalamic area (MBH) was disconnected from the remainder of the brain, did not interfere with oestrogen-induced gonadotropin release, menstrual cyclicity nor tonic gonadotropin secretion [3, 4]. Such results clearly indicate that, in the rhesus monkey, the oestrogen-responsive sites involved in the control of the menstrual cycle must reside within the MBH–pituitary unit. This is in sharp contrast with results obtained in rodents [5] and sheep [6] using a similar experimental approach. In these species, complete MBH "islands" resulted in anovulation and neural inputs entering the MBH rostrally are required for the LH ovulatory surge. After complete deafferentation in the monkey, these rostral connections were interrupted and should, therefore, not play a role. However, Norman *et al.*[7] reported that surgical or electrochemical lesions within the rostral hypothalamus blocked the oestrogen-induced LH release in the rhesus monkey and would therefore presumably interrupt spontaneous menstrual cyclicity. These results are difficult to reconcile with the fact that complete deafferentation did not interfere with normal menstrual cyclicity. We are presently exploring this problem in our laboratories [8]. In this experiment, 9 monkeys were subjected to unilateral or bilateral anterior hypothalamic surgical disconnection. During the first 75 days following surgery, all 3 animals with unilateral anterior hypothalamic lesions had a normal menstrual cycle, and responded to an oestrogen challenge by releasing gonadotropins. In contrast, none of the 6 animals with bilateral lesions had either spontaneous or oestrogen-induced gonadotropin surges during that same observation period, although they underwent identical surgical trauma. Animals undergoing complete deafferentation, however, resumed "cyclic" release of gonadotropin within this period. However, observation of 3 of the animals with bilateral anterior hypothalamic lesions over extended periods of time revealed that they spontaneously resumed menstrual cycles 4 to 7 months following surgery, and responded to an oestrogen challenge by releasing both LH and FSH. The possibility that return of menstrual cyclicity may be a result of neuronal regeneration across the cut can be reasonably excluded, for a silastic membrane had been inserted into the knife cut.

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We conclude from these experiments that, in contrast to other species, the MBH–pituitary area of the primate contains all elements necessary to control gonadotropin secretion and that inputs from structures outside this area are not essential for menstrual cyclicity. In fact, gonadotropin surges can be induced in monkeys following removal of all neural tissues dorsal and anterior to the optic chiasm [9]. The role of structures situated outside the MBH, either within the anterior hypothalamic–preoptic area or within other central structures whose axons pass through this area, however, can not be eliminated entirely for they appear to play a modulatory influence on gonadotropin secretion. But their absence, after a period of readjustment, does not prevent normal gonadotropin cyclic function. Supporting this hypothesis is the fact that, contrary to the female rat [10], oestradiol implanted into the anterior hypothalamus in the female monkey did not result in a release of LH [11].

ROLE OF THE PITUITARY GLAND

Because of the close anatomical and functional relationship between hypothalamic structures and the anterior pituitary, *in vivo* experiments to differentiate direct pituitary effects from those relayed through the hypothalamus are difficult to design. As a model to approach this problem, we have used monkeys in which the pituitary stalk had been sectioned [12]. To prevent revascularization of the pituitary, a silastic sheeting had been inserted to cover the diaphragma sellae. Isolation of the pituitary from the hypothalamus resulted in an alteration of its secretory pattern. The hormonal profile following pituitary stalk section is illustrated in Fig. 1. Prolactin secretion increased rapidly within the first few days following surgery and was accompanied by galactorrhea. Both persisted for the duration of the experimental observation period (up to 14 months) [13]. LH and oestrogen levels decreased rapidly following stalk section and menstrual cyclicity was arrested. Immunocytochemical observations of the anterior pituitary confirmed the changes in secretory patterns: prolactin cells were abundant, but LH cells disappeared within 2 weeks following stalk section [14].

Oestradiol and the gonadotroph

In rodents, oestrogens have been shown to induce histological changes within the pituitary [15] and to modulate gonadotropin secretion *in vitro* [16]. In most species, including the rhesus monkey, anterior pituitary cells bind 17β-oestradiol [17, 18]. In our laboratories, we tested whether 17β-oestradiol could affect gonadotropin release in stalk sectioned monkeys by a direct positive feedback effect at the pituitary level [19]. An oestrogen challenge, producing circulating oestradiol levels similar to those seen at the time of the endogenous midcycle gonadotropin surge, was administered to animals 8–12 h following pituitary stalk section. As illustrated in Fig. 2 in 2 representative animals, oestradiol induced LH and FSH surges, which did not differ significantly in amount or timing from similarly induced surges in intact monkeys. These results clearly indicate that the iso-

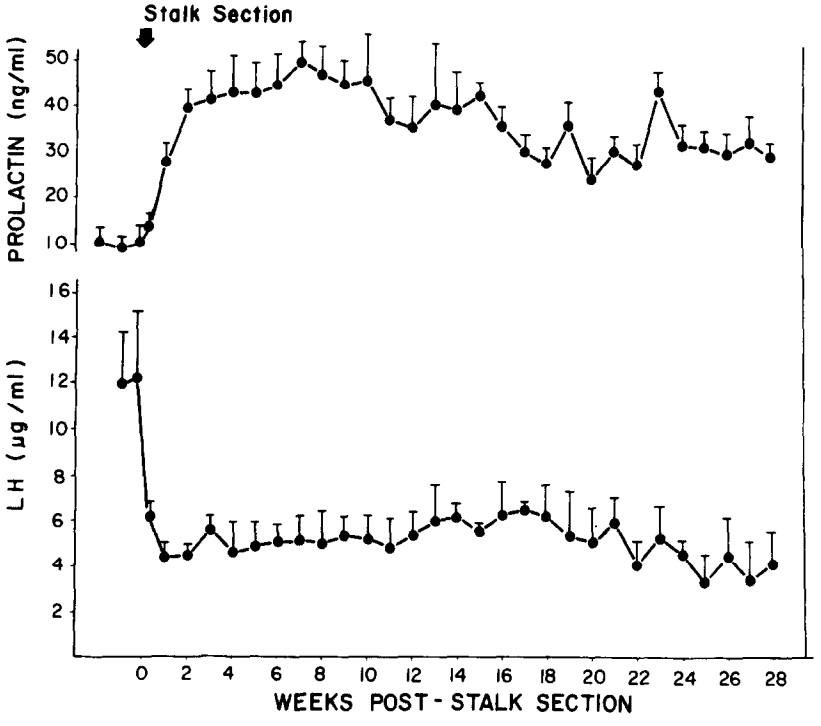

Fig. 1. Changes in prolactin and LH secretion, as measured by radioimmunoassay, in rhesus monkeys following pituitary stalk section.

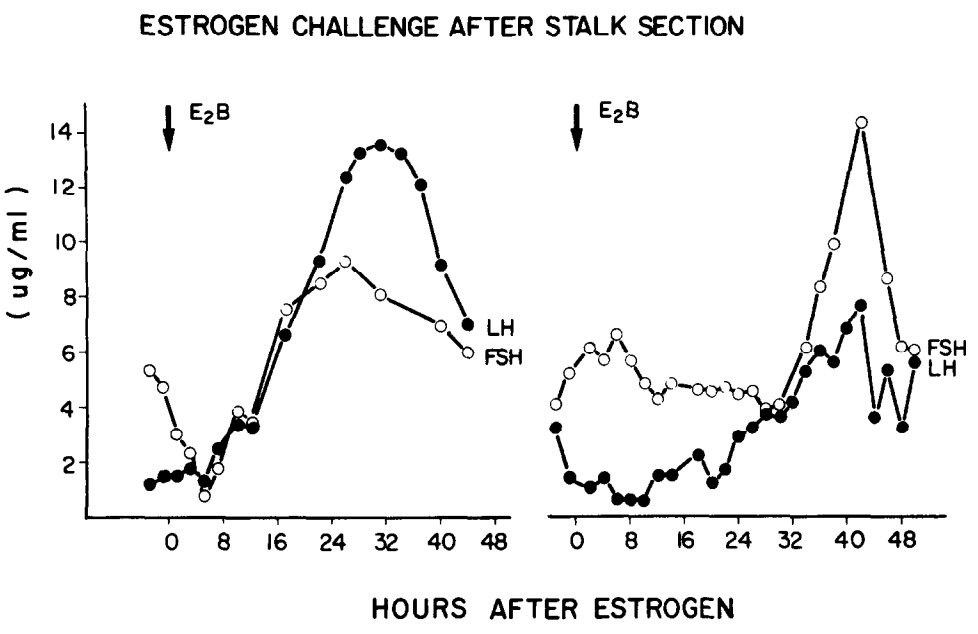

Fig. 2. LH and FSH responses to an oestrogen challenge in 2 rhesus monkeys, 8–10 h following pituitary stalk section.

lated pituitary can respond to oestrogens by releasing gonadotropins.

Were oestradiol to induce the midcycle surge by a direct pituitary effect in the intact animal as well, the question as to the role of gonadotropin releasing hormone (GnRH) in controlling gonadotropin secretion remains to be elucidated. Neill *et al.*[20] reported that the midcycle gonadotropin surge in rhesus monkeys is accompanied by an increased secretion of GnRH into the stalk portal blood circulation. Although this GnRH increase remains to be precisely quantified, a role of the hypothalamus in the gonadotropin surge can *a priori* not be discounted. However, in our experiments in stalk-sectioned animals, the pituitary stalk had been cut prior to oestradiol administration and therefore prior to an expected GnRH surge. In contrast to the doubtful role of GnRH in the control of cyclic gonadotropin release, several experiments have established that the decapeptide is essential in maintaining the secretory capacity of the gonadotroph in monkeys: after passive immunization to GnRH [21], after pituitary stalk section (Fig. 1) or after lesions of the arcuate region [22], LH levels decreased precipitously. In the animals with arcuate lesions, secretion by the gonadotroph could be reactivated by GnRH pulsatile infusions; LH and FSH concentrations increased to presurgical levels and, as in stalk-sectioned animals, an oestrogen stimulus resulted in a gonadotropin surge [22]. Administration of the decapeptide in a pulsatile fashion was found to be essential in maintaining appropriate tonic gonadotropin levels over prolonged periods of time [22]; by contrast, continuous GnRH infusions, which initially increased LH and FSH release, resulted in decrease in the secretory activity of the gonadotroph [23], presumably through a "down" regulation effect at the pituitary receptor level. In fact, physiological release of GnRH into stalk portal vessels was also found to be pulsatile, pulse frequency and amplitude varying with the endocrine milieu [24]. One can speculate as to whether a pulsatile type of GnRH release is crucial to maintain appropriate tonic gonadotropin levels as well as LH/FSH ratios.

Oestradiol and the lactotroph

Oestrogens are known to have a sensitizing effect on the release of prolactin. In rats, an increase in prolactin accompanies the preovulatory oestrogen surge [25]. Basal levels of prolactin are lower in ovariectomized monkeys (range: 1–4 ng/ml) than in intact controls (range: 4–12 ng/ml). Prolactin release to TRH is higher in human females than males and, in hypogonadal males or females, it can be increased with oestrogen treatment [26, 27]. The site at which oestradiol acts to modulate prolactin secretion remains to be elucidated. It is known that oestrogens cause an increased turnover in hypothalamic dopamine, a catecholamine inhibitory to prolactin release [28]. On the other hand, evidence from experiments related below supports a direct action by oestradiol on the pituitary. In these experiments, pituitary stalk sectioned monkeys were placed under a prolonged oestrogen environment; changes in basal prolactin secretion and in TRH-induced prolactin responses were monitored. First, one oestradiol-containing capsule, producing peripheral levels of oestradiol in the range of those seen during the early follicular phase, was implanted subcutaneously for a period of 3 weeks. Then a second capsule was added for an additional 3 weeks to increase oestrogen levels to those seen during the late follicular phase. Significant increases in prolactin, over the already elevated base-

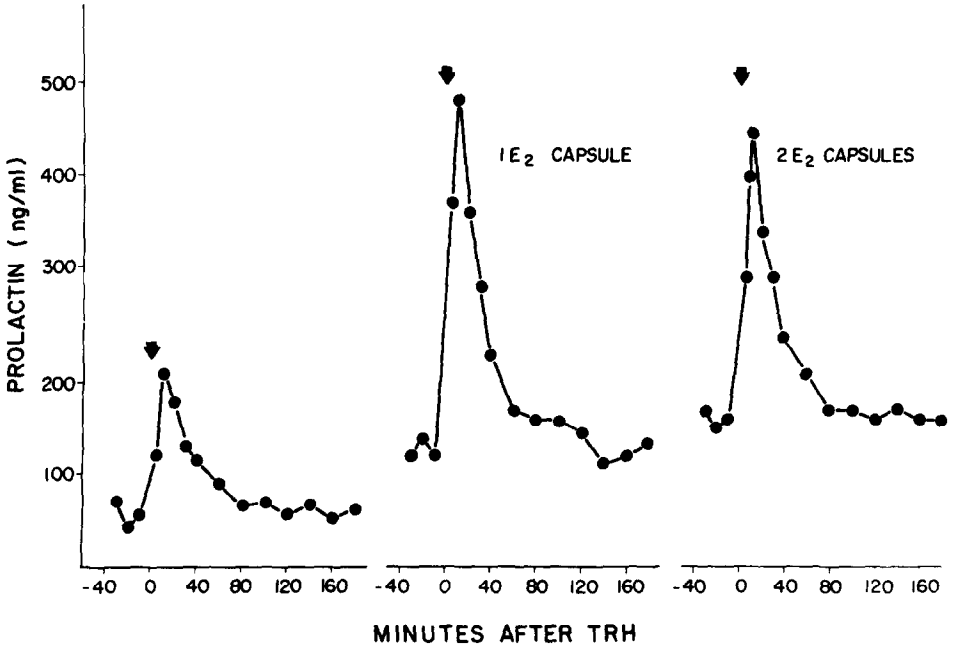

Fig. 3. Prolactin response to TRH after subcutaneous implant of 1 or 2 oestradiol-containing capsules.

line (300%) were seen within 2–3 days following implant of the first capsule. No significant changes were observed after further increasing oestrogen levels. The prolactin response to TRH (50 μg) was also significantly increased by oestrogen; peak response after implant of one oestrogen capsule was twice that in the oestrogen-deficient stalk sectioned control (Fig. 3). Our *in vivo* data support similar results obtained *in vitro*, where oestrogens were shown to stimulate both basal and TRH-induced prolactin release from rat pituitary cells in culture [29, 30]. These oestrogen effects on prolactin secretion may result from a potent antagonism to that of dopamine [31], a compound secreted into pituitary portal vessels [32] and shown to exert prolactin inhibitory effects at the level of the pituitary gland [33].

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