

60 YEARS OF NEUROENDOCRINOLOGY

MEMOIR: Harris' neuroendocrine revolution: of portal vessels and self-priming**George Fink**

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to G Fink**Email**george.fink@florey.edu.au or
georgefink1@hotmail.com**Abstract**

Geoffrey Harris, while still a medical student at Cambridge, was the first researcher (1937) to provide experimental proof for the then tentative view that the anterior pituitary gland was controlled by the CNS. The elegant studies carried out by Harris in the 1940s and early 1950s, alone and in collaboration with John Green and Dora Jacobsohn, established that this control was mediated by a neurohumoral mechanism that involved the transport by hypophysial portal vessel blood of chemical substances from the hypothalamus to the anterior pituitary gland. The neurohumoral control of anterior pituitary secretion was proved by the isolation and characterisation of the 'chemical substances' (mainly neuropeptides) and the finding that these substances were released into hypophysial portal blood in a manner consistent with their physiological functions. The new discipline of neuroendocrinology – the way that the brain controls endocrine glands and *vice versa* – revolutionised the treatment of endocrine disorders such as growth and pubertal abnormalities, infertility and hormone-dependent tumours, and it underpins our understanding of the sexual differentiation of the brain and key aspects of behaviour and mental disorder. Neuroendocrine principles are illustrated in this Thematic Review by way of Harris' major interest: hypothalamic–pituitary–gonadal control. Attention is focussed on the measurement of GnRH in hypophysial portal blood and the role played by the self-priming effect of GnRH in promoting the onset of puberty and enabling the oestrogen-induced surge or pulses of GnRH to trigger the ovulatory gonadotrophin surge in humans and other spontaneously ovulating mammals.

Key Words

- ▶ neurohormones
- ▶ hypophysial portal vessel blood
- ▶ gonadotrophin-releasing hormone (GnRH)
- ▶ self-priming effect of GnRH
- ▶ oestrogen-induced ovulatory GnRH surge
- ▶ oestrogen-induced increase in pituitary responsiveness to GnRH

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(2015) 226, T13–T24**Introduction**

The discipline of neuroendocrinology was launched by the publication of Geoffrey Harris' 1955 monograph *Neural Control of the Pituitary Gland*. The monograph consolidated the physiological evidence for the neurohumoral control of the anterior pituitary hormones as well as the regulation of the neurohypophysial hormones

vasopressin and oxytocin. In this Thematic Review, I outline some aspects of the neurohumoral control of anterior pituitary (adenohypophysial) hormone secretion, especially that of the gonadotrophins, because the neuroendocrine control of reproduction was of major interest for Harris. In this context, I also review the major

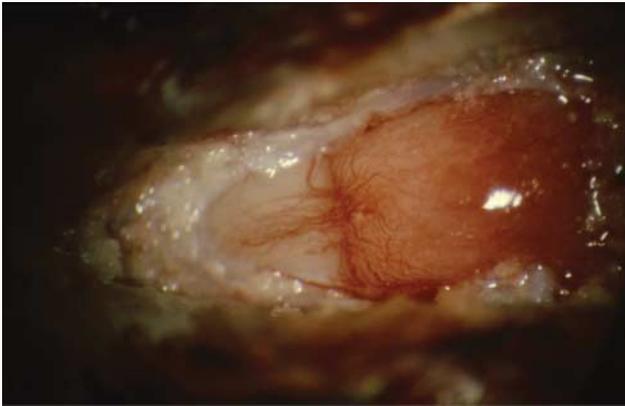


Figure 1

High-power view through a dissecting microscope of the hypophysial portal vessels on the anterior surface of the pituitary stalk (left) of an anaesthetised rat. The portal vessels (veins) arise from the primary capillary bed on the median eminence (pink area to the left) and fan out over the anterior pituitary gland (right) at the pituitary stalk junction to the right. The tubero-infundibular artery, a branch of the superior hypophysial artery, can be seen arching across the top of the stalk-pituitary junction, where it enters the anterior pituitary gland. This artery passes through the anterior pituitary gland to supply arterial blood to the neurohypophysis. Reproduced from *Handbook of Neuroendocrinology*, Fink G, Neural control of the anterior lobe of the pituitary gland (pars distalis), pp 97–138, copyright (2012), with permission from Elsevier. Note: The contentious history of the discovery and function of the hypophysial portal vessels is detailed in chapter 2 of Harris' (1955) monograph, to which the interested reader is referred. Popa & Fielding (1930, 1933), who first discovered the hypophysial portal system, posited that the direction of blood flow was centripetal: that is, from the anterior pituitary gland towards the hypothalamus. The direction of portal vessel blood flow (centrifugally from the hypothalamus to the anterior pituitary gland) was ultimately resolved by microscopic visualisation of the vessels in the living anaesthetised rat (Green & Harris 1949). In fact, Nobel Laureate (1947) Bernardo Houssay and his team had reported the centrifugal direction of portal vessel blood flow in the living toad (Houssay *et al.* 1935), but because their publication was in French, it was ignored until the late 1940s. The functional importance of the hypophysial portal vessels involved Harris in a conflict with the influential Sir Solly Zuckerman, who, on the basis of studies in the ferret, challenged the neurohumoral hypothesis of anterior pituitary control. The debate between Zuckerman and Harris was the subject of letters to *Nature* (Thomson & Zuckerman 1953, Donovan & Harris 1954). Before publishing his 1954 reply to Zuckerman, Harris submitted a draft of his letter to the regents of the Maudsley Hospital. After several months, the regents gave Harris permission to publish, but they cautioned him that if he did so, he would 'have a powerful enemy for life' (Geoffrey Harris, 1971, personal communication).

advances made after Harris' death in November 1971 in our understanding of oestrogen positive and negative feedback control of the hypothalamic–pituitary–gonadal system.

The hypophysial portal vessel system (Fig. 1) is the key to the story. The portal vessels (veins) transport neurohormones from the hypothalamus to the anterior pituitary gland, where they then stimulate or inhibit the release of

anterior pituitary hormones. The fenestrated primary capillary plexus of the hypophysial portal vessels located in the median eminence (Fig. 2) constitute one of several neurohaemal junctions that comprise the circumventricular organs, including the pineal gland and neurohypophysis. These junctions facilitate the release of chemical messengers from nerve terminals into the bloodstream, and *vice versa*. As 'windows' in the blood–brain barrier, the neurohaemal junctions subservise essential regulatory functions in diverse physiological systems (Fink 1986, 2012).

In addition to revolutionising our understanding of the mechanisms that control the key endocrine systems and peptide synthesis, release and action, the neuroendocrine revolution led to major developments in the clinical diagnosis and treatment of conditions such as infertility, central precocious puberty (CPP), acromegaly, dwarfism, Cushing's syndrome, neuroendocrine and hormone dependent cancers, hypertension and other cardiovascular disorders and metabolic syndrome (e.g. Fink 1976, Fink *et al.* 2012, Fliers *et al.* 2014).

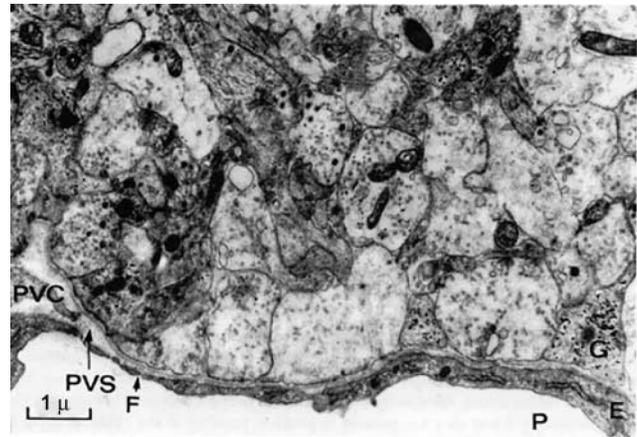


Figure 2

Electromicrograph ($\times 13\,200$) of the external layer of the median eminence of a rat at the first postnatal day. Note the high density of nerve terminals around part of a primary portal capillary vessel (P), which is fenestrated (F). Note also the large number of agranular and granular vesicles in the nerve terminals. These vesicles contain the packets (quanta) of neurohormone or neurotransmitter that are released upon nerve depolarisation as a consequence of action potentials. The neurohormones are released into the perivascular space, and from there, they move rapidly into portal vessel blood for transport to the pituitary gland. This arrangement is typical of the neurohaemal junctions found in the several circumventricular organs of the brain (see text). E, endothelial cell; F, fenestration; G, glial process; P, portal vessel; PVC, perivascular cell; PVS, perivascular space. Reproduced from Fink G & Smith GC 1971 Ultrastructural features of the developing hypothalamo–hypophysial axis in the rat: a correlative study. *Zeitschrift für Zellforschung und Mikroskopische Anatomie* 119 208–226, with kind permission from Springer Science and Business Media.

Brief history

The history of neuroendocrinology has been the subject of several reviews (Harris 1955, Guillemin 1967, 1978, 2011, Harris 1972, Fink 1976, 1986, 1988, 2012, Charlton 2008). Friedgood (1936) and Hinsey (1937) were arguably the first to postulate formally that the anterior pituitary gland was controlled by substances liberated into the hypophysial portal vessels from nerve terminals in the median eminence. However, this hypothesis was not accepted until work from Harris' laboratory in Cambridge established that: i) the direction of blood flow in the portal vessels of living mammals was from the hypothalamus to the pituitary (Green & Harris 1949); ii) after severance of the pituitary stalk, the function of the anterior pituitary gland could be correlated with the degree of its revascularisation by the hypophysial portal vessels (Harris 1950); and iii) the morphological and functional integrity of pituitary grafts were maintained (or developed in the case of tissues from immature donor animals) when these grafts were vascularised by the hypophysial portal but not the systemic circulation (Harris & Jacobsohn 1952). The results of the last study, which constituted the most important biological evidence for the neurohumoral hypothesis, were soon confirmed by the equally elegant pituitary grafting experiments of Nikitovitch-Winer & Everett (1958, 1959).

The neurohumoral hypothesis was supported by evidence of the existence in hypophysial portal vessel blood of luteinising hormone (LH) and corticotrophin-releasing activity (Porter & Rumsfeld 1963, Fink 1967, Fink *et al.* 1967, 1971, Fink & Harris 1970), and it was clinched by the isolation and sequence determination by Andrew Schally and Roger Guillemin of three hypothalamic regulatory peptide neurohormones: thyrotrophin-releasing factor (now thyrotrophin-releasing hormone, TRH), LH- and follicle-stimulating hormone (FSH)-releasing factor (now gonadotrophin-releasing hormone, GnRH) and somatostatin (Guillemin *et al.* 1971, Harris 1972, Schally *et al.* 1972, 1973, Guillemin 1978, 2011). Peptide sequencing was difficult and expensive because of the infinitesimally small peptide concentrations in the hypothalamus, the protected N and C terminals of TRH and GnRH, and the fact that the hypothalamus contains more pharmacologically active substances than any other tissue does. The energetic competition between Schally and Guillemin was legend. However, their 'inadvertent collaboration' led to success and to them being awarded the 1977 Nobel Prize in Physiology or Medicine (Fink 1977).

Schally, with Murray Saffran at McGill, and Guillemin in Houston, started their isolation studies on

corticotrophin-releasing factor (CRF). But two decades passed before the indomitable Wylie Vale at the Salk obtained the amino acid residue sequences of CRF-41 and the related urocortins (Vale *et al.* 1981, Bale & Vale 2004). Vale's success in sequencing CRF was a special landmark in neuroendocrine history, and it was perhaps brought into sharper focus by his untimely and tragic death on January 3, 2012 (Fink 1981, Bale & Chen 2012).

By now, most hypothalamic-regulatory neurohormones that mediate neural control of the anterior pituitary hormones have been isolated, sequenced and shown to be released into hypophysial portal vessel blood in a manner consistent with their physiological function (reviewed by Fink (2012)). I say 'most' because surprise is one of the most exciting features of neuroendocrinology (and much of science). This is exemplified by the recent discovery of kisspeptin neurons, which, rather than GnRH neurons, seem to serve as the 'grandmother neurons' in the control of gonadotrophin release (Fink 2012). The discovery of leptin and the several other peptides that control food intake and metabolism is also illustrative of the unexpected nature of neuroendocrinology (Farooqi & O'Rahilly 2014, Tan *et al.* 2014, Friedman & Mantzoros 2015).

Neuroendocrine control mechanisms tend to be complex. Thus, for example, pulsatile growth hormone (GH) secretion is stimulated by GH-releasing hormone, potentiated by the GH secretagogue, ghrelin, and inhibited by somatostatin (Veldhuis *et al.* 2012). Arginine vasopressin potentiates CRF-41 action in the release of corticotrophin (Fink *et al.* 1988, Tannahill *et al.* 1988, 1991, Antoni 1993, Aguilera 2011). Pulsatile GnRH, and therefore gonadotrophin secretion, in mammals might be generated by a subpopulation of kisspeptin neurons within the arcuate nucleus that co-express neurokinin B (the gene product of the tachykinin family member TAC3) and dynorphin. By forming an 'autonsynaptic feedback loop' within the hypothalamus, these 'KNDy neurons' are thought to modulate GnRH pulsatility and subsequent LH release (Hu *et al.* 2014). However, this hypothesis, together with the concept of a gonadotrophin surge-inhibiting hormone (Vega *et al.* 2015) awaits confirmation. We seem to be still far from fully understanding the hypothalamic gonadotrophin regulatory system.

In addition to controlling anterior pituitary hormone release, hypothalamic neurohormones are also essential for pituitary hormone synthesis. This has been obvious since the original graft experiments of Harris & Jacobsohn (1952), and it was reinforced, for example, by the hypogonadal (*hpg*) mouse, which has an autosomal

recessive mutation in the *GnRH* gene that results in an isolated, massive deficiency in LH and FSH (Cattanach *et al.* 1977).

Reproductive neuroendocrinology

Harris' first papers were on the induction of pseudopregnancy in the rat and ovulation in the rabbit (Harris 1936, 1937), and this major interest in reproductive physiology continued throughout his life. Thus, after adducing physiological evidence for the neurohumoral hypothesis of anterior pituitary control, Harris' personal research interests focused largely on the neural control of gonadotrophin secretion. And it is on this topic that the remainder of my review focuses, because reproductive neuroendocrinology encompassed my graduate research under Harris' supervision as well my subsequent research at Monash University Australia and then at Oxford University. Furthermore, it has heuristic value for other neuroendocrine and neurotransmitter mechanisms and ultimately led to research on the steroid hormone control of central neurotransmission, which has relevance for our understanding of mental disorders (Fink 1995a, Fink & Sumner 1996, Fink *et al.* 1996, 1999, Sumner *et al.* 2007).

In his Oxford laboratory, Harris promoted research on the isolation and characterisation of LH-releasing factor, now GnRH, and the existence of GnRH-activity in hypophysial portal vessel blood. The latter was investigated in blood collected from the cut pituitary stalk of the urethane-anaesthetised rat using the transpharyngeal approach (Worthington 1966, Fink 1967, Fink *et al.* 1967). Our findings showed significant GnRH activity in unextracted and acid-ethanol extracts of portal blood from intact and hypophysectomised (to exclude LH contamination) rats, as assessed by three different bioassays, including the induction of ovulation in the rabbit (Fink & Harris 1970).

GnRH in hypophysial portal blood determined by RIA and HPLC: effects of preoptic stimulation and oestrogen

Our bioassay findings (see the previous section) were confirmed by specific GnRH RIA, which showed that the concentration of GnRH in rat hypophysial portal was significantly greater than that in systemic jugular venous blood (Fink & Jamieson 1976). Using HPLC and two specific anti-GnRH sera, we found that a single immunoreactive peak was present in rat hypophysial portal blood and in hypothalamic extracts from rats and normal mice,

and it corresponded in retention time to synthetic GnRH (Sheward *et al.* 1985). No GnRH immunoreactivity was detected in hypothalamic extracts from the *hpg* mouse (Sheward *et al.* 1985).

Hypophysial portal plasma GnRH concentrations in urethane-anesthetised male and female rats were increased by electrical stimulation of the preoptic area in an approximately rectilinear manner with respect to the strength of the stimulating current (Fink & Jamieson 1976). The responsiveness of the preoptic GnRH release system to electrical stimulation was reduced by ovariectomy and restored or augmented (depending on dose) by oestrogen and testosterone but not by 5 α -dihydrotestosterone or progesterone. The inactivity of 5 α -dihydrotestosterone suggests that the effect of testosterone may depend on its aromatase conversion to oestrogen (Fink & Jamieson 1976). Similar findings were obtained in an independent study focused on comparing the effects of preoptic with median eminence stimulation in female rats (Sherwood *et al.* 1976). The precise mechanism of the oestrogen-induced increase in sensitivity of the preoptic area to stimulation remains unclear, but it is consonant with several subsequent findings that oestrogen can facilitate neural excitability in experimental rodent models (e.g. Bless *et al.* 1997, Good *et al.* 1999, Kow *et al.* 2005, Lee *et al.* 2008, Galankin *et al.* 2010, Rønnekleiv *et al.* 2012, Babayan & Kramár 2013). In the human, oestrogen influences sensory, motor and pain responses in women depending on the phase of the menstrual cycle (Barbosa *et al.* 2013), and it reduces the threshold corticospinal response to transcranial magnetic stimulation (Bonifazi *et al.* 2004).

Ovulatory gonadotrophin surge: neural control mediated by GnRH and an exponential increase in pituitary responsiveness to GnRH

The spontaneous ovulatory GnRH surge

As in other spontaneously ovulating mammals, such as rodents, sheep and other primates, the human basal gonadotrophin release, which is usually pulsatile, is interrupted in females by a massive surge of LH accompanied by a surge of FSH (Fink 1979a,b, 1988). The major physiological question that needed to be answered was whether the ovulatory gonadotrophin surge was triggered by a surge of GnRH. The main impediment to investigating this in rodents was the fact that commonly used anaesthetics, such as urethane or sodium pentobarbitone, which are required for transpharyngeal exposure of the

hypophysial portal vessels in the rat, also block the spontaneous ovulatory LH surge and are therefore likely to block the spontaneous ovulatory GnRH surge. The breakthrough came when we discovered that Althesin, a steroid anaesthetic in which alphaxalone is the active compound, provided perfect surgical analgesia, but when it is administered during the critical period of pro-oestrus, it does not block the spontaneous LH surge or normal ovulation (Sarkar *et al.* 1976, Sarkar & Fink 1979a). Figure 3 shows that the peripheral plasma LH surge in similarly maintained rats of the same strain begins shortly after the GnRH surge. Plasma LH concentrations then fall in parallel with portal plasma GnRH concentrations. The smaller peak of portal plasma GnRH concentration

between 2230 of pro-oestrus and 0200 of oestrus may be related to the pre-ovulatory surge of plasma FSH, which continues to rise after the cessation of the LH surge and reaches a peak at about 0500 of oestrus.

The GnRH surge is triggered by a positive feedback cascade that is initiated by an increased secretion of oestradiol-17 β (E₂). The importance of oestrogen in triggering the GnRH surge is demonstrated by the fact that ovariectomy on dioestrus, the day before pro-oestrus, abolishes the GnRH surge, which can be restored by administering E₂ immediately after ovariectomy (Fig. 4; Sarkar & Fink 1979b, Sherwood *et al.* 1980). Our findings of the oestrogen-triggered GnRH surge were confirmed in an independent study in rats by Ching (1982) and in subsequent studies in sheep (Clarke *et al.* 1987, 1989, Caraty *et al.* 1989, Moenter *et al.* 1991) and the rhesus monkey (Xia *et al.* 1992). The spontaneous pro-oestrous GnRH surge in intact rats and the oestrogen-induced GnRH surge in ovariectomised rats were inhibited by ovine CRF administered via the lateral ventricle, which suggests a possible mechanism for the stress inhibition of gonadotrophin secretion (Petraglia *et al.* 1987). This last finding was perhaps complemented by the recent report that overexpression of CRF in the central nucleus of the amygdala advances puberty and disrupts reproductive cycles in female rats (Li *et al.* 2014).

The exponential increase in pituitary responsiveness to GnRH triggered by oestrogen and the self-priming effect of GnRH

In addition to triggering the GnRH surge, the spontaneous surge of E₂ that is secreted by ovarian follicles in response to plasma gonadotrophins also induces a massive increase in pituitary gonadotroph responsiveness to GnRH. We have shown this to be the case for synthetic as well as endogenous GnRH released by preoptic stimulation (Aiyer & Fink 1974, Aiyer *et al.* 1974a, Fink & Aiyer 1974). Figure 5 shows that the responsiveness of the pituitary gland, in terms of mean maximal LH increments, increases 20- to 50-fold over a period of about 28 h between dioestrus and the afternoon of pro-oestrus in female rats. A similar 20- to 50-fold increase in pituitary responsiveness to GnRH also occurs in the human between the early follicular and mid-cycle phases (Yen *et al.* 1972). The increase in pituitary responsiveness initiated by oestrogen is further augmented by progesterone secreted during the early part of the LH surge and, more importantly, by the self-priming effect of GnRH, the unique capacity of the decapeptide to increase pituitary responsiveness to itself

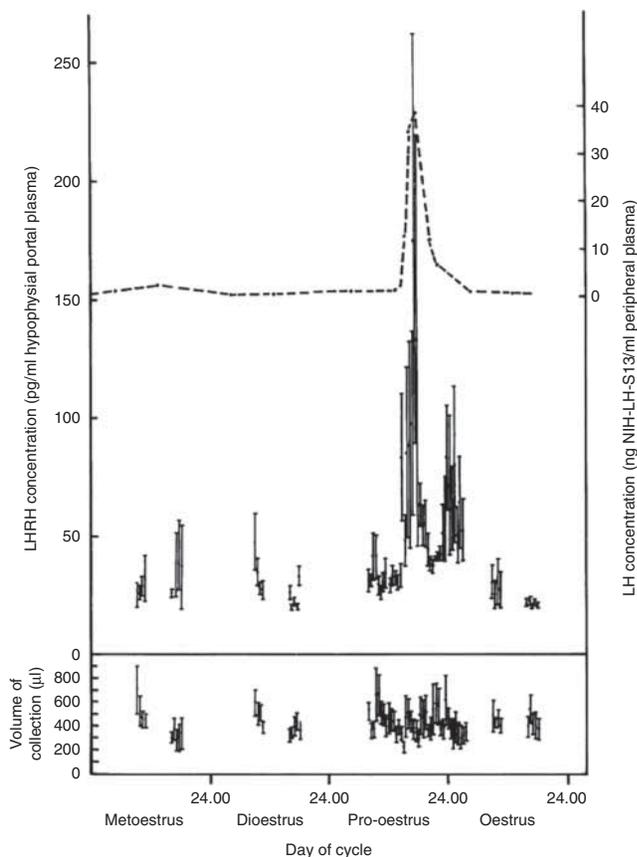
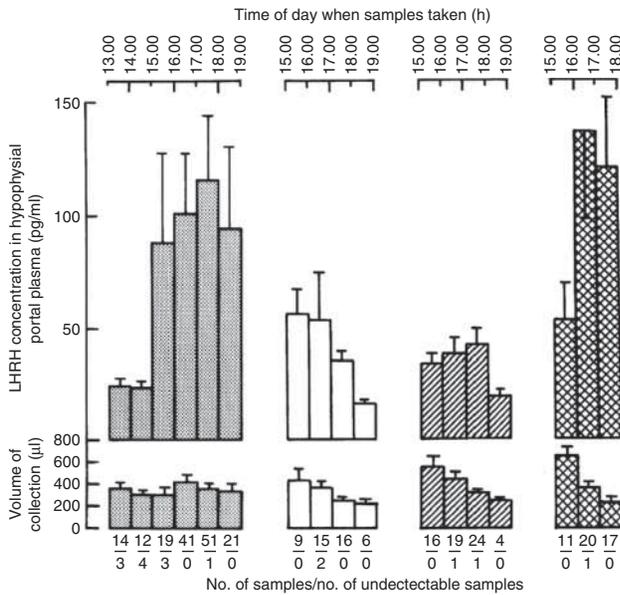


Figure 3

Mean \pm S.E.M. concentrations of LHRH (GnRH) in hypophysial portal plasma collected from female rats that were anaesthetised with alphaxalone at various stages of the oestrous cycle. For most of the cycle, the concentrations of GnRH are low, but just before and during the surge of LH (dashed line), there is a surge of GnRH. The volumes of portal blood collected are shown in the lower panel. Reproduced from Sarkar DK, Chiappa SA, Fink G & Sherwood NM 1976 Gonadotropin-releasing hormone surge in pro-oestrous rats. *Nature* 264 461–463, with permission from Macmillan Journals.

**Figure 4**

Mean \pm S.E.M. concentrations of LHRH (GnRH) in hypophysial portal plasma and volumes of portal blood collected at various times (indicated at top) on the expected day of pro-oestrus. The animals were either intact (filled bars) or ovariectomised at 1000–1100 h of dioestrus and given an s.c. injection of oil (open bars), 2.5 mg progesterone (diagonally hatched bars) or 10 μ g oestradiol benzoate (cross-hatched bars). Values below the bars refer to the total number of samples of each time/number of samples in which GnRH was not detectable. The GnRH surge was abolished by ovariectomy and was re-established by treatment at the time of ovariectomy with oestrogen but not progesterone. Reproduced, with permission, from Sarkar DK & Fink G 1979b Effects of gonadal steroids on output of luteinizing hormone releasing factor into pituitary stalk blood in the female rat. *Journal of Endocrinology* 80 303–313.

(Aiyer *et al.* 1974b, Fink *et al.* 1976, Fink 1988, 1995b). The role of the GnRH self-priming effect appears to be crucial for ensuring the coincidence of the GnRH surge with the increase in pituitary responsiveness to GnRH so that both events reach a peak simultaneously and, as a consequence, produce a massive ovulatory surge of LH.

The importance of the increase in pituitary responsiveness to GnRH: an unexpected finding that challenged accepted dogma

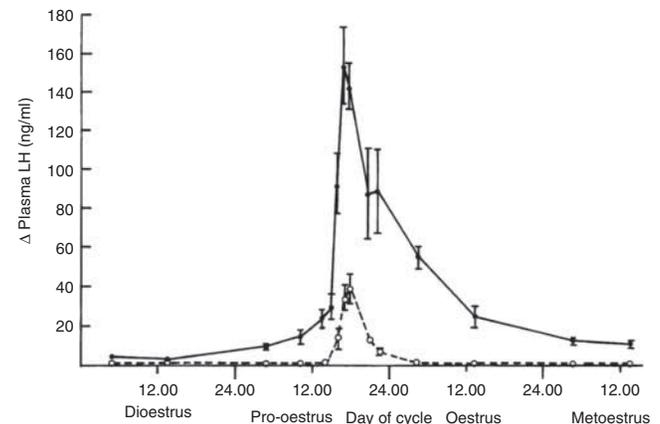
By June 1971, when Andrew Schally's laboratory first published the sequence of the decapeptide GnRH (Matsuo *et al.* 1971), accepted dogma had reversed from 'all pituitary' (as maintained from the 1920s to the early 1950s) to 'all hypothalamus'. That is, the anterior pituitary had been relegated from being a former 'conductor of the endocrine orchestra' to being the 'second fiddle' of the hypothalamus. Thus, for example,

Neena Schwartz's (1969)' model for the regulation of ovulation in the rat' did not allow for changes in pituitary responsiveness to neural drive.

The availability of synthetic GnRH made it possible to determine whether changes in pituitary responsiveness might modulate the amount of gonadotrophin released in response to GnRH. We were surprised by the massive increase (Fig. 5), which we first reported to the Society for Endocrinology at its 1972 joint meeting with the Dutch Society in Hull (Aiyer *et al.* 1973). The importance of the increase in pituitary responsiveness is that it amplifies the signal of the spontaneous oestrogen-triggered GnRH surge, which in the female rat, sheep and rhesus monkey (~ 200 pg GnRH/ml per ~ 170 pM/l) is far too small to trigger an ovulatory LH surge without the 20- to 50-fold pre-ovulatory increase in pituitary responsiveness to GnRH (Fink 1979a,b, 1988, Sherwood *et al.* 1980, Clarke *et al.* 1987).

GnRH self-priming, GnRH pulses, puberty and CPP

The self-priming effect of GnRH enables low-amplitude GnRH pulses, which are too small by themselves to evoke LH release, to trigger a massive increase in pituitary responsiveness and hence an LH surge (Fink *et al.* 1976).

**Figure 5**

Changes in pituitary responsiveness to LHRH (GnRH) during the oestrous cycle of the rat. The figure shows the mean \pm S.E.M. pre-injection concentrations (dashed line) and mean maximal increments (continuous line) in plasma LH concentrations (ng NIH-LH-S13/ml) in animals that were anaesthetised with sodium pentobarbitone 30–60 min before the i.v. injection of 50 ng LHRH/100 g body weight at different stages of the oestrous cycle. Reproduced, with permission, from Aiyer MS, Fink G & Greig F 1974a Changes in sensitivity of the pituitary gland to luteinizing hormone releasing factor during the oestrous cycle of the rat. *Journal of Endocrinology* 60 47–64.

The self-priming effect explains how GnRH pulses, rather than (or as well as) a GnRH surge, can trigger an ovulatory gonadotrophin surge in the rhesus monkey and possibly in other primates, including the human (Fink 1976, Fink *et al.* 1976, Wang *et al.* 1976, Knobil 1980).

The onset of puberty in humans is thought to be the result of an increase in the amplitude and frequency of the pulsatile secretion of GnRH that, by way of GnRH self-priming, results in increased gonadotrophin secretion (Richter & Terasawa 2001, Abreu *et al.* 2013, Macedo *et al.* 2014). Premature activation of the hypothalamic–pituitary–gonadal axis results in CPP, which is thought to reflect premature activity of the GnRH pulse generator and GnRH self-priming. The standard treatment of CPP has been to administer GnRH agonists, which suppress gonadotrophin secretion by desensitisation (tachyphylaxis), thereby stopping premature pubertal development and normalising growth and skeletal maturation rates (Trueman *et al.* 2002, Lee *et al.* 2012, Silverman *et al.* 2015).

Mechanism of oestrogen negative and positive feedback control of GnRH release

Oestrogen has two major effects on the GnRH/LH release system: low plasma concentrations of oestrogen inhibit (negative feedback), whereas high plasma concentrations of E_2 stimulate (positive feedback) GnRH release (as outlined earlier in this review). The negative feedback action of oestrogen occurs so fast that it may not necessarily involve ‘classical’ nuclear oestrogen receptors and genome-induced protein synthesis (Sarkar & Fink 1980). Conceivably, oestrogen could inhibit GnRH by a direct action on the membranes and ion channels involved in GnRH release (for a detailed review, see Fink (2012)). Whether the G protein-coupled oestrogen receptor 1, GPR30 (Almey *et al.* 2014, Anchan *et al.* 2014) and/or some other oestrogen-membrane mechanism is involved remains to be established.

In contrast to its negative (inhibitory) feedback effects, the time it takes for increased oestrogen levels to induce the positive feedback stimulation of GnRH release (the GnRH surge) is ~26–28 h (Aiyer & Fink 1974, Fink 1979a,b, 1988, 2012). This, of course, is more than sufficient time for nuclear receptor activation, transcription, translation, protein synthesis and structural changes in neuronal cytoskeleton, processes and synapses to occur. There is no robust evidence that progesterone potentiates oestrogen in triggering the GnRH surge (Sarkar & Fink 1980). However, progesterone and its receptors are involved in the

oestrogen-induced increase in pituitary responsiveness to GnRH (Aiyer & Fink 1974, Attardi *et al.* 2007).

Histochemical and gene knock-out experiments have shown that the E_2 -triggered GnRH surge is not a result of direct action on GnRH neurons; rather, it is mediated by intermediate neurons that express oestrogen receptor α (Shivers *et al.* 1983, Wintermantel *et al.* 2006). Although ‘classical’ neurotransmitter (noradrenergic, dopaminergic, serotonergic, opioid, GABAergic or glutamatergic) neurons may play a role, kisspeptin neurons appear to be pivotal in mediating the positive as well as the negative effects of oestrogen. Thus, the long-held belief that GnRH was the ‘grandmother’ neuron for the neural control of gonadotrophins has been revised in favour of kisspeptin (de Roux *et al.* 2003, Seminara *et al.* 2003, Clarkson & Herbison 2009, Oakley *et al.* 2009, Caraty *et al.* 2010, Kirby *et al.* 2010, Clarke 2011, Fink 2012).

Mechanisms involved in GnRH self-priming

GnRH self-priming is a servomechanism that is apparently unique for GnRH, possibly because, apart from the oxytocin uterine contraction system, which operates during parturition, the ovulatory surge of LH is the only positive endocrine feedback that operates under physiological conditions (Fink 1995b). Because of its importance for the ovulatory gonadotrophin surge, whether it is triggered by a GnRH surge or small, repetitive GnRH pulses, it is perhaps worth outlining the mechanism of the self-priming effect of GnRH. As noted earlier, GnRH self-priming can be demonstrated *in vivo* by different modes of exogenous GnRH administration as well as by electrical stimulation of the preoptic area, which releases endogenous GnRH (Fink *et al.* 1976).

The self-priming effect of GnRH can also be elicited *in vitro*, and this has enabled a comparison to be made between the mechanisms of the self-priming effect and the releasing action of GnRH. The key differences between the releasing and self-priming actions of GnRH are that: i) GnRH priming, but not releasing, is dependent on protein synthesis (Fink & Pickering 1975, Pickering & Fink 1976a, 1979, Curtis *et al.* 1985); ii) in contrast to the GnRH-releasing action, GnRH self-priming cannot be mimicked by K^+ depolarisation or Ca^{2+} ionophores (Pickering & Fink 1976b); iii) priming involves potentiation of the IP₃ intracellular Ca^{2+} mechanisms and protein kinase C (Johnson *et al.* 1988); and iv) priming involves activation of mitogen-activated protein (MAP) kinase (Pickering & Fink 1979, Curtis *et al.* 1985, Mobbs *et al.* 1990).

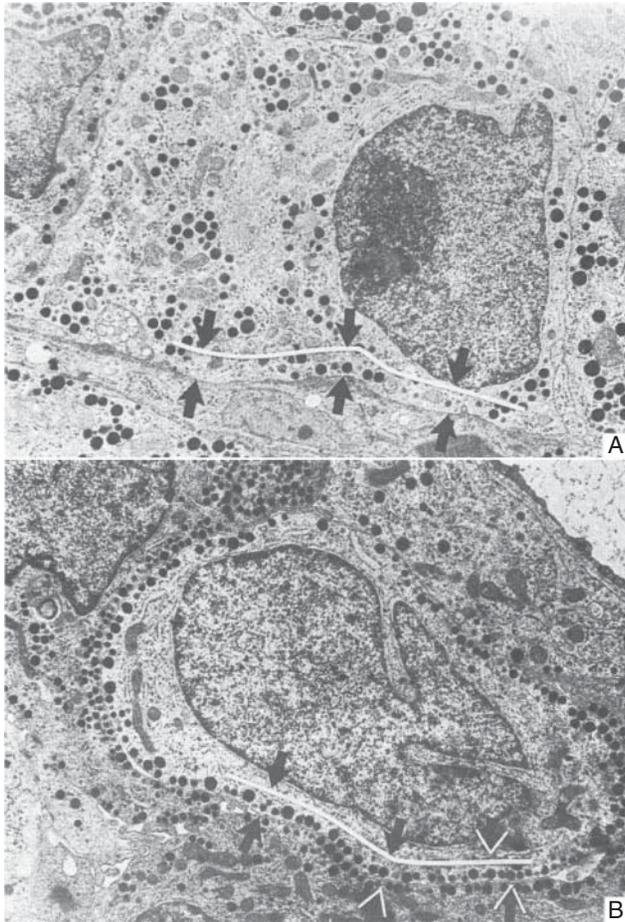


Figure 6

Electron micrographs ($\times 10\,000$) of immunoidentified gonadotrophs from the anterior pituitary glands of hypogonadal mice. The glands were pre-incubated for 2 h in medium alone and then incubated for two successive periods of 1 h each either in medium alone (A) or 8.5 nmol GnRH/1 of medium (B). A marginal zone, which is indicated by the line and arrows, has been arbitrarily defined as the region of the cytoplasm within 500 nm of the plasmalemma. In (A), the secretory granules are generally distributed in the cytoplasm, but in (B), there is a concentration of granules within the marginal zone. Reproduced, with permission, from Lewis CE, Morris JF, Fink G & Johnson, M 1986 Changes in the granule population of gonadotrophs of hypogonadal (*hpg*) and normal female mice associated with the priming effect of LH-releasing hormone *in vitro*. *Journal of Endocrinology* **109** 35–44.

GnRH self-priming also obtains with respect to FSH release (Pickering & Fink 1977).

Ultrastructural studies have shown that GnRH self-priming involves an increase in length and a change in the angle of the microfilaments in gonadotrophs as well as a migration of secretory granules towards the plasmalemma of the gonadotroph (Lewis *et al.* 1985, 1986; Fig. 6). This migration of granules ('margination') leads to an increase in the pool of LH that is available for release, so that when

the gonadotrophs are exposed for a second time to a secretagogue such as K^+ depolarisation, Ca^{2+} ionophores or GnRH itself, a massive second release of LH occurs. Full GnRH priming in mice depends on active progesterone receptors, but the basis for this molecular crosstalk remains unresolved (Chappell *et al.* 1999, Turgeon & Waring 2006, Attardi *et al.* 2007).

In sum, the mechanisms involved in GnRH self-priming are broadly understood, but some key molecular questions remain unanswered and offer the opportunity for future in-depth research. Although it has yet to predict unknown mechanisms or incisive future experiments, an elegant mathematical model of the GnRH self-priming has been generated by Gareth Leng and associates (Scullion *et al.* 2004).

'And also, what benefits will this work and knowledge confer on human welfare?' (Geoffrey Harris, 1971 Dale Lecture)

The short answer to Geoffrey Harris' question is 'many'. If that were not the case, the *Journal of Endocrinology* would not be celebrating his 1955 monograph. Neuroendocrinology has developed coincidentally with molecular genetics, genomics and, recently, optogenetics, and it has therefore played a key role in our understanding of gene regulation, transcription, translation and post-translational processing, which has helped it also to further other biomedical disciplines. The neurohumoral peptides were rapidly harnessed for the diagnosis and treatment of endocrine, neuroendocrine and related conditions, and GnRH analogues have been used extensively in IVF as well as in the breeding of domestic animals and fish. Often as not, it is the unexpected that provides benefits. For example, the use of GnRH agonists as powerful inhibitors of gonadotrophin and thereby of ovarian steroid hormone secretion for the treatment of several major disorders, including hormone-dependent cancers, might not have been immediately obvious in 1971. Similarly, the unpredicted self-priming effect of GnRH plays a key role in puberty and serves as a target for the treatment of CPP with GnRH agonists.

These are simply snippets of the vast field of neuroendocrine endeavours that began in 1936 with a young Cambridge medical student who also attained a Blue in Squash, Geoffrey Wingfield Harris.

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References

- Abreu AP, Dauber A, Macedo DB, Noel SD, Brito VN, Gill JC, Cukier P, Thompson IR, Navarro VM & Gagliardi PC 2013 Central precocious puberty caused by mutations in the imprinted gene MKRN3. *New England Journal of Medicine* **368** 2467–2475. (doi:10.1056/NEJMoa1302160)
- Aguilera G 2011 HPA axis responsiveness to stress: implications for healthy aging. *Experimental Gerontology* **46** 90–95. (doi:10.1016/j.exger.2010.08.023)
- Aiyer MS & Fink G 1974 The role of sex steroid hormones in modulating the responsiveness of the anterior pituitary gland to luteinizing hormone releasing factor in the female rat. *Journal of Endocrinology* **62** 553–572. (doi:10.1677/joe.0.0620553)
- Aiyer MS, Greig F & Fink G 1973 Changes in sensitivity of the anterior pituitary gland to synthetic luteinizing hormone releasing factor during the oestrous cycle of the rat. *Journal of Endocrinology* **57**(Supplement) xxvi. (doi:10.1677/joe.0.0570001)
- Aiyer MS, Fink G & Greig F 1974a Changes in sensitivity of the pituitary gland to luteinizing hormone releasing factor during the oestrous cycle of the rat. *Journal of Endocrinology* **60** 47–64. (doi:10.1677/joe.0.0600047)
- Aiyer MS, Chiappa SA & Fink G 1974b A priming effect of luteinizing hormone releasing factor on the anterior pituitary gland in the female rat. *Journal of Endocrinology* **62** 573–588. (doi:10.1677/joe.0.0620573)
- Almeida A, Cannell E, Bertram K, Filardo E, Milner TA & Brake WG 2014 Medial prefrontal cortical estradiol rapidly alters memory system bias in female rats: ultrastructural analysis reveals membrane-associated estrogen receptors as potential mediators. *Endocrinology* **155** 4422–4432. (doi:10.1210/en.2014-1463)
- Anchan D, Gafur A, Sano K, Ogawa S & Vasudevan N 2014 Activation of the GPR30 receptor promotes lordosis in female mice. *Neuroendocrinology* **100** 71–80. (doi:10.1159/000365574)
- Antoni FA 1993 Vasopressinergic control of pituitary adrenocorticotropin secretion comes of age. *Frontiers in Endocrinology* **14** 76–122.
- Attardi B, Scott R, Pfaff D & Fink G 2007 Facilitation or inhibition of the oestradiol-induced gonadotrophin surge in the immature female rat by progesterone: effects on pituitary responsiveness to gonadotrophin-releasing hormone (GnRH), GnRH self-priming and pituitary mRNAs for the progesterone receptor A and B isoforms. *Journal of Neuroendocrinology* **19** 988–1000. (doi:10.1111/j.1365-2826.2007.01613.x)
- Babayan AH & Kramár EA 2013 Rapid effects of oestrogen on synaptic plasticity: interactions with actin and its signalling proteins. *Journal of Neuroendocrinology* **25** 1163–1172. (doi:10.1111/jne.12108)
- Bale TL & Vale WW 2004 CRF and CRF receptors: role in stress responsivity and other behaviors. *Annual Review of Pharmacology and Toxicology* **44** 525–557. (doi:10.1146/annurev.pharmtox.44.101802.121410)
- Bale TL & Chen A 2012 Minireview: CRF and Wylie Vale: a story of 41 amino acids and a Texan with grit. *Endocrinology* **153** 2556–2561. (doi:10.1210/en.2012-1273)
- Barbosa MB, Guirro EC & Nunes FR 2013 Evaluation of sensitivity, motor and pain thresholds across the menstrual cycle through medium-frequency transcutaneous electrical nerve stimulation. *Clinics* **268** 901–908. (doi:10.6061/clinics/2013(07)03)
- Bless EP, McGinnis KA, Mitchell AL, Hartwell A & Mitchell JB 1997 The effects of gonadal steroids on brain stimulation reward in female rats. *Behavioural Brain Research* **82** 235–244. (doi:10.1016/S0166-4328(96)00129-5)
- Bonifazi M, Ginanneschi F, della Volpe R & Rossi A 2004 Effects of gonadal steroids on the input–output relationship of the corticospinal pathway in humans. *Brain Research* **1011** 187–194. (doi:10.1016/j.brainres.2004.03.022)
- Caraty A, Locatelli A & Martin GB 1989 Biphasic response in the secretion of gonadotrophin-releasing hormone in ovariectomized ewes injected with oestradiol. *Journal of Endocrinology* **123** 75–82. (doi:10.1677/joe.0.1230375)
- Caraty A, Franceschini I & Hoffman GE 2010 Kisspeptin and the preovulatory gonadotrophin-releasing hormone/luteinising hormone surge in the ewe: basic aspects and potential applications in the control of ovulation. *Journal of Neuroendocrinology* **22** 710–715.
- Cattanach BM, Iddon CA, Charlton HM, Chiappa SA & Fink G 1977 Gonadotrophin-releasing hormone deficiency in a mutant mouse with hypogonadism. *Nature* **269** 338–340. (doi:10.1038/269338a0)
- Chappell PE, Schneider JS, Kim P, Xu M, Lydon JP, O'Malley BW & Levine JE 1999 Absence of gonadotropin surges and gonadotropin-releasing hormone self-priming in ovariectomized (OVX), estrogen (E2)-treated, progesterone receptor knockout (PRKO) mice. *Endocrinology* **140** 3653–3658. (doi:10.1210/endo.140.8.6895)
- Charlton H 2008 Hypothalamic control of anterior pituitary function: a history. *Journal of Neuroendocrinology* **20** 641–646. (doi:10.1111/j.1365-2826.2008.01718.x)
- Ching M 1982 Correlative surges of LHRH, LH and FSH in pituitary stalk plasma and systemic plasma of rat during proestrus. Effect of anesthetics. *Neuroendocrinology* **34** 279–285. (doi:10.1159/000123313)
- Clarke IJ 2011 Control of GnRH secretion: one step back. *Frontiers in Endocrinology* **32** 367–375. (doi:10.1016/j.yfrne.2011.01.001)
- Clarke IJ, Thomas GB, Yao B & Cummins JT 1987 GnRH secretion throughout the ovine estrous cycle. *Neuroendocrinology* **46** 82–88. (doi:10.1159/000124800)
- Clarke IJ, Cummins JT, Jenkin M & Phillips DJ 1989 The oestrogen-induced surge of LH requires a 'signal' pattern of gonadotrophin-releasing hormone input to the pituitary gland in the ewe. *Journal of Endocrinology* **122** 127–134. (doi:10.1677/joe.0.1220127)
- Clarkson J & Herbison AE 2009 Oestrogen, kisspeptin, GPR54 and the pre-ovulatory luteinising hormone surge. *Journal of Neuroendocrinology* **21** 305–311. (doi:10.1111/j.1365-2826.2009.01835.x)
- Curtis A, Lyons V & Fink G 1985 The priming effect of LH-releasing hormone: effects of cold and involvement of new protein synthesis. *Journal of Endocrinology* **105** 163–167. (doi:10.1677/joe.0.1050163)
- Donovan BT & Harris GW 1954 Effect of pituitary stalk section on light-induced oestrus in the ferret. *Nature* **174** 503–504. (doi:10.1038/174503a0)
- Farooqi IS & O'Rahilly S 2014 20 years of leptin: human disorders of leptin action. *Journal of Endocrinology* **223** T63–T70. (doi:10.1530/JOE-14-0480)
- Fink G 1967 Nature of luteinizing hormone releasing factor in hypophysial portal blood. *Nature* **215** 159–161. (doi:10.1038/215159a0)
- Fink G 1976 The development of the releasing factor concept. *Clinical Endocrinology* **5**(Supplement s1) S245–S260. (doi:10.1111/j.1365-2265.1976.tb03833.x)
- Fink G 1977 Inadvertent collaboration. *Nature* **269** 747–748. (doi:10.1038/269747a0)
- Fink G 1979a Feedback actions of target hormones on hypothalamus and pituitary with special reference to gonadal steroids. *Annual Review of Physiology* **41** 571–585. (doi:10.1146/annurev.ph.41.030179.003035)
- Fink G 1979b Neuroendocrine control of gonadotrophin secretion. *British Medical Bulletin* **35** 155–160.
- Fink G 1981 Has corticotropin-releasing factor finally been found? *Nature* **294** 511–512. (doi:10.1038/294511a0)
- Fink G 1986 The external layer of the median eminence: a neurovascular synapse. *Neurochemistry International* **9** 141–153. (doi:10.1016/0197-0186(86)90043-4)
- Fink G 1988 The G W Harris lecture. Steroid control of brain and pituitary function. *Quarterly Journal of Experimental Physiology* **73** 257–293. (doi:10.1113/expphysiol.1988.sp003145)

- Fink G 1995a The psychoprotective action of estrogen is mediated by central serotonergic as well as dopaminergic mechanisms. In *Serotonin in the Central Nervous System and Periphery*, pp 175–187. Eds A Takada & G Curzon. Amsterdam, The Netherlands: Elsevier.
- Fink G 1995b The self-priming effect of LHRH: a unique servomechanism and possible cellular model for memory. *Frontiers in Neuroendocrinology* **16** 183–190. (doi:10.1006/frne.1995.1006)
- Fink G 2012 Neural control of the anterior lobe of the pituitary gland (pars distalis). In *Handbook of Neuroendocrinology*, pp 97–138. Eds G Fink, DW Pfaff & JE Levine. London, Waltham, San Diego: Academic Press, Elsevier.
- Fink G & Harris GW 1970 The luteinizing hormone releasing activity of extracts of blood from the hypophysial portal vessels of rats. *Journal of Physiology* **208** 221–241. (doi:10.1113/jphysiol.1970.sp009115)
- Fink G & Smith GC 1971 Ultrastructural features of the developing hypothalamo–hypophysial axis in the rat: a correlative study. *Zeitschrift für Zellforschung und Mikroskopische Anatomie* **119** 208–226. (doi:10.1007/BF00324522)
- Fink G & Aiyer MS 1974 Gonadotrophin secretion after electrical stimulation of the preoptic area during the oestrous cycle of the rat. *Journal of Endocrinology* **62** 589–604. (doi:10.1677/joe.0.0620589)
- Fink G & Pickering A 1975 Dependence of the priming effect of luteinizing hormone releasing factor (LRF) on RNA and protein synthesis. *Journal of Physiology* **252** 73–75P.
- Fink G & Jamieson MG 1976 Immunoreactive luteinizing hormone releasing factor in rat pituitary stalk blood: effects of electrical stimulation. *Journal of Endocrinology* **68** 71–87. (doi:10.1677/joe.0.0680071)
- Fink G & Sumner BEH 1996 Oestrogen and mental state. *Nature* **383** 306. (doi:10.1038/383306a0)
- Fink G, Nallar R & Worthington C Jr 1967 The demonstration of luteinizing hormone releasing factor in hypophysial portal blood of pro-oestrous and hypophysectomized rats. *Journal of Physiology* **191** 407–416. (doi:10.1113/jphysiol.1967.sp008258)
- Fink G, Smith JR & Tibballs J 1971 Corticotrophin releasing factor in hypophysial portal blood of rats. *Nature* **230** 467–468. (doi:10.1038/230467a0)
- Fink G, Chiappa SA & Aiyer MS 1976 Priming effect of luteinizing hormone releasing factor elicited by preoptic stimulation and by intravenous infusion and multiple injections of the synthetic decapeptide. *Journal of Endocrinology* **69** 359–372. (doi:10.1677/joe.0.0690359)
- Fink G, Robinson ICAF & Tannahill LA 1988 Effects of adrenalectomy and glucocorticoids on the peptides CRF-41, AVP and oxytocin in rat hypophysial portal blood. *Journal of Physiology* **401** 329–345. (doi:10.1113/jphysiol.1988.sp017165)
- Fink G, Sumner BEH, Rosie R, Grace O & Quinn JP 1996 Estrogen control of central neurotransmission: effect on mood, mental state, and memory. *Cellular and Molecular Neurobiology* **16** 325–344. (doi:10.1007/BF02088099)
- Fink G, Sumner B, Rosie R, Wilson H & McQueen J 1999 Androgen actions on central serotonin neurotransmission: relevance for mood, mental state and memory. *Behavioural Brain Research* **105** 53–68. (doi:10.1016/S0166-4328(99)00082-0)
- Fink G, Pfaff DW & Levine JE (Eds) 2012 *Handbook of Neuroendocrinology*, pp 3–871. Eds G Fink, DW Pfaff & JE Levine. London, Waltham, San Diego: Academic Press, Elsevier.
- Fliers E, Korbonsits M & Romijn JA (Eds) 2014 *Clinical Neuroendocrinology. Handbook of Neurology Series, Vol 124*. pp 2–432. London, Waltham, San Diego: Academic Press, Elsevier. (doi:10.1016/B978-0-444-59602-4.09985-8)
- Friedgood HB 1936 Studies on the sympathetic nervous control of the anterior hypophysis with special reference to a neuro-humoral mechanism. Symposium on Endocrine Glands; Harvard Tercentenary Celebrations. Cited from *Textbook of Endocrinology*, Ed RH Williams, ch 10, pp 635–698, 1950. Philadelphia, PA, USA: Saunders.
- Friedman JM & Mantzoros CS 2015 20 years of leptin: from the discovery of the leptin gene to leptin in our therapeutic armamentarium. *Metabolism* **64** 1–4. (doi:10.1016/j.metabol.2014.10.023)
- Galankin T, Shekunova E & Zvartau E 2010 Estradiol lowers intracranial self-stimulation thresholds and enhances cocaine facilitation of intracranial self-stimulation in rats. *Hormones and Behavior* **58** 827–834. (doi:10.1016/j.yhbeh.2010.08.006)
- Good M, Day M & Muir JL 1999 Cyclical changes in endogenous levels of oestrogen modulate the induction of LTD and LTP in the hippocampal CA1 region. *European Journal of Neuroscience* **11** 4476–4480. (doi:10.1046/j.1460-9568.1999.00920.x)
- Green JD & Harris GW 1949 Observation of the hypophysio-portal vessels of the living rat. *Journal of Physiology* **108** 359–361. (doi:10.1113/jphysiol.1949.sp004339)
- Guillemin R 1967 The adenohypophysis and its hypothalamic control. *Annual Review of Physiology* **29** 313–348. (doi:10.1146/annurev.ph.29.030167.001525)
- Guillemin R 1978 Control of adenohypophysial functions by peptides of the central nervous system. *Harvey Lectures* **71** 71–131.
- Guillemin R 2011 Neuroendocrinology: a short historical review. *Annals of the New York Academy of Sciences* **1220** 1–5. (doi:10.1111/j.1749-6632.2010.05936.x)
- Guillemin R, Burgus R & Vale W 1971 The hypothalamic hypophysiotropic thyrotropin-releasing factor. *Vitamins and Hormones* **29** 1–39.
- Harris GW 1936 The induction of pseudopregnancy in the rat by electrical stimulation through the head. *Journal of Physiology* **88** 361–367. (doi:10.1113/jphysiol.1936.sp003446)
- Harris GW 1937 The induction of ovulation in the rabbit, by electrical stimulation of the hypothalamo–hypophysial mechanism. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **122** 374–394. (doi:10.1098/rspb.1937.0031)
- Harris GW 1950 Oestrous rhythm. Pseudopregnancy and the pituitary stalk in the rat. *Journal of Physiology* **111** 347–360. (doi:10.1113/jphysiol.1950.sp004484)
- Harris GW 1955 *Neural Control of the Pituitary Gland*, pp 298. London, UK: Edward Arnold.
- Harris GW 1972 Humours and hormones. *Journal of Endocrinology* **53** ii–xxiii. (doi:10.1677/joe.0.0530001)
- Harris GW & Jacobsohn D 1952 Functional grafts of the anterior pituitary gland. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **139** 263–276. (doi:10.1098/rspb.1952.0011)
- Hinsey JC 1937 The relation of the nervous system to ovulation and other phenomena of the female reproductive tract. *Cold Spring Harbor Symposia on Quantitative Biology* **5** 269–279. (doi:10.1101/SQB.1937.005.01.027)
- Houssay BA, Biasotti A & Sammartino R 1935 Modifications fonctionelles de l'hypophyse après les lésions infundibulotuberiennes chez le crapaud. *Comptes Rendus des Seances et Memoires de la Societe de Biologie* **120** 725–727.
- Hu G, Lin C, He M & Wong AO 2014 Neurokinin B and reproductive functions: "KNDy neuron" model in mammals and the emerging story in fish. *General and Comparative Endocrinology* **208** 94–108. (doi:10.1016/j.ygcen.2014.08.009)
- Johnson MS, Mitchell R & Fink G 1988 The role of protein kinase C in LHRH-induced LH and FSH release and LHRH self-priming in rat anterior pituitary glands *in vitro*. *Journal of Endocrinology* **116** 231–239. (doi:10.1677/joe.0.1160231)
- Kirby HR, Maguire JJ, Colledge WH & Davenport AP 2010 International Union of Basic and Clinical Pharmacology. LXXVII. Kisspeptin receptor nomenclature, distribution, and function. *Pharmacological Reviews* **62** 565–578. (doi:10.1124/pr.110.002774)
- Knobil E 1980 The neuroendocrine control of the menstrual cycle. *Recent Progress in Hormone Research* **36** 53–88.
- Kow L-M, Easton A & Pfaff DW 2005 Acute estrogen potentiates excitatory responses of neurons in rat hypothalamic ventromedial

- nucleus. *Brain Research* **1043** 124–131. (doi:10.1016/j.brainres.2005.02.068)
- Lee AW, Kyrozis A, Chevaleyre V, Kow LM, Devidze N, Zhang Q, Eten G & Pfaff DW 2008 Estradiol modulation of phenylephrine-induced excitatory responses in ventromedial hypothalamic neurons of female rats. *PNAS* **105** 7333–7338. (doi:10.1073/pnas.0802760105)
- Lee PA, Klein K, Mauras N, Neely EK, Bloch CA, Larsen L, Mattia-Goldberg C & Chwalisz K 2012 Efficacy and safety of leuprolide acetate 3-month depot 11.25 milligrams or 30 milligrams for the treatment of central precocious puberty. *Journal of Clinical Endocrinology and Metabolism* **97** 1572–1580. (doi:10.1210/jc.2011-2704)
- Lewis CE, Morris JF & Fink G 1985 The role of microfilaments in the priming effect of LH-releasing hormone: an ultrastructural study using cytochalasin B. *Journal of Endocrinology* **106** 211–218. (doi:10.1677/joe.0.1060211)
- Lewis CE, Morris JF, Fink G & Johnson M 1986 Changes in the granule population of gonadotrophs of hypogonadal (hpg) and normal female mice associated with the priming effect of LH-releasing hormone *in vitro*. *Journal of Endocrinology* **109** 35–44. (doi:10.1677/joe.0.1090035)
- Li XF, Hu MH, Li SY, Geach C, Hikima A, Rose S, Greenwood MP, Greenwood M, Murphy D, Poston L *et al.* 2014 Overexpression of corticotropin releasing factor in the central nucleus of the amygdala advances puberty and disrupts reproductive cycles in female rats. *Endocrinology* **155** 3934–3944. (doi:10.1210/en.2014-1339)
- Macedo DB, Brito VN & Latronica AC 2014 New causes of central precocious puberty: the role of genetic factors. *Neuroendocrinology* **100** 1–8. (doi:10.1159/000366282)
- Matsuo H, Baba Y, Nair RM, Arimura A & Schally AV 1971 Synthesis of the porcine LH- and FSH-releasing hormone by the solid-phase method. *Biochemical and Biophysical Research Communications* **43** 1334–1339. (doi:10.1016/S0006-291X(71)80019-0)
- Mobbs CV, Fink G & Pfaff DW 1990 HIP-70: an isoform of phosphoinositol-specific phospholipase C- α . *Science* **249** 566–567. (doi:10.1126/science.2382136)
- Moenter SM, Caraty A, Locatelli A & Karsch FJ 1991 Pattern of gonadotropin releasing hormone (GnRH) secretion leading up to ovulation in the ewe: existence of a preovulatory GnRH surge. *Endocrinology* **129** 1175–1182. (doi:10.1210/endo-129-3-1175)
- Nikitovitch-Winer M & Everett JW 1958 Functional restitution of pituitary grafts re-transplanted from kidney to median eminence. *Endocrinology* **63** 916–930. (doi:10.1210/endo-63-6-916)
- Nikitovitch-Winer M & Everett JW 1959 Histochemical changes in grafts of rat pituitary on the kidney and upon re-transplantation under the diencephalon. *Endocrinology* **65** 357–368. (doi:10.1210/endo-65-3-357)
- Oakley AE, Clifton DK & Steiner RA 2009 Kisspeptin signaling in the brain. *Endocrine Reviews* **230** 713–743. (doi:10.1210/er.2009-0005)
- Petraglia F, Sutton S, Vale W & Plotsky P 1987 Corticotropin-releasing factor decreases plasma luteinizing hormone levels in female rats by inhibiting gonadotropin-releasing hormone release into hypophysial-portal circulation. *Endocrinology* **120** 1083–1088. (doi:10.1210/endo-120-3-1083)
- Pickering A & Fink G 1976a Priming effect of luteinizing hormone releasing factor; *in vitro* and *in vivo* evidence consistent with its dependence upon protein and RNA synthesis. *Journal of Endocrinology* **69** 373–379. (doi:10.1677/joe.0.0690373)
- Pickering A & Fink G 1976b Priming effect of luteinizing hormone releasing factor: *in vitro* studies with raised potassium ion concentrations. *Journal of Endocrinology* **69** 453–454. (doi:10.1677/joe.0.0690453)
- Pickering AJMC & Fink G 1977 A priming effect of luteinizing hormone releasing factor with respect to release of follicle stimulating hormone *in vitro* and *in vivo*. *Journal of Endocrinology* **75** 155–159. (doi:10.1677/joe.0.0750155)
- Pickering AJMC & Fink G 1979 Priming effect of luteinizing hormone releasing factor *in vitro*: role of protein synthesis, contractile elements, Ca²⁺ and cyclic AMP. *Journal of Endocrinology* **81** 223–234. (doi:10.1677/joe.0.0810223)
- Popa GT & Fielding U 1930 A portal circulation from the pituitary to the hypothalamic region. *Journal of Anatomy* **65** 88–91.
- Popa GT & Fielding U 1933 Hypophysio-portal vessels and their colloid accompaniment. *Journal of Anatomy* **67** 227–232.
- Porter JC & Rumsfeld HW, Jr 1963 Chemistry of neuroendocrine mediators: discussion. In *Advances in Neuroendocrinology*, pp 334–342. Ed. AV Nalbandov. Urbana, IL, USA: University of Illinois Press.
- Richter TA & Terasawa E 2001 Neural mechanisms underlying the pubertal increase in LHRH release in the rhesus monkey. *Trends in Endocrinology and Metabolism* **12** 353–359. (doi:10.1016/S1043-2760(01)00442-8)
- Rønnekleiv OK, Bosch MA & Zhang C 2012 17 β -oestradiol regulation of gonadotrophin-releasing hormone neuronal excitability. *Journal of Neuroendocrinology* **24** 122–130.
- de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL & Milgrom E 2003 Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *PNAS* **100** 10972–10976. (doi:10.1073/pnas.1834399100)
- Sarkar DK & Fink G 1979a Mechanism of the first spontaneous gonadotrophin surge and that induced by pregnant mare serum and effects of neonatal androgen. *Journal of Endocrinology* **83** 339–354. (doi:10.1677/joe.0.0830339)
- Sarkar DK & Fink G 1979b Effects of gonadal steroids on output of luteinizing hormone releasing factor into pituitary stalk blood in the female rat. *Journal of Endocrinology* **80** 303–313. (doi:10.1677/joe.0.0800303)
- Sarkar DK & Fink G 1980 Luteinizing hormone releasing factor in pituitary stalk plasma from long-term ovariectomized rats: effects of steroids. *Journal of Endocrinology* **86** 511–524. (doi:10.1677/joe.0.0860511)
- Sarkar DK, Chiappa SA, Fink G & Sherwood NM 1976 Gonadotropin-releasing hormone surge in pro-oestrous rats. *Nature* **264** 461–463. (doi:10.1038/264461a0)
- Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG *et al.* 2003 The GPR54 gene as a regulator of puberty. *New England Journal of Medicine* **349** 1614–1627. (doi:10.1056/NEJMoa035322)
- Schally AV, Kastin AJ & Arimura A 1972 FSH-releasing hormone and LH-releasing hormone. *Vitamins and Hormones* **30** 83–164. (doi:10.1016/S0083-6729(08)60795-5)
- Schally AV, Arimura A & Kastin AJ 1973 Hypothalamic regulatory hormones. *Science* **179** 341–350. (doi:10.1126/science.179.4071.341)
- Schwartz NB 1969 A model for the regulation of ovulation in the rat. *Recent Progress in Hormone Research* **25** 1–55.
- Scullion S, Brown D & Leng G 2004 Modelling the pituitary response to luteinizing hormone-releasing hormone. *Journal of Neuroendocrinology* **16** 265–271. (doi:10.1111/j.0953-8194.2004.01169.x)
- Sherwood NM, Chiappa SA & Fink G 1976 Immunoreactive luteinizing hormone releasing factor in pituitary stalk blood from female rats; sex steroid modulation of response to electrical stimulation of preoptic area or median eminence. *Journal of Endocrinology* **70** 501–511. (doi:10.1677/joe.0.0700501)
- Sherwood NM, Chiappa SA, Sarkar DK & Fink G 1980 Gonadotropin-releasing hormone (GnRH) in pituitary stalk blood from proestrous rats: effects of anesthetics and relationship between stored and released GnRH and luteinizing hormone. *Endocrinology* **107** 1410–1417. (doi:10.1210/endo-107-5-1410)
- Sheward WJ, Harmar AJ & Fink G 1985 LH-RH in the rat and mouse hypothalamus and rat hypophysial portal blood: confirmation of identity by high performance liquid chromatography. *Brain Research* **345** 362–365. (doi:10.1016/0006-8993(85)91017-0)
- Shivers BD, Harlan RE, Morell JI & Pfaff DW 1983 Absence of oestradiol concentration in cell nuclei of LHRH-immunoreactive neurons. *Nature* **304** 345–347. (doi:10.1038/304345a0)
- Silverman LA, Neely EK, Kletter GB, Lewis K, Chitra S, Terleckyj O & Eugster EA 2015 Long-term continuous suppression with once-yearly histrelin subcutaneous implants for the treatment of central precocious puberty: a final report of a phase 3 multicenter trial.

- Journal of Clinical Endocrinology and Metabolism* **100** 2354–2363. (doi:10.1210/jc.2014-3031)
- Sumner BE, Grant KE, Rosie R, Hegele-Hartung C, Fritzsche KH & Fink G 2007 Raloxifene blocks estradiol induction of the serotonin transporter and 5-hydroxytryptamine 2A receptor in female rat brain. *Neuroscience Letters* **417** 95–99. (doi:10.1016/j.neulet.2007.02.039)
- Tan K, Knight ZA & Friedman JM 2014 Ablation of AgRP neurons impairs adaptation to restricted feeding. *Molecular Metabolism* **3** 694–704. (doi:10.1016/j.molmet.2014.07.002)
- Tannahill LA, Dow RC, Fairhall KM, Robinson ICAF & Fink G 1988 Comparison of adrenocorticotropin control in Brattleboro, Long-Evans and Wistar rats. *Neuroendocrinology* **48** 650–657. (doi:10.1159/000125077)
- Tannahill LA, Sheward WJ, Robinson IC & Fink G 1991 Corticotrophin-releasing factor-41, vasopressin and oxytocin release into hypophysial portal blood in the rat: effects of electrical stimulation of the hypothalamus, amygdala and hippocampus. *Journal of Endocrinology* **129** 99–107. (doi:10.1677/joe.0.1290099)
- Thomson APD & Zuckerman S 1953 Functional relations of the adenohypophysis and hypothalamus. *Nature* **171** 970. (doi:10.1038/171970a0)
- Trueman JA, Tillmann V, Cusick CF, Foster P, Patel L, Hall CM, Price DA & Clayton PE 2002 Suppression of puberty with long-acting goserelin (Zoladex-LA): effect on gonadotrophin response to GnRH in the first treatment cycle. *Clinical Endocrinology* **57** 223–230. (doi:10.1046/j.1365-2265.2002.01571.x)
- Turgeon JL & Waring DW 2006 Differential expression and regulation of progesterone receptor isoforms in rat and mouse pituitary cells and β 2 gonadotropes. *Journal of Endocrinology* **190** 837–846. (doi:10.1677/joe.1.06923)
- Vale W, Spiess J, Rivier C & Rivier J 1981 Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β -endorphin. *Science* **213** 1394–1397. (doi:10.1126/science.6267699)
- Vega MG, Zarek SM, Bhagwat M & Segars JH 2015 Gonadotropin surge-inhibiting/attenuating factors: a review of current evidence, potential applications, and future directions for research. *Molecular Reproduction and Development* **82** 2–16. (doi:10.1002/mrd.22439)
- Veldhuis JD, Iranmanesh A, Erickson D, Roelfsema F & Bowers CY 2012 Lifetime regulation of growth hormone (GH) secretion. In *Handbook of Neuroendocrinology*, pp 237–257. Eds G Fink, DW Pfaff & JE Levine. London, Waltham, San Diego: Academic Press, Elsevier.
- Wang CF, Lasley BL, Lein A & Yen SS 1976 The functional changes of the pituitary gonadotrophs during the menstrual cycle. *Journal of Clinical Endocrinology and Metabolism* **42** 718–728. (doi:10.1210/jcem-42-4-718)
- Wintermantel TM, Campbell RE, Porteous R, Bock D, Gröne HJ, Todman MG, Korach KS, Greiner E, Pérez CA, Schütz G *et al.* 2006 Definition of estrogen receptor pathway critical for estrogen positive feedback to gonadotropin-releasing hormone neurons and fertility. *Neuron* **52** 271–280. (doi:10.1016/j.neuron.2006.07.023)
- Worthington WC 1966 Blood samples from the pituitary stalk of the rat: method of collection and factors determining volume. *Nature* **210** 710–712. (doi:10.1038/210710a0)
- Xia L, Van Vugt D, Alston EJ, Luckhaus J & Ferin M 1992 A surge of gonadotropin-releasing hormone accompanies the estradiol-induced gonadotropin surge in the rhesus monkey. *Endocrinology* **131** 2812–2820. (doi:10.1210/endo.131.6.1446619)
- Yen SSC, Vandenberg G, Rebar R & Ehara Y 1972 Variation of pituitary responsiveness to synthetic LRF during different phases of the menstrual cycle. *Journal of Clinical Endocrinology and Metabolism* **35** 931–934. (doi:10.1210/jcem-35-6-931)

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