J. clin. Path., 1974, **27**, 173-184

Hypothalamic regulatory hormones: A review

G. M. BESSER AND C. H. MORTIMER

From the Medical Professorial Unit, St Bartholonew's Hospital, London

It has long been suggested that the secretion of the anterior pituitary hormones is under the control of the hypothalamus (Green and Harris, 1947; Harris, 1955). Early studies of the vasculature of the hypothalamic-hypophyseal system in birds and mammals described a portal system of capillaries in close contact with neurosecretory cells of the median eminence of the hypothalamus and draining to the anterior pituitary (Scharrer and Scharrer, 1954). It was therefore assumed that neurosecretory transmitters from the hypothalamus drained via this portal system to their target cells in the pars anterior. The anatomical relationship between the hypothalamus and posterior pituitary was, however, recognized to be different since the nerve fibres were found to extend from the supraoptic and paraventricular hypothalamic nuclei actually into the posterior pituitary. These nerve endings are closely applied to the capillaries and contain storage granules and direct neurosecretion of posterior pituitary hormones into the systemic circulation occurs at these endings. The absence of a significant neural connexion but the presence of the portal capillary circulation linking the hypothalamus and anterior pituitary clearly suggested that the control of the secretion of the anterior pituitary hormones was different from that of the posterior, and the alteration in function of the target organs when the pituitary stalk was cut leaving the pituitary intact implied that the hypothalamus secreted regulatory substances. Despite the abundant evidence for the existence of hypothalamic substances controlling pituitary function, it is only recently that the chemical structures of some of these have been established and their effects demonstrated. They are conventionally referred to as factors (F) if the activity of simple extracts of the hypothalamus is being studied, or as hormones (H) if their structures have been elucidated (Schally, Arimura, Bowers, Kastin, Sawano, and Redding, 1968). Since they have been shown to have either stimulatory or inhibitory effects the term 'hypothalamic regulatory hormones' would seem to be appropriate although early work resulted in the discovery mainly of releasing hormones. Each of the hormones will be considered individually and aspects of their structure and function will be discussed in the light of recent knowledge.

Received for publication 11 January 1974.

Thyrotrophin-releasing Hormone (TRH)

The existence of a hypothalamic factor important for the regulation of thyrotrophin (TSH) release from the pituitary was first demonstrated in rats by producing lesions in the median eminence of the hypothalamus. These resulted in a decrease in circulating TSH and thyroid hormone levels (Greer, 1951), and conversely electrical stimulation of sites in the medial-basal and paraventricular areas of the hypothalamus resulted in a rapid increase in circulating levels of TSH (Martin and Reichlin, 1970; Guillemin, 1970). There followed a period of intense search for the nature of thyrotrophin-releasing hormone. Increasingly elaborate techniques of purification of extracts of many hundreds of thousands of animal hypothalami yielded material of pure TSH-releasing activity. Finally the hypothalamic origin of TRH was confirmed by the isolation and subsequent synthesis of a tripeptide, pyro-GLU-HIS-PROamide (see table), with thyrotrophin-releasing properties (Schally, Bowers, Redding, and Barrett, 1966; Folkers, Enzman, Boler, Bowers, and Schally, 1969; Schally, Redding, Bowers, and Barrett, 1969a; Nair, Barrett, Bowers, and Schally, 1970; Burgus, Dunn, Desiderio, Ward, Vale, and Guillemin, 1970). Porcine, ovine, bovine, and human TRH appear to have the same structures. This major achievement—the isolation, analysis, and synthesis of the first hypothalamic regulatory hormone—marked a major turning point in the understanding of the control of the endocrine system. The biosynthesis *in vitro* of TRH from its three constituent amino acids by fragments of the median eminence, as well as the ventral and dorsal hypothalamus, has been demonstrated. Its synthesis appears to be under the influence of the non-ribosomal enzyme, TRH-synthetase, the activity of which is enhanced by incubation with thyroxine, although a resulting increase in TRH production has not yet been demonstrated. Endogenous TRH also appears to be dependent on catecholaminergic and possibly monoaminergic neurones for its synthesis and release (Reichlin, Martin, Mitnick, Boshans, Grimm,

Releasing hormones or factors[1] for:		
TSH	PYRO-GLU-HIS-PRO-NH_2	TRH
(Prolactin FSH[2])		
LH and FSH	PYRO-GLU-HIS-TRP-SER-TYR-GLY-LEU-ARG-PRO-GLY-NH_2	LH/FSH-RH
GH	Structure uncertain	GRH (?F)
ACTH	Structure uncertain	CRF
MSH	Structure uncertain	MRF
Prolactin	Structure uncertain	PRF
Release-inhibiting hormones or factors for:		
GH	H-ALA-GLY-CYS-LYS-ASN-PHE-PHE-TRP-LYS-THR-PHE-THR-SER-CYS-OH	
(TSH, FSH, glucagon, insulin)[2]	|_____|	GH-RIH
MSH	? PRO-LEU-GLY-NH_2	MIH (?F)
Prolactin	Structure uncertain	PIF

Table *Hypothalamic regulatory hormones and factors*

[1]Hypothalamic regulatory substances are called 'hormones' (H) when their structures have been elucidated; otherwise they are referred to as 'factors' (F).
[2]Anterior pituitary hormones which are secondarily affected—see text.

Bollinger, Gordon, and Malacra, 1972). Recently it has been shown that tritiated TRH is specifically bound by pituitary membrane receptors (Wilber and Seibel, 1973) and, since the adenyl cyclase system is also associated with these structures, it seems likely that cyclic AMP may be the mediator of the actions of the tripeptide on the pituitary thyrotrophs. Further evidence for this is the demonstration of an increase in pituitary adenyl cyclase activity produced by TRH (Kaneko, Saito, Oka, Oda, and Yanaihara, 1972). The stimulatory effect of TRH is blocked by an increase in circulating thyroid hormone levels acting directly on the pituitary. Although the mechanisms involved in hypothalamic-pituitary thyroid regulation are complex it seems likely that the thyrotroph is primarily regulated by circulating levels of triiodothyronine (T_3) and thyroxine (T_4), or possibly by T_3 only. These provide a negative feedback at the pituitary level. The inhibitory actions of the thyroid hormones modulate and oppose the stimulatory effects of thyrotrophin-releasing hormone. It is not yet certain whether TRH secretion is altered by the level of circulating thyroid hormones but it seems likely that the negative feedback also operates at the hypothalamic level so that TRH secretion increases when thyroid hormone levels fall, and vice versa. Various analogues of TRH have been synthesized, including a proline methyl derivative with enhanced biological action. This appears to be due to increased binding properties at the pituitary cell receptor site (Burgus, Monahan, Rivier, Vale, Ling, Grant, Amoss, and Guillemin, 1973).

Apart from the fascinating theoretical considerations of the biosynthesis and mode of action of TRH at the cellular level, the wide availability of this material has enabled extensive clinical observations to be made. It has been shown to cause a dose-related TSH response when administered as a single dose orally or intravenously (Hall, Amos, Garry, and Buxton, 1970; Ormston, 1972), and to result in elevation of circulating T_3 and T_4 (Lawton, 1972).

A standard intravenous TRH test has been developed which will differentiate both hyperthyroidism and hypothyroidism from normality. When 200 μg TRH is given intravenously the serum TSH normally rises to reach a peak at about 20 minutes and then falls again. In the TRH test, serum immunoreactive TSH is measured at 0, 20, and 60 minutes; unfortunately TSH rather than serum thyroxine or protein-bound iodine has to be measured since the changes in circulating thyroid hormone levels are too small to be easily followed and the effects are delayed for up to eight hours. The TSH responses to TRH are slightly, but significantly, greater in women than in men unless the latter are treated with oestrogens (Ormston, Garry, Cryer, Besser, and Hall, 1971; Faglia, Beck-Pecioz, Ferrari, Ambrosi, Spada, and Travaglini, 1973).

In hyperthyroidism, whether due to Graves' disease or to an autonomous thyroid nodule, the high circulating T_3 and T_4 levels interfere with the action of TRH on the pituitary thyrotroph cell and impair the TSH response (see fig.). Using the standard intravenous 200 μg TRH test, we have never seen a significant (> 1 μU/ml) TSH response in a thyrotoxic patient, although it has been reported that with very large doses of TRH some TSH secretion may occur. Clearly this simple test is most valuable since it provides confirmatory evidence of the clinically suspected diagnosis even when other thyroid function tests are normal or equivocal such as in T_3-toxicosis or borderline T_4-toxicosis. Before the advent of this test the only equivalent test procedure was the T_3-suppression test which required a radioactive iodine uptake study before and after

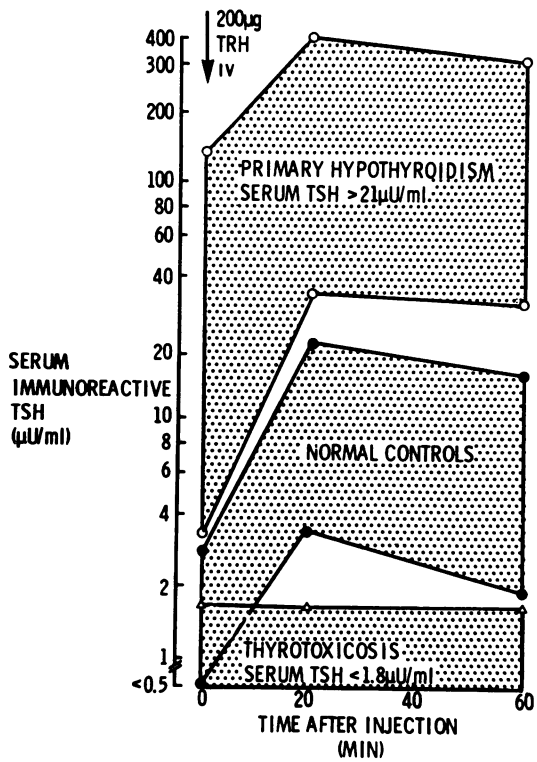

Fig *Ranges of the serum TSH responses to the standard TRH test (200 μg iv) in euthyroid, hyperthyroid, and primary hypothyroid patients.*

Ninety-five per cent confidence intervals are shown for the data of Ormston et al (1971). The diagnostic TSH levels quoted refer to the values obtained 20 minutes after TRH administration.

seven days' treatment with a supraphysiological dose of T_3 (80-120 μg/day). Not only was this test time consuming it was also potentially dangerous as it could induce an exacerbation of the thyrotoxicosis, especially the cardiac manifestations. The TRH test takes only one hour, can be used on an outpatient basis without any preparation, and is completely safe. It may cause transient flushing, a feeling of nausea, and an odd brief urethral discomfort due to contraction of the smooth muscle of the internal meatus, but none of these symptoms are particularly unpleasant. The same information is obtained as from the T_3-suppression test. Like the latter test, occasional euthyroid patients with Graves' disease or nodular goitres give abnormal results, since all the TRH test is detecting is an abnormal thyroid state, not controlled by pituitary TSH. Basal TSH levels cannot distinguish these conditions, for the assays are not good enough at the lower end of the normal range and many euthyroid patients have undetectable levels. Clinically euthyroid patients on replacement therapy may also show no TSH secretion in response to TRH (Evered, Young, Ormston, Menzies, Smith, and Hall, 1973b), but no evidence has yet been presented to show that these patients are in any way overtreated and for the moment this observation remains a biochemical curiosity of unknown significance.

In primary hypothyroidism, usually the basal serum TSH levels are elevated but again in mild cases there is often considerable overlap with the normal range. The TRH test will allow the distinction to be made, however, since the TSH response will be excessive (see fig). This test has replaced the TSH stimulation test for the confirmation of mild primary hypothyroidism. Some patients are seen who are euthyroid both clinically and in terms of the levels of circulating thyroid hormone concentrations, but who have mildly elevated basal serum TSH levels or simply an excessive response to TRH (Evered, Ormston, Smith, Hall, and Bird, 1973a). The term 'subclinical hypothyroidism' has been applied to these patients who usually have thyroiditis or have undergone a recent thyroidectomy. This seems a misnomer, however, since there is no evidence of thyroid insufficiency symptomatically on clinical examination or on testing the patients in any other way other than by measuring serum TSH. These patients clearly have a compensated state in which the thyroid gland with a reduced capacity for hormone production is enabled to produce normal amounts of T_3 and T_4 by being driven harder by TSH, ie, a state of compensated euthyroidism exists. Evidence has yet to be presented that these patients are at a disadvantage and require treatment with thyroid hormone.

Secondary (pituitary) or tertiary (hypothalamic) hypothyroidism may also be investigated using TRH (Hall, Ormston, Besser, Cryer, and McKendrick, 1972). If the patient is hypothyroid and the TSH response to TRH is not excessive then the hypothyroidism is not primary. Usually secondary hypothyroidism is associated with an impaired 20 and 60 minutes' TSH response. However, in patients with tertiary hypothyroidism TSH secretion after TRH is usually normal and the 60-minute value is higher than the 20-minute value and may be excessive. This 'delayed' pattern of response is never seen in normal patients and is characteristic of hypothalamic lesions. Exceptions to the classical types of response in pituitary and hypothalamic disease are, however, often seen and the responses may be normal in secondary or tertiary hypothyroid patients; the test has not proved as valuable in this area as in patients with primary thyroid disease.

As well as being of great diagnostic value, repeated

administration of TRH has been used in the treatment of thyroid carcinoma (Fairclough, Cryer, McAllister, Hawkins, Jones, McKendrick, Hall, and Besser, 1973). Patients received infusions or oral administration of TRH to raise circulating TSH levels before tracer or therapy doses or radioactive ^{131}I were given. It is hoped the serum TSH levels rise into the range found in primary hypothyroidism to increase incorporation of ^{131}I into the thyroid tissues and metastases without it being necessary for the patients to be rendered grossly hypothyroid.

The actions of TRH are not specific to TSH alone, however, since it has been shown to release prolactin in man as well as in animals (Jacobs, Snyder, Wilber, Utiger, and Daughaday, 1971). Indeed its use in the stimulation of milk production in cows is currently being investigated. It is evident that the pituitary cell receptors for the actions of TRH to stimulate TSH and prolactin are separate since the effects can easily be dissociated (Sachson, Rosen, Cuatrecasas, Roth, and Frantz, 1972; Hall, Besser, Schally, Coy, Evered, Goldie, Kastin, McNeilly, Mortimer, Phenekos, Tunbridge, and Weightman, 1973; and Mortimer, Besser, Goldie, Hook, and McNeilly, 1974a), and TRH does not appear to be the physiological prolactin-releasing hormone. It may be that the prolactin-releasing action of TRH is simply a pharmacological side effect. However, TRH may be used to test for the integrity of the reserve prolactin secretory capacity of the pituitary in cases of pituitary or hypothalamic dysfunction. A rise in serum follicle-stimulating hormone (FSH) has also been reported following TRH (Mortimer, Besser, McNeilly, Tunbridge, Gomez-Pan, and Hall, 1973d); this occurs in men but not in women and LH levels are unaffected. Following the administration of oestrogen, the serum TSH response to TRH is enhanced but the FSH levels are suppressed. Stimulation of LH at the preovulatory phase of the menstrual cycle has been reported in some normal women (Franchimont, 1972). The biological significance of these observations on the gonadotrophins is not known.

Luteinizing Hormone- and Follicle-stimulating Hormone-releasing Hormone (LH/FSH-RH)

The role of the central nervous system in the control of gonadotrophin secretion was first demonstrated by the fact that an electrical current passed through the heads of oestrous rabbits could elicit ovulation and pseudopregnancy (Marshall and Verney, 1936). Others demonstrated that stimulation of the tuber cinereum and the preoptic-suprachiasmatic region produced release of gonadotrophins (Harris, 1937). The same stimulus applied to the pituitary stalk or pituitary itself was ineffective. It was also noted that section of the pituitary stalk with the insertion of a barrier between the median eminence and pituitary gland resulted in ovarian atrophy in monkeys (Harris, 1950). However, if the barrier was not positioned satisfactorily rapid regeneration of the portal vessels in the stalk was noted with recovery of some gonadotrophic activity. This correlated well with the level of vascular reconstruction (Harris and Johnson, 1950). Such evidence, together with the fact that the gonadotrophic function of an isolated pituitary will be re-established if implanted in intimate contact with the median eminence, provided strong evidence of hypothalamic control of gonadotrophin secretion. The conclusions from the early classical experiments were confirmed when two apparently separate extracts of rat stalk median eminence (SME) were shown to release LH and FSH from the pituitary (McCann, 1962; McCann and Dhariwal, 1966). It was concluded therefore that the hypothalamus regulated the release of LH and FSH by means of two releasing hormones (LH-RH and FSH-RH), transported to the anterior pituitary via the hypothalamic portal circulation. Early evidence of the importance of neurostimulatory mechanisms in the secretion of gonadotrophin-releasing hormones themselves and the known actions of various central nervous transmitters has led to the realization that dopamine stimulates the release of the gonadotrophin-releasing hormone(s) from the hypothalamus.

Although the existence of two separate releasing hormones for LH and FSH had been suggested by this work, it proved possible to isolate only a single hormone, a decapeptide, from many thousands of porcine hypothalami, and the controversial suggestion was made that only one gonadotrophin-releasing hormone exists, not two (Schally, Arimura, Baba, Nair, Matsuo, Redding, Debeljuk, and White, 1971). This decapeptide released both LH and FSH *in vitro* and *in vivo* in animals and its actions were indistinguishable from that of natural hypothalamic LH-RF. The synthetic decapeptide also caused a dose-related increase in circulating levels of LH and FSH in man, although less FSH was secreted, and it was suggested therefore that the hormone be referred to as LH/FSH-RH (Besser, McNeilly, Anderson, Marshall, Harsoulis, Hall, Ormston, Alexander, and Collins, 1972a). The time course of release of the two gonadotrophins has been shown to differ. During continuous infusions of the decapeptide the rise in FSH precedes that of LH and the levels of both hormones rise in an asynchronous and often pulsatile fashion suggesting that there are occasions when the gonadotroph is refractory to the LH- or FSH-releasing actions of the hormone (Mortimer,

Besser, Goldie, Hook, and McNeilly, 1973a). The ability of a single gonadotrophin-releasing hormone to produce such independent changes in LH and FSH is important since it suggests that the normal spontaneous fluctuations in basal levels of LH and FSH (Naftolin, Yen, and Tsai, 1972) may be due to intermittent activity of the pituitary cells themselves under constant stimulation by the hypothalamic hormone, rather than to intermittent and asynchronous secretion of an LH- and FSH-releasing hormone(s). Further, the cyclical gonadotrophin secretion seen during the menstrual cycle does not necessarily imply that there are separate releasing hormones for LH and FSH. It is possible that the feedback effects of circulating levels of gonadal steroids may play a part in determining the pattern of gonadotrophin secretion by interaction with one hypothalamic regulatory hormone. In male rats treatment with testosterone proprionate decreases the release of LH and FSH occurring after the administration of LH/FSH-RH (Debeljuk, Arimura, and Schally, 1972) whereas the administration of small doses of oestrogen enhances the response to the releasing hormone in female but not male rats (Arimura and Schally, 1971); progesterone in large doses will suppress the response to LH/FSH-RH in cycling rats (Arimura and Schally, 1970); oestrogen administration in normal human males will markedly reduce the LH and FSH responses to continuous infusions of LH/FSH-RH (Mortimer, Besser, McNeilly, and Goldie, 1973b) whereas we find only a slight suppression after large doses of testosterone proprionate. The relationship of circulating hormones to the regulation of hypothalamic-pituitary-gonadal function remains uncertain but in females it would appear possible that the rise in circulating oestrogen at midcycle increases pituitary responsiveness to LH/FSH-RH facilitating the mid-cycle LH and FSH surge (Yen, VandenBerg, Rebar, and Ehara, 1972). Following ovulation oestrogen in combination with progesterone results in diminished pituitary sensitivity. In males, since the administration of small doses of oestrogen produces a much greater reduction in pituitary response to LH/FSH-RH than does testosterone, oestrogens may be the more important in any rapid feedback mechanism. The role of inhibin, a hormone presumably derived from spermatozoa, remains undecided although clinically we have frequently seen a normal LH, but a grossly exaggerated FSH response to LH/FSH-RH in patients with oligo- or azoo-spermia. Further investigation using the gonadotrophin-releasing hormone, together with the elucidation of the modulatory properties of sex hormone binding globulin (Anderson, 1974), may yet further clarify the complex feedback mechanisms concerned with the regulation of sex hormones. The actions of this releasing hormone are specific to gonadotrophin release and it does not cause release of TSH, ACTH, growth hormone, or prolactin; no interaction is seen with the mechanisms of release of other pituitary hormones. Thus when the gonadotrophin-releasing hormone is administered along with TRH or insulin-induced hypoglycaemia, the pituitary hormone responses are the same as they are without LH/FSH-RH (Mortimer *et al*, 1973d).

Apart from causing the rapid release of LH and FSH from the pituitary there is evidence that the decapeptide is capable also of stimulating gonadotrophin synthesis. The addition of the hormone to cultures of rat anterior pituitary glands was shown to increase the total content of LH and FSH in both the tissue and medium with increased incorporation of ^{3}H-glucosamine into the LH and FSH glycoproteins (Redding, Schally, Arimura, and Matsuo, 1972). It is thought that the action of LH/FSH-RH is produced by stimulating adenyl cyclase activity in the gonadotrophs.

The value of the material as a diagnostic tool in clinical endocrinology is not as clear as with TRH. It was hoped that a test using this material would allow gonadal, pituitary, and hypothalamic causes of hypogonadism to be differentiated, and a simple test has been described involving intravenous administration of 100 μg LH/FSH-RH with blood sampling for LH and FSH at 0, 20 (about the time of the LH peak), and 60 minutes, and such a procedure requires no preparation and is associated with no side effects (Besser *et al*, 1972a). The initial studies with this test showed that the condition of so called 'isolated gonadotrophin deficiency', in which patients show absent or partial puberty, low or absent circulating gonadotrophin levels with no response to clomiphene, a normal gonadal steroid response to exogenous gonadotrophins, and no evidence of other pituitary hormone deficiency, was in fact due to a hypothalamic defect with deficiency of the gonadotrophin-releasing hormone rather than to a pituitary cell dysfunction, since these patients show LH and FSH responses to exogenous LH/FSH-RH (Marshall, Harsoulis, Anderson, McNeilly, Besser, and Hall, 1972; Mortimer, Besser, McNeilly, Marshall, Harsoulis, Tunbridge, Gomez-Pan, and Hall, 1973c). The few patients with this condition, who do not respond to the first injection of LH/FSH-RH, do respond after repeated administration, providing additional evidence that the decapeptide can promote synthesis as well as release of LH and FSH. Further studies, however, suggest that it is not possible to differentiate between acquired pituitary and hypothalamic dysfunction using LH/FSH-RH. In a recent review of 155 such patients tested in this way

(Mortimer *et al*, 1973c), it proved possible to increase circulating levels of either LH or FSH in all but nine, whether the primary abnormality was at the pituitary or hypothalamic level despite the fact that 137 were clinically hypogonadal at the time of testing. Although primary gonadal failure could be differentiated since it resulted in an exaggerated response, it was not possible to differentiate between hypothalamic or pituitary causes of hypogonadism. It was particularly interesting that pituitaries containing tumours uniformly responded.

The evidence that the majority of these patients, although not secreting LH and FSH spontaneously, had gonadotrophs capable of stimulation by LH/FSH-RH and the fact that it can promote synthesis of both LH and FSH, has led to the suggestion that this material might be used to promote fertility in cases of hypogonadotrophic hypogonadism. The correct regime for this has not yet been established. It has been shown that the time courses of the effects of the decapeptide are the same whether given intravenously, subcutaneously, or intramuscularly. Elevation of circulating levels of LH persist for five to seven hours and FSH for three to five hours after 100 μg of the material. Although the decapeptide is partially absorbed when given via the nasal mucosa, the effects on circulating gonadotrophin levels are more marked after 100 μg given parenterally than when 2 mg was given intranasally (Mortimer, Besser, Hook, and McNeilly, 1973b). It is suggested, therefore, that repeated subcutaneous injection of at least 100 μg of the hormone six hourly may be of therapeutic value in the treatment of hypogonadotrophic infertility (Mortimer, Besser, and McNeilly, 1974c). Preliminary reports of the induction of ovulation (Zarate, Canales, Schally, Ayala-Valdes, and Kastin, 1972) provide further encouragement for the future. The use of the material is also being explored in domestic animals for the induction of ovulation and egg production in chickens and enhancement of the reproductive capabilities of sheep and cattle. As well as promoting fertility the development of an analogue of the gonadotrophin-releasing hormone which would block the pituitary receptors and act as a contraceptive agent is being explored. The raising of antisera reacting specifically with the endogenously secreted hormone has been achieved in rabbits and guinea pigs (Schally, Arimura, and Kastin, 1973; Kerdelhué, Jutisz, Studer, Gillessen, and Künzi, 1973; Barker, Isles, Fraser, and Gunn, 1973), and such immunized rabbits showed testicular atrophy and a diminution in the pituitary LH content. It has not yet been possible to detect LH/FSH-RH activity by radioimmunoassay convincingly in the circulation and it may be that the levels are below the limits of detection by this method. However, immunoreactive LH/RSH-RH has been detected in urine. The half life of the disappearance of exogenous decapeptide from the circulation in man is reported as five minutes for the first phase and 45 minutes for the second phase of the biexponential disappearance curve (Jeffcoate, Greenwood, and Holland, 1974).

Growth Hormone-releasing Hormone (GRH) and Growth Hormone-release Inhibiting Hormone (GHR-IH)

Growth retardation following the destruction of the ventral hypothalamus in rats was first demonstrated by Hetherington and Ranson in 1940, but it was 24 years before there was direct evidence of the existence of an extractable hypothalamic factor responsible for pituitary release of growth hormone (GH) in female rats (Deuben and Meites, 1964). Similar releasing factors have been identified in the hypophyseal portal blood (Wilber and Porter, 1970) and infusion of SME extracts into rats has confirmed the hypothalamic hormonal control of pituitary GH secretion (Sandow, Arimura, and Schally, 1972). Isolation of a polypeptide with apparent GH-releasing properties was achieved by extracting 200 000 porcine hypothalami (Schally, Sawano, Arimura, Barrett, Wakabayashi, and Bowers, 1969b) and characterization of its decapeptide structure revealed that it bore a similarity to the terminal amino acid sequence of the β chain of porcine haemoglobin (Veber, Bennett, Milkowski, Gal, Denkewalter, and Hirschmann, 1971). Comparison of the effects of this synthetic decapeptide and the natural porcine extract in rats revealed that only biologically assayable GH was released and no increase in GH levels was seen when specific immunoassays for this hormone were used. Since it is also inactive in man it is unlikely that this material is the physiological GRH (Kastin, Gual, and Schally, 1972a). However, an extractable form of GH-releasing substance has been demonstrated and shown to result in increased levels of immunoassayable GH *in vitro* (Wilber, Nagel, and White, 1971). Recent work therefore suggests the existence of a growth hormone-releasing hormone although the exact structure remains to be elucidated.

Evidence for an inhibitory hypothalamic hormone controlling GH secretion was provided when gel filtration of both sheep and rat hypothalamic extracts on Sephadex resulted in the elution from the column of two zones which influenced the release of GH from rat pituitaries *in vitro*. Although the first zone increased the release of GH, incubation with material eluted from the second zone inhibited

the secretion of GH to about half that released by control glands (Krulich, Dhariwal, and McCann, 1968). Recently a cyclic tetradecapeptide (table I) has been isolated from ovine hypothalami and a synthetic linear form of the substance was shown to inhibit the release of GH from rat and human pituitary cells *in vitro* and rats *in vivo* (Brazeau, Vale, Burgus, Ling, Butcher, Rivier, and Guillemin, 1973). The cyclic tetradecapeptide was subsequently synthesized using solid phase methods and purified by partition chromatography using Sephadex (Coy, Coy, Arimura, and Schally, 1973) and we have recently studied its effects in human subjects *in vivo* (Hall *et al*, 1973). This growth hormone-release inhibiting hormone (GHR-IH) inhibits the growth hormone rise during insulin-induced hypoglycaemia although its effects are shortlived, lasting for only the duration of the intravenous infusion. The normal increase in prolactin and corticosteroids during the hypoglycaemia were unaffected. This substance was also found to inhibit TSH and FSH release induced by TRH but not to affect the concomitant prolactin secretion. There was no interaction with LH/FSH-RH and circulating levels of LH and FSH remained unaffected in normal male subjects basally and in response to LH/FSH-RH. It remains to be seen whether TSH, LH, and FSH secretion under the influence of endogenous natural hypothalamic releasing hormones are inhibited. From this recent work it seems possible that this tetradecapeptide may have great value in the treatment of diseases associated with excessive GH secretion such as acromegaly, gigantism, and diabetes mellitus. The first studies in acromegaly showed that the circulating growth hormone levels in three such patients were markedly suppressed during and for 30 minutes after the infusion of GHR-IH. Circulating prolactin levels were unaffected. The effects of long-term treatment are not yet known. Longer-acting preparations of GHR-IH will doubtless be developed. In addition to its action on the pituitary, GHR-IH appears partially to inhibit secretion of glucagon and insulin by a direct effect on the pancreas (Mortimer, Carr, Lind, Bloom, Mallinson, Schally, Tunbridge, Yeomans, Coy, Kastin, Besser, and Hall, 1974d).

Corticotrophin-releasing Factor (CRF)

The isolation of n.aterial with ACTH-releasing activity from extracts of the median eminence and posterior pituitary was the first successful demonstration of a hypothalamic hormone (Saffran, Schally, and Benfey, 1955). The secretion of CRF in stressed rats and the differentiation from vasopressin by a biological assay gave early promise of the elucidation of the structure of this hormone (Anderson, 1966). However, repeated attempts to isolate sufficient amounts for further investigations have been unsuccessful since it is extremely labile in a pure form. However a partial amino acid sequence of a tentative corticotrophin-releasing hormone has been suggested (Schally and Bowers, 1964). Whether this represents the structure of endogenous CRH remains uncertain and so the structure of historically the most important hypothalamic hormone still remains an elusive prize.

Melanocyte-stimulating Hormone Release-inhibiting Hormone (MIH) and Melanocyte-stimulating Hormone-releasing Hormone (MRH)

The secretion of melanocyte-stimulating hormone (MSH) by the pars intermedia is responsible for the background adaptation to light seen in certain species of frogs. The demonstration that section of the pituitary stalk or destruction of the hypothalamus in frogs led to their progressive pigmentation suggested a hypothalamic inhibitory mechanism controlling the secretion of MSH (Etkin, 1962). This was confirmed when aqueous extracts of hypothalami from both light- and dark-adapted frogs were shown to inhibit the secretion of MSH from the pars intermedia of the dark-adapted frog. In 1971, an extract of bovine hypothalami was purified over 11 000 times by Sephadex filtration and thin-layer chromatography and shown to consist of two active peptides (Nair, Kastin, and Schally, 1971). The tripeptide (table I) was shown to have greater biological activity and also to have electrophoretic mobility identical to that of natural bovine MIF. The other fraction was a pentapeptide which was far less effective in promoting skin lightening after direct application to the pituitary in a pigmented frog with a destroyed hypothalamus; a second pentapeptide, tocinoic acid, with MIH activity, has also been described. Although the amino acids constituting the various isolated forms of MIH differ, there is a common link in that all are contained within the molecule of oxytocin, in the appropriate sequences. There is, however, evidence of species specificity among the related peptides with MIH activity. Recent work also reveals the existence of a melanocyte-releasing hormone (MRH) (Kastin, Miller, and Schally, 1968; Kastin, Schally, Viosca, and Miller, 1969; Kastin, Schally, Gual, Glick, and Arimura, 1972b), which is a pentapeptide consisting of the N-terminal amino acids of oxytocin except that the structure is linear and not cyclic. It has been suggested that oxytocin in fact is functioning as a pro-hormone for MIH and MRH. The action of intrahypothalamic peptides is thought to cleave the oxytocin molecule to form the active substances responsible for the control of pigmentation (Celis, Taleisnik,

and Walter, 1971). However, MRH has not been isolated from hypothalamic tissues.

Various pharmacological and physical stimuli have been shown to influence MSH secretion in animals. By and large MSH secretion follows that of ACTH and is secreted from the same anterior pituitary cells in man. Plasma and pituitary MSH has been measured in albino rats and an increase in plasma MSH activity and a decrease in pituitary content was shown to occur following the administration of trifluoperazine, ether, and lysine vasopressin. Exposure to darkness and pinealectomy elevated pituitary MSH content but left plasma levels unaffected. A combination of Na pentobarbital and morphine was particularly effective in causing pituitary release of MSH but this action was inhibited by the injection of MIH. The tripeptide MIH does not appear to alter MSH levels in man (Kastin *et al*, 1972b) and it is unlikely to be of benefit in the treatment of MSH-dependent pigmentary diseases such as Nelson's syndrome. The tripeptide MIH has, however, been used in Parkinsonism but this action does not require the presence of the pituitary gland (Schally *et al*, 1973).

Prolactin Release-inhibiting Factor (PIF) and Prolactin-releasing Factor (PRF)

The secretion of prolactin was the first pituitary hormone shown to be predominantly under the control of a hypothalamic inhibitory influence (Desclin, 1950; Everett, 1954). These workers observed that removal of the pituitary and transplantation to the renal capsule resulted in maintenance of the function of the corpora lutea and mammary glands. Following this, it was noted that median eminence lesions or pituitary stalk section would result in continuous release of prolactin in rats *in vivo* (Meites, Nicoll, and Talwalker, 1963; Meites and Nicoll, 1966; Welsch, Squiers, Cassell, Chen, and Meites, 1971) and that crude hypothalamic extracts would inhibit prolactin release from rat pituitary glands *in vitro* (Talwalker, Ratner, and Meites, 1963); extracts from sheep, cattle, and pigs were shown to have similar effects. Physiological studies using a method *in vitro* to measure PIF activity (Kragt and Meites, 1967) have shown a decrease in biological activity following the administration of oestrogen, progesterone, testosterone, cortisol, norethyrodrel-mestranol combinations, Na pentobarbital, reserpine, perphenazine, chlorpromazine, haloperidol, methyldopa, α methyl para-and meta-tyrosine, and amphetamine. These stimuli together with suckling and stress all resulted in increased circulating levels of prolactin. Increased secretion of PIF with consequent reduction in circulating prolactin in rats occurred with 1-dopa, iproniazid, pargyline, pyrogalol, and prolactin itself. As well as these substances, various ergot derivatives have been shown to inhibit prolactin release notably 2-brom-α-ergocryptine which has been used successfully in the treatment of patients with the galactorrhoea-amenorrhoea syndrome (Besser, Parke, Edwards, Forsyth, and McNeilly, 1972b) and to terminate puerperal lactation (Del Pozo, Brun del Re, Varga, and Freisen, 1972). Studies *in vitro* with ergocornine showed depressed pituitary release with accumulation of prolactin when this compound was incubated directly with cultures of rat pituitary gland. It also prevented the stimulatory effect of oestradiol (Lu and Meites, 1971). As well as having a direct inhibitory effect on the prolactin-secreting cells of the pituitary, this compound was also shown to increase PIF activity in the hypothalamus and increase dopamine in the median eminence (Wuttke, Gelato, and Meites, 1972). The hypothalamic stimulus for PIF secretion probably depends on dopaminergic transmitters, since following the injection of dopamine into the third ventricle of rats, PIF secretion occurred with a fall in serum prolactin (Kamberi, Mical, and Porter, 1971). Although the structure of PIF remains to be elucidated, current evidence indicates that it is a polypeptide of low molecular weight.

It was several years after the demonstration of an inhibitory mechanism controlling prolactin secretion that evidence of a stimulatory hypothalamic material was first described. This was achieved following injections of crude rat hypothalamic extracts into oestrogen-primed female rats which showed an increase in circulating levels of prolactin (Meites, Talwalker, and Nicoll, 1960); purification showed that the extracts contained a prolactin-releasing factor (PRF) distinct from the tripeptide, TRH (Valverde, Chieffo, and Reichlin, 1972). The secretion of a PRF, probably a polypeptide, may be dependent on serotonin as the intrahypothalamic transmitter (Schally *et al*, 1973), since a rise in serum prolactin may be achieved by the injection of serotonin into the third ventricle of rats although systemic administration fails to produce any change, presumably because a blood-brain barrier exists. However, a single intraperitoneal injection of 5-hydroxytryptophan and melatonin have been shown to increase serum prolactin levels (Meites, 1973). The addition of serotonin to pituitary cultures does not result in prolactin release (Talwalker *et al*, 1963). Although mammalian hypothalami have been shown to have both PIF and PRF activity, studies in birds have shown that all extracts obtained to date stimulate prolactin release. This may be the predominant control in the avian species although further

studies will be required before this can be accepted.

A number of observations suggest that the stimulatory effect of TRH on the release of prolactin is distinct from PRF in man, although both materials act on the pituitary. In isolated TSH deficiency, prolactin secretion after TRH occurs without TSH release (Sachson *et al*, 1972); G-R-IH will block the TSH-induced rise to TRH but not the prolactin response (Hall *et al*, 1973); oestrogen administration to normal males results in elevation of serum TSH and prolactin levels basally and in response to infusions of TRH but the secretion of the two trophic hormones is asynchronous, with smooth rises in TSH but marked pulsatile variations in prolactin; insulin-induced hypoglycaemia results in a rise in prolactin but not TSH (Mortimer *et al*, 1973d); the intrahypothalamic control of TRH differs in that its synthesis is reduced by reserpine and serotonin, while being increased by catecholamines (Reichlin *et al*, 1972). However, although PRF is not TRH it seems likely that they share some similarities in structure. This may be the reason for the increased incidence of galactorrhoea in hypothyroidism since there may be an increase in TRH secretion in thyroid deficiency.

Although prolactin appears to be regulated by the dynamic equilibrium of PIF and PRF through the secretion of hypothalamic neurotransmitters, the exact feedback mechanisms are largely unknown. Oestrogen administration in rats produces a characteristic rise in prolactin and this effect has been demonstrated in normal males. Although changes in prolactin secretion have been described during the menstrual cycle in women (Robyn, Delroye, Nokin, Vekemans, Badawi, Perez-Lopez, and L'Hermite, 1973) other studies have not confirmed this (McNeilly and Chard, 1974); levels do rise during pregnancy. The nature of the hormonal feedback system remains in doubt although it would seem reasonable to expect prolactin to exert an inhibitory effect itself on the hypothalamus. Experimental data suggest that this concept may be valid. High circulating levels of prolactin or implantation of small amounts of prolactin into the median eminence will decrease prolactin in the pituitary and inhibit normal mammary development in lactation in the rat. This has been shown to occur in conjunction with an increase in PIF activity in the hypothalamus and dopamine concentration in the median eminence (Chen, Minaguchi, and Meites, 1967). The precise interaction of prolactin with the gonadotrophins, however, remains far from clear. LH/FSH-RH does not release prolactin nor interfere with its secretion during insulin-induced hypoglycaemia with or without TRH (Mortimer *et al*, 1973d). In rats hyperprolactinaemia has been shown to stimulate the secretion of LH and FSH resulting in the re-emergence of oestrous cycles in early pregnant rats (Voogt and Meites, 1971). There is also evidence that there is competition between secretion of the gonadotrophins and prolactin in non-gravid rats (Ben-David, Danon, and Sulman, 1971). This may also appear to be the case in women in whom raised prolactin levels result in the cessation of periods. However, abundant gonadotrophin stores in the human pituitary seem to exist in hypogonad, hyperprolactinaemic patients, since normal or even excessive amounts of LH and FSH can be released by the administration of LH/FSH-RH (Mortimer *et al*, 1973b). Some patients in fact have normal or high circulating gonadotrophin levels when compared with those of normal women in the follicular phase of the menstrual cycle. It would appear therefore in human subjects at least that hyperprolactinaemia results in the failure of cyclical release of pituitary stores of gonadotrophins, possibly by inhibiting the secretion or actions of endogenous gonadotrophin-releasing hormone; there is also a possibility that prolactin may have antigonadotrophic actions at the gonadal level. The feedback of blood electrolytes on prolactin secretion is currently being investigated (Horrobin, Burstyn, Lloyd, Durkin, Lipton, and Muiruri, 1971) but it will be some time before the full story of prolactin, its hypothalamic-pituitary control, and biological significance are known.

Summary

There is little doubt that the hypothalamic-pituitary-target organ system provides a sophisticated amplifier, whereby the brain can cause a tiny amount of hypothalamic hormone to be released which in turn activates the pituitary and causes large amounts of target gland hormones to be secreted. These can not only profoundly alter the subject's physical and behavioural functioning, but also, by influencing his reactivity with his surroundings, alter his environment. By sensing the circulating hormone levels as well as the physical and environmental changes, the brain modulates the secretion of its own hormones and the system as a whole.

The isolation and synthesis of various hypothalamic regulatory hormones has revolutionized the field of endocrinology and led to a greater understanding of the pathophysiology of many disease processes. The feedback mechanisms operating between the hypothalamus, pituitary, and target organ secretions which have been revealed already will no doubt be further clarified when sensitive assays for the regulatory hormones themselves are developed. Mean-

while recent experience with these biologically active polypeptides has provided encouraging opportunities in both diagnosis and treatment of endocrine disorders. The discovery and synthesis of further regulatory hormones and their application in clinical medicine is therefore eagerly awaited.

References

Anderson, E. (1966). Adrenocorticotrophin-releasing hormone in peripheral blood: increase during stress. *Science*, **152**, 379-380.

Anderson, D. C. (1974). Sex-hormone-binding globulin. *Clin. Endocr.*, **3**, 69-96.

Arimura, A., and Schally, A. V. (1970). Progesterone suppression of LH-releasing hormone-induced stimulation of LH release in rats. *Endocrinology*, **87**, 653-657.

Arimura, A., and Schally, A. V. (1971). Augmentation of pituitary responsiveness to LH-releasing hormone (LH-RH) by estrogen. *Proc. Soc. exp. Biol. (N.Y.)*, **136**, 290-293.

Barker, H. M., Isles, T. E., Fraser, H. M., and Gunn, A. (1973). Radioimmunoassay of luteinizing hormone releasing hormone. *Nature (Lond.)*, **242**, 527-528.

Ben-David, M., Danon, A., and Sulman, F. G. (1971). Evidence of antagonism between prolactin and gonadotrophin secretion: effect of methallibure on perphenazine-induced prolactin secretion in ovariectomised rats. *J. Endocr.*, **51**, 719-725.

Besser, G. M., McNeilly, A. S., Anderson, D. C., Marshall, J. C., Harsoulis, P., Hall, R., Ormston, B. J., Alexander, L., and Collins, W. P. (1972a). Hormonal responses to synthetic luteinizing hormone and follicle stimulating hormone releasing hormone in man. *Brit. med. J.*, **3**, 267-271.

Besser, G. M., Parke, L., Edwards, C. R. W., Forsyth, I. A., and McNeilly, A. S. (1972b). Galactorrhoea: successful treatment with reduction of plasma prolactin levels by brom-ergocryptine. *Brit. med. J.*, **3**, 669-672.

Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier J., and Guillemin, R. (1973). Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science*, **179**, 77-79.

Burgus, R., Dunn, T. F., Desiderio, D., Ward, D. N., Vale, W., and Guillemin, R. (1970). Characterisation of ovine hypothalamic hypophysiotropic TSH-releasing factor. *Nature (Lond.)*, **226**, 321-325.

Burgus, R., Monahan, M., Rivier, J., Vale, W., Ling, N., Grant, G., Amoss, M., and Guillemin, R. (1973). Structure-biological activity relationships of thyrotropin and luteinizing hormone releasing factor analogues. In *Hypothalamic Hypophysiotropic Hormones* (Excerpta Medica Int. Congr. Ser. No. 263), edited by C. Gual and E. Rosemberg, pp. 12-23. Excerpta Medica, Amsterdam.

Celis, M. E., Taleisnik, S., and Walter, R. (1971). Release of pituitary melanocyte stimulating hormone by the oxytocin fragment, H-Cys-Tyr-Ile-Glu-Asn-OH. *Biochem. biophys. Res. Commun.*, **45**, 564-569.

Chen, C. L., Minaguchi, H., and Meites, J. (1967). Effects of transplanted pituitary tumors on host pituitary prolactin secretion. *Proc. Soc. exp. Biol. (N.Y.)*, **126**, 317-320.

Coy, D. H., Coy, E. J., Arimura, A., and Schally, A. V. (1973). Solid phase synthesis of growth hormone-release inhibitory factor. *Biochem. biophys. Res. Commun.*, **54**, 1267-1273.

Debeljuk, L., Arimura, A., and Schally, A. V. (1972). Effect of testosterone and estradiol on the LH and FSH release induced by LH-releasing hormone (LH-RH) in intact male rats. *Endocrinology*, **90**, 1578-1581.

Del Pozo, E., Brun del Re, R., Varga, L., and Freisen, H. (1972). The inhibition of prolactin secretion in man by CB 154 (2 brom α ergocryptine). *J. clin. Endocr.*, **35**, 768-771.

Desclin, L. (1950). A propos du mecanisme d'action des ostrogènes sur le lobe antérieur de l'hypophyse chez le rat. *Ann. Endocr. (Paris)*, **11**, 656-659.

Deuben, R. R., and Meites, J. (1964). Stimulation of pituitary growth hormone release by a hypothalamic extract *in vitro*. *Endocrinology*, **74**, 408-414.

Etkin, W. (1962). Neurosecretory control of the pars intermedia. *Gen. comp. Endocr.*, **2**, 161-169.

Evered, D. C., Ormston, B. J., Smith, P. A., Hall, R., and Bird, T. (1973a). Grades of hypothyroidism. *Brit. med. J.*, **1**, 657-661.

Evered, D. C., Young, E. T., Ormston, B. J., Menzies, R., Smith' P. A., and Hall, R. (1973b). Treatment of hypothyroidism: a reappraisal of thyroxine therapy. *Brit. med. J.*, **3**, 131-134.

Everett, J. W. (1954). Luteotrophic function of autografts of the rat hypophysis. *Endocrinology*, **54**, 685-690.

Faglia, G., Beck-Pocioz, P., Ferrari, C., Ambrosi, B., Spada, A., and Travaglini, P. (1973). Enhanced plasma thyrotrophin response to thyrotrophin releasing hormone following oestradiol administration in man. *Clin. Endocr.*, **2**, 207-210.

Fairclough, P. D., Cryer, R. J., McAllister, J., Hawkins, L., Jones, A. E., McKendrick, M., Hall, R., and Besser, G. M. (1973). Serum TSH responses to intravenously and orally administered TRH after thyroidectomy for carcinoma of the thyroid. *Clin. Endocr.*, **2**, 351-359.

Folkers, K., Enzman, F., Boler, J., Bowers, C. Y., and Schally, A. V. (1969). Discovery of modification of the synthetic tripeptide sequence of the thyrotropin releasing hormone having activity. *Biochem. biophys. Res. Commun.*, **37**, 123-126.

Franchimont, P. (1972). Discussion on effects of TRH on other pituitary hormones. In *Thyrotrophin Releasing Hormone* (Frontiers of Hormone Research, Vol. 1), edited by R. Hall, I. Werner, and H. Holgate, pp. 139-140. H. Karger, Basle.

Green, J. D., and Harris, G. W. (1947). The neurovascular link between the neurohypophysis and adenohypophysis. *J. Endocr.*, **5**, 136-146.

Greer, M. A. (1951). Evidence of hypothalamic control of the pituitary release of thyrotrophin. *Proc. Soc. exper. Biol. (N.Y.)*, **77**, 603-608.

Guillemin, R., and Vale, W. (1970). Bioassays of the hypophysiotropic hormones: *in vitro* systems. In *Hypophysiotropic Hormones of the Hypothalamus*, edited by J. Meites, pp. 21-35. Williams and Wilkins, Baltimore.

Hall, R., Amos, J., Garry, R., and Buxton, R. L. (1970). Thyroid stimulating hormone response to synthetic thyrotrophic releasing hormone in man. *Brit. med. J.*, **2**, 274-277.

Hall, R., Besser, G. M., Schally, A. V., Coy, D. W., Evered, D. Goldie, D. J., Kastin, A. J., McNeilly, A. S., Mortimer, C. H., Phenekos, C., Tunbridge, W. M. G., and Weightman, D. (1973). Action of growth-hormone-release inhibitory hormone in normal men and in acromegaly. *Lancet*, **2**, 581-584.

Hall, R., Ormston, B. J., Besser, G. M., Cryer, R. J., and McKendrick, M. (1972). The thyrotrophin-releasing hormone test in diseases of the pituitary and hypothalamus. *Lancet*, **1**, 759-763.

Harris, G. W. (1937). The induction of ovulation in the rabbit by electrical stimulation of the hypothalamo-hypophysial mechanism. *Proc. roy. Soc. B*, **122**, 374-394.

Harris, G. W. (1950). Oestrous rhythm, pseudopregnancy, and the pituitary stalk in the rat. *J. Physiol. (Lond.)*, **111**, 347-360.

Harris, G. W. (1955). *Neural Control of the Pituitary Gland*. Arnold, London.

Harris, G. W., and Johnson, R. T. (1950). Regeneration of the hypophysial portal vessels after section of the hypophysial stalk in the monkey (*Macacus rhesus*). *Nature (Lond.)*, **165**, 819-820.

Hetherington, A. W., and Ranson, S. W. (1940). Hypothalamic lesions and adiposity in the rat. *Anat. Rec.*, **78**, 149-172.

Horrobin, D. F., Burstyn, P. G., Lloyd, I. J., Durkin, N., Lipton, A., and Muiruri, K. L. (1971). Actions of prolactin on human renal function. *Lancet*, **2**, 352-354.

Jacobs, L. S., Snyder, P. J., Wilber, J. F., Utiger, R. D., and Daughaday, W. H. (1971). Increased scrum prolactin after administration of synthetic TRH in man. *J. clin. Endocr.*, **33**, 996-998.

Jeffcoate, S. L., Greenwood, R. H., and Holland, D. J. (1974). Blood and urine clearance of luteinizing hormone releasing hormone measured by radioimmunoassay. *J. Endocr.*, in press.

Kamberi, I. A., Mical, R. S., and Porter, J. C. (1971). Effect of anterior pituitary perfusion and intraventricular injection of catecholamines on prolactin release. *Endocrinology*, **88**, 1012-1020.

Kaneko, T., Saito, S., Oka, H., Oda, T., and Yanaihara, N. (1973). Effects of synthetic releasing hormones on cyclic AMP levels and hormone release from rat anterior pituitary tissue. In *Hypothalamic Hypophysiotrophic Hormones* (Excerpta Medica Int. Congr. Ser. No. 263), edited by C. Gual and E. Rosemberg, pp. 198-203. Excerpta Medica, Amsterdam.

Kastin, A. J., Gual, C., and Schally, A. V. (1972a). Clinical experience with hypothalamic releasing hormones. 2. Luteinizing hormone-releasing hormone and other hyphophysiotropic releasing hormones. *Rec. Progr. Hormone Res.*, **28**, 201-227.

Kastin, A. J., Miller, M. C., III, and Schally, A. V. (1968). MSH acti-

vity in the rat pituitary after treatment with nembutal and morphine: a new bioassay for MSH-release inhibiting factor (MIF) *Endocrinology*, **83**, 137-140.

Kastin, A. J., Schally, A. V., Gual, C., Glick, S., and Arimura, A. (1972b). Clinical evaluation in man of a substance with growth hormone-releasing activity in rats. *J. clin. Endocr.*, **35**, 326-329.

Kastin, A. J., Schally, A. V., Viosca, S., and Miller, M. C. (1969). MSH activity in plasma and pituitaries of rats after various treatments. *Endocrinology*, **84**, 20-27.

Kerdelhué, B., Jutisz, M., Studer, R. O., Gillessen, D., and Künzi, H. (1973). Comparison between the *in vitro* LH-releasing activity and radioimmunological activity of some structural analogs of luteinizing hormone releasing hormone. (Abstr.) *Acta endocr. (kbh.)*, Suppl. **177**, 197.

Kragt, C. L., and Meites, J. (1967). Dose-response relationships between hypothalamic PIF and prolactin release by rat pituitary tissue *in vitro*. *Endocrinology*, **80**, 1170-1173.

Krulich, L., Dhariwal, A. P. S., and McCann, S. M. (1968). Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary *in vitro*. *Endocrinology*, **83**, 783-790.

Lawton, N. F. (1972). Effects of TRH on thyroid hormone release. In *Thyrotrophin Releasing Hormone. Front* (Frontiers of Hormone Research, Vol. 1), edited by R. Hall, I. Werner, and H. Holgate, pp. 91-113. Karger, Basle.

Lu, K. H., and Meites, J. (1971). Inhibition by l-dopa and monoamine oxidase inhibitors of pituitary prolactin release: Stimulation by methyldopa and α-amphetamine. *Proc. Soc. exp. Biol. (N.Y.)*, **137**, 480-483.

McCann, S. M. (1962). A hypothalamic luteinizing hormone-releasing factor. *Amer. J. Physiol.*, **202**, 395-400.

McCann, S. M., and Dhariwal, A. P. S. (1966). Hypothalamic releasing factors and the neurosecretory link between the brain and anterior pituitary. In *Neuroendocrinology*, Vol. 1, edited by L. Martini and W. F. Ganong, pp. 261-296. Academic Press, New York.

McNeilly, A. S., and Chard, T. (1974). Circulating levels of prolactin during the menstrual cycle. *Clin. Endocr.*, in press.

Marshall, F. H. A., and Verney, E. G. (1936). The occurrence of ovulation and pseudopregnancy in the rabbit as a result of central nervous stimulation. *J. Physiol. (Lond.)*, **86**, 327-336.

Marshall, J. C., Harsoulis, P., Anderson, D. C., McNeilly, A. S., Besser, G. M., and Hall, R. (1972). Isolated pituitary gonadotrophin deficiency: gonadotrophin secretion after synthetic luteinizing hormone and follicle stimulating hormone releasing hormone. *Brit. med. J.*, **4**, 643-645.

Martin, J. B., and Reichlin, S. (1970). Thyrotrophin secretion in rats after hypothalamic electrical stimulation or injection of synthetic TSH-releasing factor. *Science*, **168**, 1366-1368.

Meites, J. (1973). Control of prolactin secretion in animals. In *International Symposium on Human Prolactin*, edited by J. L. Pasteels, and C. Robyn, pp. 52-64. *Excperta Medica Foundation*, Amsterdam.

Meites, J., and Nicoll, C. S. (1966). Adenohypophysis: prolactin. *Ann. Rev. Physiol.*, **28**, 57-88.

Meites, J., Nicoll, C. S., and Talwalker, P. I. L. (1963). The central nervous system and the secretion and release of prolactin. In *Advances in Neuroendocrinology*, ch. 8, edited by A. V. Nalbanov, p. 238. University of Illinois Press, Urbana, Illinois.

Meites, J., Talwalker, P. K., and Nicoll, C. S. (1960). Initiation of lactation in rats with hypothalamic or cerebral tissue. *Proc. Soc. exp. Biol. (N.Y.)*, **103**, 298-300.

Mortimer, C. H., Besser, G. M., Goldie, D. J., Hook, J., and McNeilly, A. S. (1973a). Asynchronous changes in circulating LH and FSH after the gonadotrophin releasing hormone. *Nature [new Biol.]*, **246**, 22-23.

Mortimer, C. H., Besser, G. M., Goldie, D. J., Hook, J., and McNeilly, A. S. (1974a). The TSH, FSH and prolactin responses to continuous infusions of TRH and the effects of oestrogen administration in normal males. *Clin. Endocr.*, in press.

Mortimer, C. H., Besser, G. M., Hook, J., and McNeilly, A. S. (1974b). Intravenous, intramuscular, subcutaneous and intranasal administration of LH/FSH-RH: the duration of effect and occurrence of asynchronous pulsatile release of LH and FSH. *Clin. Endocr.*, **3**, 19-25.

Mortimer, C. H., Besser, G. M., and McNeilly, A. S. (1974c). The induction of gonadotrophin secretion with repeated subcutaneous injections of LH/FSH-RH in males with hypogonadotrophic hypogonadism. (Abstr.) *J. Endocr.*, in press.

Mortimer, C. H., Besser, G. M., McNeilly, A. S., and Goldie, D. J. (1973b). Asynchronous pulsatile LH and FSH responses during LH/FSH-RH and TRH infusions. (Abstr.) *J. Endocr.*, **59**, xii.

Mortimer, C. H., Besser, G. M., McNeilly, A. S., Marshall, J. C., Harsoulis, P., Tunbridge, W. M. G., Gomez-Pan, A., and Hall, R. (1973c). The LH and FSH releasing hormone test in patients with hypothalamic-pituitary-gonadal dysfunction. *Brit. med. J.*, **4**, 73-77.

Mortimer, C. H., Besser, G. M., McNeilly, A. S., Tunbridge, W. M. G., Gomez-Pan, A., and Hall, R. (1973d). Interaction between secretion of the gonadotrophins, prolactin, growth hormone, thyrotrophin and corticosteroids in man: the effects of LH/FSH-RH, TRH and hypoglycaemia alone and in combination. *Clin. Endocr.*, **2**, 317-326.

Mortimer, C. H., Carr, D., Lind, T., Bloom, S. R., Mallinson, C. N., Schally, A. V., Tunbridge, W. M. G., Yeomans, L., Coy, D. H., Kastin, A. J., Besser, G. M., and Hall, R. (1974d). Growth hormone-release-inhibiting hormone: effects on circulating glucagon, insulin and growth hormone in normal, diabetic, acromegalic and hypopituitary patients. *Lancet*, in press.

Naftolin, F., Yen, S. S. C., and Tsai, C. C. (1972). Rapid cycling of plasma gonadotrophins in normal man as demonstrated by frequent sampling. *Nature [new Biol.]*, **236**, 92-93.

Nair, R. M. G., Barrett, J. F., Bowers, C. Y., and Schally, A. V. (1970). Structure of porcine thyrotrophin releasing hormone. *Biochemistry*, **9**, 1103-1106.

Nair, R. M. G., Kastin, A. J., and Schally, A. V. (1971). Isolation and structure of hypothalamic MSH release-inhibiting hormone. *Biochem. biophys. Res. Comman.*, **43**, 1376-1381.

Ormston, B. J. (1972). Clinical effects of TRH on TSH release after i.v. and oral administration in normal volunteers and patients with thyroid disease. In *Front. Hormone Res.*, Vol. 1, edited by R. Hall, I. Werner, and H. Holgate, pp. 45-75. Karger, Basle.

Ormston, B. J., Garry, R., Cryer, R. J., Besser, G. M., and Hall, R. (1971). Thyrotrophin-releasing hormone as a thyroid-function test. *Lancet*, **2**, 10-14.

Redding, T. W., Schally, A. V., Arimura, A., and Matsuo, H. (1972). Stimulation of release and synthesis of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in tissue cultures of rat pituitaries in response to natural and synthetic LH and FSH releasing hormone. *Endocrinology*, **90**, 764-770.

Reichlin, S., Martin, J. B., Mitnick, M. A., Boshans, R. L., Grimm, Y., Bollinger, J., Gordon, J., and Malacra, J. (1972). The hypothalamus in pituitary thyroid regulation. *Rec. Progr. Hormone Res.*, **28**, 229-286.

Robyn, C., Delroye, P., Nokin, J., Vekemans, M., Badawi, M., Perez-Lopez, F. P., and L'Hermite, M. (1973). Prolactin and human reproduction. In *International Symposium on Human Prolactin*, edited by J. L. Pasteels and C. Robyn, pp. 98-119. Excerpta Medica, Amsterdam.

Sachson, R., Rosen, S. W., Cuatrecasas, P., Roth, J., and Frantz, A. G. (1972). Prolactin stimulation by thyrotrophin-releasing hormone in a patient with isolated thyrotrophin deficiency. *New Engl. J. Med.*, **287**, 972-973.

Saffran, M., Schally, A. V., and Benfey, B. G. (1955). Stimulation of the release of corticotropin from the adenohypophysis by a neurohypophysial factor. *Endocrinology*, **57**, 439-444.

Sandow, J., Arimura, A., and Schally, A. V. (1972). Stimulation of growth hormone release by anterior pituitary perfusion in the rat. *Endocrinology*, **90**, 1315-1319.

Schally, A. V., Arimura, A., Baba, Y., Nair, R. M. G., Matsuo, H., Redding, T. W., Debeljuk, L., and White, W. F. (1971). Isolation and properties of the FSH and LH releasing hormone. *Biochem. biophys. Res. Commun.*, **43**, 393-399.

Schally, A. V., Arimura, A., Bowers, C. Y., Kastin, A. J., Sawano, S., and Redding, T. W. (1968). Hypothalamic neurohormones regulating anterior pituitary function. *Rec. Progr. Hormone Res.*, **24**, 497-588.

Schally, A. V., Arimura, A., and Kastin, A. J. (1973). Hypothalamic regulatory hormones. *Science*, **179**, 341-350.

Schally, A. V., and Bowers, C. Y. (1964). Corticotropin-releasing factor and other hypothalamic peptides. *Metabolism*, **13**, 1190-1205.

Schally, A. V., Bowers, C. Y., Redding, T. W., and Barrett, J. F. (1966). Isolation of thyrotropin-releasing factor (TRF) from porcine hypothalamus. *Biochem. biophys. Res. Commun.*, **25**, 165-169.

Schally, A. V., Redding, T. W., Bowers, C. Y., and Barrett, J. F. (1969a). Isolation and properties of porcine thyrotropin-releasing hormone. *J. biol. Chem.*, **244**, 4077-4088.

Schally, A. V., Sawano, S., Arimura, A., Barrett, J. F., Wakabayashi,

I., and Bowers, C. Y. (1969b). Isolation of growth hormone-releasing hormone (GRH) from porcine hypothalami. *Endocrinology*, **84**, 1493-1506.

Scharrer, E., and Scharrer, B. (1954). Hormones produced by neurosecretory cells. *Rec. Progr. Hormone Res.*, **10**, 183-240.

Talwalker, P. K., Ratner, A., and Meites, J. (1963). *In vitro* inhibition of pituitary prolactin synthesis and release by hypothalamic extract. *Amer. J. Physiol.*, **205**, 213-218.

Valverde, R., Chieffo, W., and Reichlin, S. (1972). Prolactin-releasing factor in porcine and rat hypothalamic tissue. *Endocrinology*, **91**, 982-993.

Veber, D. F., Bennett, C. D., Milkowski, J. D., Gal, G., Denkewalter, R. G., and Hirschmann, R. (1971). Synthesis of a proposed growth hormone releasing factor. *Biochem. biophys. Res. Commun.*, **45**, 235-239.

Voogt, J. L., and Meites, J. (1971). Effects of an implant of prolactin in median eminence of pseudopregnant rats on serum and pituitary LH FSH and prolactin. *Endocrinology*, **88**, 286-292.

Welsch, C. W., Squiers, M. D., Cassell, E., Chen, C. L., and Meites, J. (1971). Median eminence lesions and serum prolactin: influence of ovariectomy and ergocornine. *Amer. J. Physiol.*, **221**, 1714-1717.

Wilber, J. F., Nagel, T., and White, W. F. (1971). Hypothalamic growth hormone-releasing activity (GRA): characterisation by the *in vitro* rat pituitary and radioimmunoassay. *Endocrinology*, **89**, 1419-1424.

Wilber, J. F., and Porter, J. C. (1970). Thyrotropin and growth hormone releasing activity in hypophysial portal blood. *Endocrinology*, **87**, 807-811.

Wilber, J. F., and Seibel, M. J. (1973). Thyrotropin releasing hormone interactions with an anterior pituitary membrane receptor. In *Hypothalamic Hypophysiotropic Hormones*. (Excerpta Medica Int. Congr. Ser. No. 263), edited by C. Gual and E. Rosemberg, pp. 182-188. Excerpta Medica, Amsterdam.

Wuttke, W., Gelato, M., and Meites, J. (1972). Effects of Napentobarbital on hypothalamic PIF, LRF and FSH-RH and on serum prolactin, LH and FSH. In *Brain Endocrine Interaction. Median Eminence: Structure and Function*, edited by K. M. Knigge, D. E. Scott, and A. Weindle, pp. 267-279. Karger, Basle.

Yen, S. S. C., VandenBerg, G., Rebar, R., and Ehara, Y. (1972). Variation of pituitary responsiveness to synthetic LRF during different phases of the menstrual cycle. *J. clin. Endocr.*, **35**, 931-934.

Zarate, A., Canales, E. S., Schally, A. V., Ayala-Valdes, L., and Kastin, A. J. (1972). Successful induction of ovulation with synthetic luteinizing hormone-releasing hormone in anovulatory infertility. *Fertil. and Steril.*, **23**, 672-674.