Hypothalamic Regulatory Hormones

At least nine substances from the hypothalamus control the secretion of pituitary hormones.

Andrew V. Schally, Akira Arimura, Abba J. Kastin

Although the existence of hypothalamic substances regulating anterior pituitary function was postulated many years ago (*1*), it is only during the past few years that sufficient progress has been made to bring this concept into the realm of practical significance. The isolation, determination of structure, and synthesis of several hypothalamic hormones have been accomplished and these hormones are being evaluated now for diagnostic and therapeutic uses. Furthermore, additional hypothalamic hormones will probably be structurally identified and synthesized during the next few years. Thus, the ability to exert a type of control over the endocrine system not previously possible appears to be at hand.

Because the hypothalamus is the part of the brain nearest to the pituitary gland, it was reasonable to envisage neural components in the control of the secretion of the pituitary hormones. The hypothalamus is an area of the diencephalon, lying at the base of the brain, ventral to the thalamus and forming the floor and part of the lateral walls of the third ventricle. It is bounded anteriorly by the optic chiasma and posteriorly by the mammillary bodies. The median eminence of the tuber cinereum, a specialized expansion in the floor of the third ventricle, is connected to the pituitary by means of a stalk (Fig. 1). The anatomical basis for the control of the anterior pituitary gland by the hypothalamus was clearly established by the work of Harris and others (*1–3*). That this control is not mediated by nerve fibers was suggested by the absence of an appreciable nerve supply to the anterior pituitary gland (*3*). A portal system of blood vessels between the median eminence and pituitary appeared as the likely pathway for the hypothalamic regulation of pituitary function (*1, 3*). Changes in function of the endocrine glands (ovary, testis, adrenal cortex, and thyroid), acted upon by anterior pituitary hormones after severing the portal blood vessels between the hypothalamus and pituitary provided additional evidence that maintenance of the normal secretory activity of the pituitary gland may depend on vascularization by these portal blood vessels (*3*). A hypophysial portal circulation was found in man and other mammals as well as in lower vertebrates. The most probable explanation of the relationship between the portal blood supply and anterior pituitary function seemed to be that hypothalamic nerve fibers of different types liberate hormonal substances from their nerve endings into the capillaries in the median eminence, and then these substances are carried by the portal vessels to the pituitary gland where they stimulate or inhibit the release of various anterior pituitary hormones (*1, 3*). This neurohumoral concept (*1*) of the regulation of secretion of anterior pituitary hormones was indirectly supported by the neurosecretory theory (*4*). This theory (*4*) suggested that posterior pituitary hormones, oxytocin and vasopressin, are synthesized in the neurosecretory cells of the supra-optic and paraventricular nuclei of the hypothalamus and that they are transported down the axons of the hypothalamic-hypophysial tract to the nerve endings in the posterior lobe of the pituitary gland which serves as a storage and release center. Neurosecretory materials in the median eminence may be related to hypothalamic releasing hormones.

Although other anatomical, physiologic, and pharmacologic data (*3, 5*) accumulated supporting the neurohumoral concept of regulation of the anterior pituitary gland, direct evidence for the existence of specific hypothalamic neurohormones involved in the release of anterior pituitary hormones was lacking for several years. Demonstration of the existence of a corticotropin-releasing factor (*6*) opened the way for subsequent discoveries of other hypothalamic regulatory substances. The existence of at least nine hypothalamic regulators of the pituitary gland is now reasonably well established (Table 1). Since some of these substances, also called factors, satisfy classical criteria for designation as hormones (*7*), in this article we use in most instances the nomenclature proposed previously (*8*). The abbreviation —RH (Table 1) could represent releasing hormone or regulating hormone, since some hypothalamic hormones appear to affect the synthesis, as well as release of respective anterior pituitary hormones. For at least three pituitary hormones there is a dual system of hypothalamic control, one system being inhibitory and one being stimulatory. The need for hypothalamic inhibitors, as well as stimulators of growth hormone, prolactin, and melanocyte-stimulating hormone, can be explained by the absence of negative feedback products from their target tissues. In the case of corticotropin, thyrotropin, luteinizing hormone, and follicle-stimulating hormone, hormones (corticosteroids, thyroxine, and sex steroids) from the target glands inhibit secretion of these anterior pituitary hormones by negative feedback action exerted on the pituitary, hypothalamus, or both (*9, 10*). We describe here the most recent biochemical, physiological, and clinical findings relating to each of the known hypothalamic hormones; other details can be found elsewhere (*8–10*).

Control of ACTH Release

The central nervous system (CNS), and the hypothalamus in particular, mediates the classical response to "stress" (*10*). Thus external environmental factors, sensory stimuli, emotions, or any interference with the body's ability to maintain homeostasis

The authors are on the staff of Veterans Administration Hospital and Tulane University School of Medicine, New Orleans, Louisiana 70146.

Table 1. Hypothalamic hormones known to control the release of pituitary hormones.

Hypothalamic hormone (or factor)	Abbreviation
Corticotropin (ACTH)-releasing hormone	CRH or CRF
Thyrotropin (TSH)-releasing* hormone	TRH or TRF
Luteinizing hormone (LH)-releasing* hormone	LH-RH or LH-RF
Follicle-stimulating hormone (FSH)–releasing* hormone	FSH-RH or FSH-RF
Growth hormone (GH)-releasing* hormone	GH-RH or GH-RF
Growth hormone (GH) release-inhibiting hormone	GH-RIH or GIF
Prolactin release-inhibiting hormone	PRIH or PIF
Prolactin-releasing hormone	PRH or PRF
Melanocyte-stimulating hormone (MSH) release-inhibiting hormone	MRIH or MIF
Melanocyte-stimulating hormone (MSH)–releasing hormone	MRH or MRF

* Or regulating hormone.

can result in the liberation of corticotropin-releasing hormone (CRH) which stimuates the release of adrenocorticotropic hormone (ACTH) from the pituitary. In turn, ACTH augments the secretion by the adrenal cortex of steroids necessary for survival. Although CRH was the first hypothalamic hormone to be demonstrated by Saffran and co-workers (*6*) and Guillemin *et al.* (*11*), its instability and the difficulty with its assays (*8*) have delayed the isolation of adequate amounts for elucidation of its structure. Indeed, little or no progress has been made on its chemistry for the past 8 years, although the existence of CRH, different from vasopressin, is well accepted (*12*). In our early attempts to purify CRH we utilized posterior pituitary powders, since hypothalami were not readily available. A tentative, partial amino acid sequence (*13*) of a corticotropin-releasing factor (CRF) purified from powdered porcine posterior pituitary tissue was reported (*14*) as

Ac-Ser-Tyr-Cys-Phe-His-[Asp-NH_2, Glu-NH_2]-
-Cys-(Pro, Val)-Lys-Gly-NH_2

It is not known whether the physiologic CRH from the hypothalamus is related to the proposed neurohypophysial CRF, but since hypothalamic CRH is destroyed by some proteolytic enzymes (*8*), it is probably a polypeptide. Polypeptides synthesized by coupling the dipeptides Ser-His or His-Ser to the free amino-terminal group of lysine vasopressin have some CRF activity (*15*). The clinical usefulness of CRH in endocrinology may be limited to diagnostic tests of pituitary function.

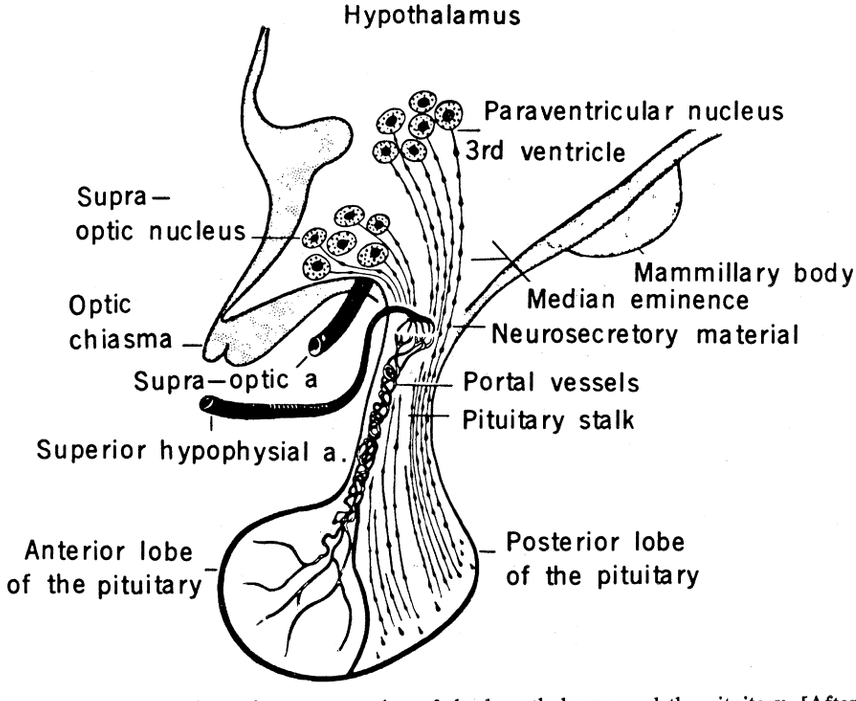


Fig. 1. Simplified schematic reconstruction of the hypothalamus and the pituitary. [After Hansel (*153*), courtesy *International Journal of Fertility*]

Control of Thyrotropin Release

The secretion of thyrotropin (TSH) by the pituitary is regulated by an interaction between hypothalamic thyrotropin-releasing hormone (TRH), which stimulates TSH release, and thyroid hormones which inhibit it (*10, 16, 17*). The existence of TRH was first demonstrated in 1961 (*18*) although earlier physiological studies (*17*) indicated the likelihood of its presence. Laborious attempts to purify TRH were made by investigators in two laboratories (*8, 19*) and in 1966 we reported that TRH isolated from porcine hypothalami contained three amino acids, histidine, proline, and glutamic acid, in equimolar ratios (*20*). This report of the chemical nature and composition of TRH, the first of the hypothalamic hormones to be isolated, was followed by investigations in which the amino acid sequence and the structure of porcine TRH was determined, and in which the synthesis of TRH was achieved (*21*). The molecular structure of porcine TRH is shown in Fig. 2. In similar experiments conducted by Burgus and co-workers (*22*), it was established that the structure of ovine TRH was also (pyro)Glu-His-Pro-amide. It is probable that bovine and human TRH have the same structure (*16*). Many analogs of TRH have been synthesized in an attempt to study the relationship between structure and activity (*23*). Most analogs have little TRH activity but one, with a methyl group in the 3-N position of the imidazole ring of histidine, has much greater activity than natural TRH itself (*23*).

The results of various physiological studies conducted since 1963 with natural preparations of TRH (*8*), have been confirmed and extended by using synthetic TRH, which has the same activity as natural TRH (*16, 22, 24*). The concentration of TSH in the plasma increases when TRH is administered intravenously, subcutaneously, intraperitoneally, or orally (*8, 16, 24*) or when TRH is infused into the hypophysial portal vessels (*25*). Even hypophysectomized rats with pituitary transplants show increases in the concentration of TSH in plasma after administration of TRH (*24*).

In vitro, TRH in picogram doses releases TSH from the pituitaries of rats, sheep, and goats (*16, 26*). A dose-response relationship, both in vivo and in vitro, is readily demonstrable—that is, increasing doses of TRH cause a progressively greater release of TSH

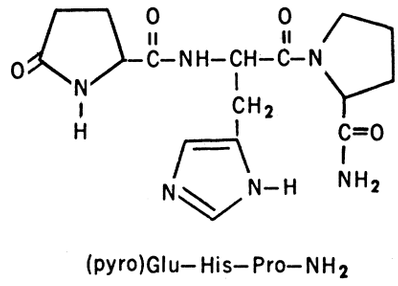
(*16, 24, 26*). In pituitary tissue cultures, TRH stimulates the synthesis as well as the release of TSH (*27*).

The confirmation that the thyroid hormones thyroxine and triiodothyronine can block the stimulatory effect of TRH by an action exerted directly on the pituitary gland (*16, 24, 26*) were especially important and supported an earlier hypothesis of Von Euler and Holmgren (*28*) that the release of TSH is regulated at the pituitary level by a negative feedback effect of thyroid hormones. Among the physiological stimuli that may release TRH is exposure to mild cold (*16, 29*). A rapid discharge of TSH occurs after electrical stimulation of the hypothalamus and is most probably mediated by the release of preformed TRH (*30*) because TRH activity in hypophysial portal blood is increased under similar conditions (*31*). Hypothalamic fragments incubated in buffer solution have been shown to synthesize TRH from glutamic acid, histidine, and proline (*32*), but the exact cellular site of formation of TRH (and other hypothalamic hormones) remains unknown.

After administration of tritiated or ^{14}C-labeled TRH to rats and mice, the radioactivity accumulates in the pituitary (*33*). Some radioactivity also becomes concentrated in kidney and liver, probably because of their role in the inactivation and excretion of TRH (*34*). Rapid inactivation of TRH by rat and human plasma (*35*) is caused mainly by the enzymatic cleavage of the amide group at the prolyl end. The half-life of TRH in the blood of the rat is about 4 minutes (*33*).

Other studies in which bovine anterior pituitary glands were used showed that tritiated TRH is specifically bound by pituitary membrane receptors (*35*). Adenylate cyclase, the enzyme that catalyzes the formation of adenosine 3′,5′-monophosphate (cyclic AMP), is associated with these membranes. Adenylate cyclase activity is stimulated by the addition of TRH, and derivatives of cyclic AMP can also stimulate TSH release in vitro. Thus, cyclic AMP may be the mediator of the action of TRH on the pituitary cell (*35*).

These studies of the metabolism, distribution, and mechanism of action of TRH have been of value because of the increasing clinical use of the hormone. The initial demonstration of the effectiveness of TRH in releasing TSH in human beings was made with a natural preparation of porcine TRH (*36*). The elucidation of the structure



(pyro)Glu–His–Pro–NH_2

Fig. 2. Molecular structure of thyrotropin-releasing hormone (TRH).

(*21, 22*) and large-scale synthesis of TRH made possible extensive clinical trials with this hormone in which it was demonstrated that TRH is a potent, safe, and nontoxic stimulant of TSH release in men, women, and children (*37*). It is also a useful diagnostic compound for testing pituitary TSH reserve and for distinguishing pituitary from hypothalamic hypothyroidism. The various concentrations of TSH in the plasma that follow the administration of TRH may also elucidate some aspects of thyroid function. Further refinements of diagnosis may come from the recent development of a radioimmunoassay for TRH (*38*). Synthetic TRH will also release prolactin in animals including human beings (*39*), but it remains to be established whether this effect is physiological or pharmacological.

Luteinizing Hormone and Follicle-Stimulating Hormone

That reproduction is influenced by seasonal, environmental, and emotional factors has been known for centuries. Studies conducted during the past 40 years have done much to clarify the diversified role of the CNS in the regulation of reproductive cycles (*3, 5, 9, 10, 40*). Investigators utilizing electrical stimulation of the hypothalamus, ablation of discrete areas of the CNS, transplantation of the pituitary, and implantation of sex steroids obtained data suggesting that the hypothalamus is involved in the control of secretion of the gonadotropins LH and FSH (*3, 9, 40*). The involvement of the CNS in this control was also demonstrated by pharmacological activation and inhibition of release of pituitary gonadotropins by centrally acting drugs and by recording changes in the electrical activity of the brain associated with pituitary stimulation (*5, 9, 40*). Thus it was shown that the CNS, including the hypothalamus, controls the secretion of LH and FSH from the pituitary and through LH and FSH, regulates gonadal function. In turn, sex hormones (estrogen, progesterone, and testosterone), secreted by the gonads, exert a feedback action on the hypothalamus and the pituitary. This action is predominantly inhibitory, but estrogen can exert a stimulatory action as well (*9*). The CNS also correlates reproductive processes with changes in the environment, especially in animals which breed seasonally, and coordinates reproductive behavior with the activity of the pituitary and the gonads (*3, 9, 40*). Both in reflex ovulators such as rabbits which ovulate after copulation, and in spontaneous (cyclic) ovulators such as rats, an accurately timed signal from the brain causes a massive discharge of LH which results in ovulation (*40*).

Overwhelming evidence is now available to support the concept that this neural impulse for the discharge of LH is mediated by a hormonal substance which originates in the hypothalamus and reaches the pituitary gland through the hypophysial portal system (*3, 8*). The presence of an LH-releasing hormone (LH-RH) in hypothalamic extracts of rats was first demonstrated in the early 1960's (*41*); LH-RH was subsequently found in similar extracts from domestic animals and human beings (*41*). Hypothalamic extracts were also found to contain an FSH-releasing hormone (FSH-RH) (*42*). The concentrations of LH-RH and FSH-RH in hypothalamic tissue and blood were then investigated. It was established that in the hypothalamic tissue, the concentration of LH-RH changes during the estrous cycle of rats (*43*). The decrease in the concentration of LH-RH that occurs before estrus suggests that the release of LH-RH is involved in the discharge of an amount of LH sufficient to cause ovulation (*43*). A sharp drop in the LH-RH and FSH-RH content of rat hypothalami at puberty indicates that LH-RH and FSH-RH play a key role in the onset of puberty (*44*). Treatment with contraceptive steroids lowers the hypothalamic content of LH-RH and FSH-RH, possibly by inhibiting their synthesis (*45*). Hypophysial portal blood collected from the cut ends of the pituitary stalks of rats at proestrus has a higher LH-RH content than peripheral blood from the same animals. Electrical stimulation of the hypothalamus increases LH-RH activity in hypophysial portal blood of

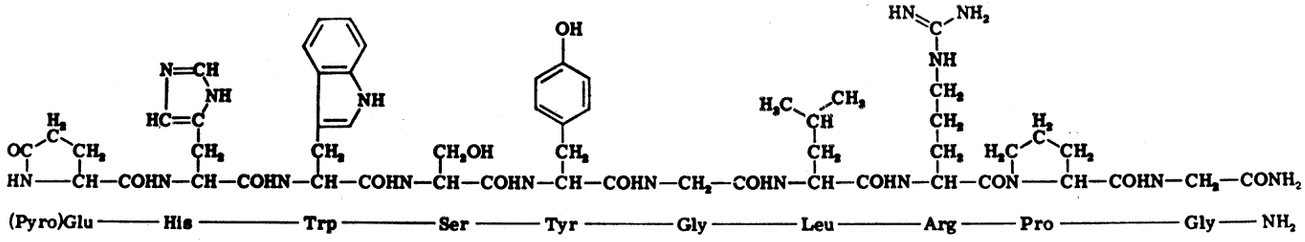


Fig. 3. Molecular structure of LH- and FSH-releasing hormone (LH-RH/FSH-RH).

rats at proestrus (*46*). In women, LH-RH activity has been detected in peripheral blood at the time of the midcycle ovulatory surge of LH release (*47*). Furthermore, FSH-RH activity has been found in blood of rats (*48*).

Recently it has been determined that dopamine, which is one of the substances present in hypothalamic neurons, may participate in the release of LH-RH and FSH-RH. Dopamine does stimulate release of LH and FSH from the pituitary but it acts indirectly through the hypothalamus (*49*). Increased concentrations of LH-RH and FSH-RH in blood from hypophysial stalk of rats after intraventricular administration of dopamine suggest that this amine stimulates the release of LH-RH and FSH-RH (*50*).

Initially, it was thought that LH-RH and FSH-RH activities were the properties of two different substances (*8, 41, 42*), but later it became necessary to question this belief (*51*). In several laboratories vigorous attempts were made to isolate both of these substances. As in the case of TRH, extracts of hundreds of thousands of hypothalami had to be laboriously processed, concentrated, and purified in order to obtain enough material for chemical characterization. The purified materials were then evaluated for their physiological and clinical effects (*8, 52*). Our own efforts were intensified when we demonstrated that highly purified LH-RH of porcine origin unequivocally stimulated both LH and FSH release in men and women under a variety of conditions and, used in combination with human menopausal gonadotropin (Pergonal), induced ovulation, proved by pregnancy in a woman with secondary amenorrhea (*52*). This work led to the isolation from porcine hypothalami of a decapeptide with both LH-RH and FSH-RH activity and the determination of its amino acid composition and sequence (*53*). The structure of LH-RH is shown in Fig. 3. This decapeptide was then synthesized (*53, 54*). After the announcement of its structure, LH-RH was synthesized by workers in many other laboratories because of its anticipated medical importance (*55, 56*). Assays in our laboratory showed that not only the decapeptide synthesized by us (*54*) but also the LH-RH preparations made by others (*56*) according to the structure we proposed (*53*) had the same LH-RH and FSH-RH activity as pure, natural LH-RH (*54, 57, 58*).

Because both natural LH-RH and the synthetic decapeptide corresponding to its structure possessed major FSH-RH as well as LH-RH activity in rats and human beings, we proposed that one hypothalamic hormone, designated LH-RH/FSH-RH, could be responsible for stimulating the release of both FSH and LH from the anterior pituitary gland (*53, 58*). This view was supported by much biochemical and physiological evidence. Chemical and enzymatic inactivation of LH-RH was always accompanied by a loss of FSH-RH activity (*53, 59*); the latter activity could not be separated from LH-RH activity even by use of the most sophisticated separation techniques. That this FSH-RH activity is intrinsic to the LH-RH molecule, was proved by the synthesis of LH-RH (*54, 58*). It is also indisputable that the natural and synthetic decapeptide in nanogram doses will stimulate the release of rat FSH and LH in vitro and in vivo (*53, 58*). The time courses of the release of LH and FSH in vitro, induced by the synthetic or natural hormone, are identical (*58*). Microgram doses of LH-RH cause a discharge of FSH, in addition to LH, in sheep (*60*), monkeys (*51, 61*), human beings (*52, 62, 63*), and other species (*64*).

Against the concept of one hypothalamic hormone controlling the discharge of both LH and FSH is the apparent occasional divergence of LH and FSH release in the human menstrual cycle, the estrous cycle in other animals, and in certain pathological conditions. Although this can be explained in part by interactions with sex steroids (*9, 58*), different biological half-lives of LH and FSH (*65*), and varying durations of pituitary stimulation by LH-RH (*66*), the possibility that another hormone which releases only or predominantly FSH is present in hypothalamic tissue cannot be excluded at present. Nevertheless, the ovulation which can be induced in golden hamsters (*64*), rabbits (*67, 68*), sheep (*60*), and amenorrheic women (*63*) after treatment with natural and synthetic LH-RH, and the histologic observations of ovaries in female rats with pituitary transplants (*69*), demonstrate that this decapeptide may release enough FSH to cause follicular ovarian maturation. In any case, the detection by radioimmunoassay of a peak of (pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 before and during the preovulatory surge of LH and FSH in sheep (*70*) suggests that this decapeptide is most probably the hypothalamic mediator responsible for stimulating the release of the ovulatory quota of LH and of FSH.

The effects of steroids on responses to LH-RH/FSH-RH are complex. Interactions of LH-RH/FSH-RH with sex steroids may be major factors in the control of LH and FSH release (*9, 58*). These interactions were studied in several species with highly purified natural preparations of LH-RH/FSH-RH as well as with the synthetic decapeptide when it became available. Since massive doses of 12 commonly used oral contraceptive preparations containing combinations of estrogen and progestin did not block the stimulatory effects of purified LH-RH on the release of LH in ovariectomized rats, we suggested that the negative feedback of contraceptive steroids was exerted mainly on the hypothalamus or another CNS center rather than on the pituitary (*71*). However, recent studies indicate that sex steroids have some direct effects on the pituitary gland. Thus, large doses of progesterone suppress the response to threshold doses of LH-RH in cyclic rats (*72*), and in rabbits (*67*). However, larger doses of

LH-RH overcome the progesterone blockade (*67, 73*). The treatment of male rats with testosterone propionate decreases the release of LH and FSH that occurs after administration of LH-RH (*74*). In contrast to the inhibitory effects of progesterone and testosterone, administration of small doses of estrogen augmented the response to LH-RH in female but not in male rats (*73, 75*). In sheep, treatment with estrogen similarly enhanced the response to LH-RH (*76*). However, the combination of estrogen and progesterone suppressed the release of LH in female rats and in anestrous ewes (*73*). These results may be correlated with events in the human menstrual cycle and the estrous cycle of animals. A peak in the concentration of estrogen in the plasma, which precedes the ovulatory surge of LH in rats, monkeys, and women, may augment responsiveness of the pituitary to LH-RH (*58, 73*). Conversely, the large amounts of estrogen and progesterone which are secreted after ovulation may lower pituitary responsiveness to LH-RH (*58, 73*). The release of FSH appears to be more susceptible to the inhibitory effects of estrogen. Studies in vitro have also shown that the regulatory effect of sex steroids is exerted partly at the pituitary level (*58*). Thus, the endogenous concentrations of sex hormones could contribute to different sensitivities of the pituitary gland to LH-RH. Changes in pituitary responsiveness to LH-RH during the reproductive cycle are in agreement with this view. In cycling ewes, the same dose of LH-RH induces a greater change in the concentration of LH in the serum when administered on the day of onset of estrus than when it is administered at any other stage of the estrous cycle (*77*). When a similar experiment was performed in golden hamsters and rats, the greatest response to LH-RH was observed during proestrus (*78*). Exogenous LH-RH also induced the release of highest concentrations of LH at midcycle in rhesus monkeys and normal women (*61, 79*). Thus, the ovulatory surge of LH release may be caused in part by the greater sensitivity of the pituitary gland to LH-RH, in addition to an increased release of endogenous LH-RH at that time.

Under experimental conditions, the concentrations of LH and FSH in the plasma can be changed by varying the duration of stimulation of the pituitary gland with LH-RH. When ovariectomized rats previously treated with estrogen and progesterone are given picogram or nanogram doses of LH-RH by rapid injection, the concentration of LH but not FSH in the plasma increases (*80*). However, intravenous infusion of LH-RH for 3 to 4 hours induces a significant increase in the concentration of FSH in the plasma as well (*69*). Normal male rats show an even greater (11-fold) increase in plasma FSH after the intravenous infusion of 200 nanograms of LH-RH for 3 to 4 hours (*66, 69*). Thus, prolonged stimulation of pituitary FSH gonadotroph cells by LH-RH seems to be necessary to effect a marked release of FSH (*66*). It is interesting that oral administration of LH-RH can increase the concentrations of LH and FSH in the plasma of ovariectomized rats treated with estrogen and progesterone, but the doses required are many thousands of times larger than those effective intravenously (*81*).

Natural and synthetic LH-RH/FSH-RH can stimulate the synthesis as well as the release of LH and FSH. This was proved by using modified organ cultures of rat anterior pituitary glands (*82*). The addition of nanogram amounts of LH-RH to the incubation medium daily for 5 days augmented the total content of LH and FSH in the stimulated tissue and medium, in comparison with controls, and increased the incorporation of ^{3}H-glucosamine into LH and FSH.

The effect of prolonged treatment with synthetic LH-RH was studied in hypophysectomized male and female rats bearing pituitary grafts under the kidney capsule (*58, 69, 83*). After 2 months, the control rats showed a severe regression of spermatogenesis, but rats injected with LH-RH exhibited a striking stimulation of the spermatogenesis. In hypophysectomized female rats with pituitary grafts, long-term treatment with synthetic LH-RH stimulated follicular development.

It was demonstrated by electron microscopy that LH-RH induces the extrusion of secretory granules from LH gonadotrophs in rats with persistent estrus (*84*). The mechanism of action of LH-RH/FSH-RH in inducing LH and FSH release is not known. However, cyclic AMP and its derivatives can stimulate LH and FSH release in vitro (*85*). Synthetic LH-RH incubated with rat anterior pituitary tissue caused increases in intracellular concentration of cyclic AMP by stimulating adenyl cyclase activity in LH- and FSH-secreting cells. This indicates that cyclic AMP may be the mediator of the action of LH-RH on the anterior pituitary.

Natural and synthetic preparations of LH-RH/FSH-RH have also been studied in other species of mammals and in chickens (*86*). The effect of LH-RH in various animals is summarized in Table 2. In golden hamsters treated with phenobarbital, the administration of LH-RH induces ovulation and increases the concentration of LH in the plasma (*64, 78*); LH-RH

Table 2. The effects of natural (N) and synthetic (S) LH-RH/FSH-RH in animals and human beings.

Species	Sex ♂	Sex ♀	Effects	References
Rats	♂	♀	Release of LH and FSH in vivo and in vitro (N, S)	(*8, 41, 42, 53, 58, 71–75, 80, 81*)
	♂		Stimulation of synthesis of LH and FSH in vitro (N, S)	(*82*)
	♂		Stimulation of spermatogenesis (S)	(*83*)
		♀	Stimulation of follicular maturation (S)	(*69, 58*)
		♀	Ovulation (N, S)	(*152, 78, 69*)
Golden hamsters		♀	Release of LH (S)	
		♀	Ovulation (S)	(*64, 78*)
Rabbits		♀	Release of LH (N, S)	
		♀	Ovulation (N, S)	(*67, 68*)
Sheep	♂	♀	Release of LH and FSH (N, S)	(*60, 76, 77, 87*)
		♀	Ovulation (S)	
Pigs		♀	Release of LH (S)	(*88*)
Cattle	♂		Release of LH (S)	(*89*)
Monkeys		♀	Release of LH and FSH (N, S)	(*61*)
Chickens		♀	Premature ovulation (S)	(*86*)
Humans	♂	♀	Release of LH and FSH (N, S)	(*52, 62, 58, 79*)
	♂		Stimulation of spermatogenesis* (S)	(*91*)
		♀	Ovulation (N, S)	(*52, 63*)

* Preliminary results.

administered to estrous rabbits produces similar effects (*67, 68*). When LH-RH is given intravenously, intramuscularly, or subcutaneously to sheep, it increases the concentration of LH and FSH in the plasma and induces ovulation in the ewes (*60, 76, 77, 87*). Hereford bulls and prepubertal pigs injected intramuscularly with synthetic LH-RH similarly show increased LH concentrations in the plasma (*88, 89*). Premature ovulation is also induced in domestic fowl given LH-RH (*86*). The effect of LH-RH in domestic animals and chickens indicates a possible application of LH-RH/FSH-RH in animal husbandry. In monkeys, LH-RH causes a small but significant increase in the concentration of LH in the plasma (*61*).

It is well documented that LH-RH/FSH-RH of natural or synthetic origin releases LH and FSH in human beings (*52, 58, 62, 63, 79*). Maximum increases in the concentration of LH and FSH in the plasma were observed 15 to 60 minutes after intravenous or subcutaneous injection of LH-RH. It has been shown that LH-RH does not stimulate the release of growth hormone, thyrotropin, and ACTH, nor does it inhibit the release of prolactin in rats or humans (*90*). When given by intravenous infusion or intramuscularly, LH-RH induced ovulation, confirmed by pregnancy, in at least six women with hypothalamic amenorrhea (*63*). This indicates that LH-RH may be useful in the treatment of sterility. Preliminary results also suggest that prolonged therapy with LH-RH may be helpful for treatment of oligospermia and azoospermia (*91*). Because LH-RH/FSH-RH is active in a number of laboratory and domestic animals, primates including man, and birds, species specificity probably does not exist for this hormone. The structure of ovine LH-RH was found to be identical to that of the previously announced porcine hormone (*92*).

Investigations in which LH-RH/FSH-RH, its structural analogs, and its other derivatives are used may lead to the development of new methods of birth control. The two principal approaches for the control of fertility may be based on the development of a specific antiserum capable of neutralizing endogenous LH-RH and on the synthesis of competitive analogs of LH-RH (*58*).

Antiserums to LH-RH have been produced recently in rabbits and in guinea pigs (*69, 70*). Antibodies to the LH-RH were assessed by the binding affinity with ^{125}I-labeled LH-RH and by complement fixation tests. Male rabbits, with serums containing antibodies to LH-RH, developed considerable testicular atrophy. The weight of their testes was only 0.3 gram, whereas the testes of control rabbits weighed 5 grams. Histological examination of the testes of the immunized rabbits indicated complete atrophy of the seminiferous tubules associated with aspermatogenesis. Pituitary content of LH was also reduced in these rabbits. This work indicates that antibodies to LH-RH can be produced in animals, but the clinical safety of purified animal antiserums to LH-RH or of complexes containing conjugated LH-RH is not known. These antiserums have been used in radioimmunoassays of LH-RH during studies of the dynamics of LH-RH secretion in vivo (*69*). By means of this system the half-life of exogenously administered LH-RH in the rat was measured and was found to be 6 to 7 minutes (*69*).

The synthesis of a large number of structural analogs of LH-RH will help to establish the structure-activity relationship for this hormone (*57*). This information would guide investigators in their attempts to create practical synthetic inhibitors of LH-RH. Such synthetic polypeptides should be devoid of LH-RH activity, but by competing with endogenous LH-RH for binding to the pituitary receptors, could lead to a decrease in the secretion of LH or FSH, or both. The synthesis of various analogs of LH-RH has been reported by several laboratories (*57, 93*). The results indicate that amino-terminal tripeptide and tetrapeptide fragments of LH-RH as well as the carboxyl-terminal nonapeptide and octapeptide of LH-RH have very little or no LH-RH activity (*57*). In contrast to early reports (*94*) the tetrapeptide (pyro)Glu-Tyr-Arg-Trp-NH_2, which is not a part of the LH-RH sequence, has only 1 part in 7800 of the activity of the LH-RH decapeptide and some FSH-RH activity. This indicates that, unlike gastrin tetrapeptide amide (*95*), very active small fragments cannot be obtained from LH-RH. However, certain amino acids can be replaced in the LH-RH molecule without major loss of activity. For instance, tyrosine can be replaced by phenylalanine (*93*). On the other hand, replacement of the hydroxyl group of serine by hydrogen or of arginine by lysine or ornithine result in a 20- to 30-fold decrease in LH-RH activity (*93*). Many modifications abolish LH-RH activity. Which amino acids in the LH-RH molecule are involved in binding to the receptors and which exert a functional effect still remains to be established. One of the analogs, des-His-2-LH-RH, was reported to antagonize competitively LH-RH in a system in vitro, when present in dosages 10,000 times greater than LH-RH (*96*). However, various observations suggest that des-His-2-LH-RH is unlikely to be a practical contraceptive (*69*) and the search for more effective inhibitors of LH-RH must continue. Irrespective of any possible success in the development of new methods of birth control based on various derivatives of LH-RH/FSH-RH, the available results indicate that LH-RH/FSH-RH should find diagnostic and therapeutic application in clinical medicine and that it may be also useful for stimulation of fertility in domestic animals.

Control of Release of Growth Hormone

Much physiological evidence indicates that the hypothalamus regulates the release of growth hormone (GH) (*8, 10*). The concept of neural control is supported by the correlation of cyclic GH secretion with electroencephalographic patterns (*97*), by the stimulation or the blockade of GH release by neuropharmacologic means (*98*), and by the demonstration that electrical stimulation of the ventromedial nucleus and the median eminence induces an increase in the concentration of GH in the plasma of rats (*99*). Thus, impulses reaching the hypothalamus may provoke a discharge of GH-RH which in turn stimulates GH secretion from the pituitary. The pituitary stalk is a pathway for the transmission of stimuli since its transection abolished the stimulatory effect of hypoglycemia on the release of GH in man (*100*). Direct proof for the existence of a GH-RH is provided by the stimulation of release of GH in vivo and in vitro by various hypothalamic preparations. Rat hypothalamic extracts or materials purified from pig or sheep hypothalami (*101*) or plasma from the cut end of the pituitary stalk (*102*) added to pituitaries incubated in vitro released GH into the incubation medium. Crude hypothalamic extracts injected into monkeys (*103*), sheep (*104*), and rats primed with estrogen and progesterone (*105*) or reserpine and urethane, induced a significant increase in the concentration of GH in

the plasma, as measured by radioimmunoassays. Hypothalamic extracts infused directly into the pituitary (*106*) or into the hypophysial portal vessel of the rat (*107*) also stimulated the release of immunoreactive GH.

Progress in the isolation of GH-RH has been hampered by the inconsistency of the methods used for its detection. For following GH-RH activity in the course of its purification, we have conducted tests in vitro and in vivo. The test in vitro is based on stimulation of release of bioassayable GH (*108*) from rat pituitaries (*101*). The materials to be tested are added to the incubation medium. For the test in vivo we use depletion of bioassayable GH in the rat pituitary after intracarotid injection, as an index for GH-RH activity (*109*). A decapeptide active in these test systems was isolated in pure form from pig hypothalami (*110*). It was structurally characterized as Val-His-Leu-Ser-Ala-Glu-Glu-Lys-Glu-Ala (*111*) and was synthesized (*112*). This amino acid sequence is similar to that of the amino-terminal sequence of the β-chain of porcine hemoglobin (*112*). The pure porcine material and the synthetic decapeptide corresponding structurally to the proposed natural GH-RH were both active in these assay systems in vitro and in vivo (*113*). We have also observed the stimulatory effect of highly purified hypothalamic GH-RH preparations on the synthesis as well as the release of GH during short-term incubation of rat pituitary glands (*8, 101*). Mittler *et al.* (*114*) investigated the effect of highly purified hypothalamic GH-RH preparations on GH synthesis in 5-day tissue cultures of rat pituitary glands. Addition of these GH-RH preparations caused an increase in bioassayable GH in both the medium and the tissue and increased the incorporation of radioactive amino acids into GH, indicating stimulation of GH synthesis. Similarly, electron microscopic studies of rat pituitary somatotrophs (*115*) showed an increase in the number of secretory granules being extruded after administration of the GH-RH preparations.

However, discrepancies between the results obtained by bioassay (*108*) and radioimmunoassay (*116*) for GH soon became evident. Administration of hypothalamic extracts to the rat caused a significant reduction in the concentration of bioassayable GH but not of immunoreactive GH in the pituitary gland (*117*). Intracarotid injection of partially purified GH-RH preparations or of pure natural decapeptide, proposed as GH-RH, increased the GH-like activity in the blood of normal rats (*118*) as measured by bioassay (*108*), but not by radioimmunoassay (*116*) in spite of the greater sensitivity of the latter. Moreover, pure preparations of the natural porcine decapeptide proposed as a GH-RH (*110, 111*), or of the corresponding synthetic decapeptide (*112*), or both, did not stimulate the release of immunoreactive GH when administered systematically to sheep, monkeys, pigs, and rats previously treated with estrogen and progesterone, or with reserpine and urethane, or when administered by intrapituitary or portal vessel infusion to untreated rats (*111, 113, 119*). Crude hypothalamic extracts were active in all these tests (*103–107*). Similarly, the synthetic decapeptide did not release GH in man (*120*), or stimulate the growth rate of female rats already at a growth plateau, newborn rats treated with glucocorticoids, or hypophysectomized rats with pituitary grafts (*119*). Consequently, athough the reasons for the discrepancy between the bioassay and radioimmunoassay for rat GH remain unsettled, it is unlikely that the decapeptide Val-His-Leu-Ser-Ala-Glu-Glu-Lys-Glu-Ala is the physiological GH-RH.

Purification of a GH-RH which releases immunoreactive GH was reported recently (*69, 121*). Wilber *et al.* (*121*) fractionated crude rat and pig hypothalamic extracts on Sephadex G-25 columns and located a GH-RH capable of stimulating the release of immunoreactive GH from rat pituitaries in vitro. The fractions containing this GH-RH were retarded on Sephadex in comparison with the other GH-RH decapeptide. Using the same assay conditions (*69*), we have also found a GH-RH activity, which augmented the release of GH as measured by radioimmunoassay, in similar fractions of porcine hypothalamic extracts. Although many criteria govern separation on Sephadex, the retardation of this GH-RH indicates that it may be smaller than a decapeptide. This work confirms the existence of a GH-RH capable of stimulating the release of immunoreactive GH.

Tests of other partially purified and highly purified fractions of porcine hypothalami revealed the presence of materials capable, in nanogram doses, of inhibiting the release of immunoreactive GH (*69*). The existence of a GH release-inhibiting hormone (GH-RIH or GIF, Table 1) was suggested by Krulich *et al.* (*122*) but until now, its existence remained unconfirmed. It is conceivable that GIF could be used in the control of some types of diabetes in man. Further work is needed for the isolation, determination of structure, and synthesis of GIF as well as of the physiological GH-RH. This GH-RH might provide a material of possible therapeutic use in inducing normal growth in children and perhaps acting as an anabolic agent free of androgenic effects.

Control of Prolactin Release

Much evidence indicates that the hypothalamus can both stimulate and inhibit prolactin secretion (*123, 124*). The inhibitory influence may predominate in man, rat, rabbit, and other mammals, and possibly in reptiles and amphibians. The galactorrhea seen in human patients after section of the pituitary stalk (*125*) or administration of certain tranquilizers (*126*) may result from removal of the inhibitory influence of the hypothalamus on prolactin secretion. Similary, in rats, transplantation of the pituitary to extracranial sites results in increased prolactin secretion (*127*). However, in birds the stimulatory influence appears to be of primary importance (*123, 124*).

That the inhibition of prolactin secretion in rats is mediated by a hypothalamic neurohumoral agent was first shown by studies conducted in vitro (*128*). Subsequent studies in vitro and in vivo revealed the presence of a prolactin release-inhibiting hormone (PRIH or PIF) in hypothalamic extracts of sheep, cattle, and pigs also (*129*). The concentration of PRIH in the hypothalamus of rats is reduced by suckling and is increased by treatment with tranquilizers; the PRIH concentration also changes during the estrous cycle (*130*). Nevertheless, the lack of a simple assay for PRIH (*124*) and prolactin delayed the isolation of PRIH and hampered many studies. Recent development of radioimmunoassays for prolactin (*131*) made possible the observation that hypothalamic extracts depress the concentration of prolactin in the plasma of rats by inhibiting the release of prolactin (*132, 133*). Infusion of dopamine into the third ventricle increased PRIH activity in hypophysial portal blood of rats and decreased the release of prolactin (*50*). Dopamine also decreased the concentration of prolactin in man (*134*). Conversely,

transplantation of the pituitaries, placement of lesions in the hypothalamus, and administration of CNS depressants increased immunoreactive prolactin in the serum of rats (*132, 133*) in agreement with earlier conclusions based on the response of the corpus luteum and mammary glands (*127*).

Administration of hypothalamic extracts did not decrease the concentration of prolactin in rats with surgically ablated hypothalami or with pharmacological blockade (*133*). This indicates that contamination of these extracts with prolactin-releasing hormone (PRH) may have obscured their PRIH effect. It is also possible that the decrease in prolactin in the plasma of intact rats after administration of hypothalamic extracts may have been caused by liberation of endogenous PRIH. Although PRH was thought, at first, to be present only in avian hypothalami (*124, 135*), recent studies indicate that hypothalamic extracts of rats (*124*) and pigs (*69*) contain this activity in addition to PRIH. A part of this prolactin-releasing activity can clearly be attributed to TRH, which is present in hypothalamic extracts. Synthetic TRH has been shown to stimulate release of prolactin as well as TSH in rats, human beings, and sheep (*39*). Nevertheless, TRH may not be the physiological PRH. Recent studies indicate that purified pig hypothalamic fractions, from which TRH was removed, still stimulate prolactin secretion in vitro (*69*).

The chemical nature of PRIH and of PRH remains unknown. Synthetic LH-RH does not have PRIH activity (*90*). From their behavior during gel filtration on Sephadex G-25 (*69*) and from other characteristics (*123*), it can be surmised that both PRIH and PRH have small molecular weights. Catecholamines can inhibit prolactin secretion in vitro (*136*), but the evidence suggests that PRIH is probably a small polypeptide. The usefulness of TRH in increasing milk production in cows is being evaluated. It is also possible that PRIH will be of clinical significance in inhibiting undesired lactation.

Melanocyte-Stimulating Hormone

Melanocyte-stimulating hormone (MSH) darkens the skin of amphibia, but its role in mammals is not clearly established. Indirect evidence accumulated for a number of years that the hypothalamus exerts an inhibitory influence upon the release of MSH in lower vertebrates (*137*). Etkin showed that destruction of the hypothalamus of frogs removed an inhibitory influence upon the release of MSH and resulted in darkening of their skin (*138*). Later Kastin and Ross (*139*) proved the validity of this hypothesis in amphibians by showing that this procedure increased the concentration of circulating MSH in frogs; these workers used the same procedure in rats (*140*). Evidence was then provided not only that the hypothalamus inhibits the release of MSH in mammals, but also that the effect is exerted by a hypothalamic hormone (*141*). This substance, designated MSH release-inhibiting factor (MIF), increased pituitary content of MSH and decreased the amount of MSH released from the pituitary into rat blood or tissue culture medium and lightened the skin of frogs previously darkened by destruction of the hypothalamus (*142*). It seems likely, moreover, that many of the other influences which modify the release of MSH do so through the hypothalamus. These include the effects of the pineal gland, light and dark, stress, morphine, tranquilizers, and a short feedback of MSH to the hypothalamus (*143*).

After MIF was purified from bovine hypothalami, two peptides were isolated and identified as Pro-Leu-Gly-NH_2 and Pro-His-Phe-Arg-Gly-NH_2 (*144*). The first of these, which happens to be the carboxyl terminal tripeptide side chain of oxytocin, had greater activity as measured by skin lightening after direct application to the pituitary of a frog which had been previously darkened by destruction of the hypothalamus (*144, 145*).

It was originally observed by Celis *et al.* (*146*) that Pro-Leu-Gly-NH_2 can be formed by incubating oxytocin with an enzyme found in hypothalamic tissue. Celis *et al.* (*146*) state that this compound inhibits MSH release in the rat also. However, Bower and coworkers (*147*) propose that tocinoic acid, Cys-Tyr-Ile-Gln-Asn-Cys-OH, the cyclic pentapeptide ring of oxytocin, or its amide, tocinamide, is more likely to be MIF. Since tocinoic acid is ineffective in lightening the darkened skin of *Rana pipiens*, the possibility of species specificity for MIF exists (*147, 148*).

There is also evidence for an MSH-releasing hormone (MRH) (*142, 149*). Celis *et al.* have suggested that the opened NH_2-terminal ring portion of oxytocin, H-Cys-Tyr-Ile-Gln-Asn-Cys-OH, constitutes MRH (*149*). If the ring is closed, however, one has tocinoic acid mentioned above as a possible MIF. Neither MRH nor tocinoic acid has yet been identified in hypothalamic tissue.

With the increasing evidence for extrapigmentary effects of MSH, particularly upon the CNS of rat and man (*150*), more attention must be given to the hypothalamic hormones affecting its release. Pro-Leu-Gly-NH_2 has been shown to be effective in several animal models of Parkinsonism and depression, but its actions do not require the presence of the pituitary (*151*). Clinical trials with this MIF are in progress.

Conclusions

The molecular structures of several polypeptides isolated from hypothalamic tissue have been established and the synthesis of these compounds has been achieved. These polypeptides selectively stimulate or inhibit the release of anterior pituitary hormones and melanocyte-stimulating hormone. Various studies indicate their important physiological role and support the concept that some of these polypeptides are hormones. Some synthetic hypothalamic hormones and their derivatives may find important clinical and veterinary applications.

References and Notes

1. J. D. Green and G. W. Harris, *J. Endocrinol.* **5**, 136 (1947).
2. G. T. Popa and V. Fielding, *J. Anat.* **65**, 88 (1930); G. B. Wislocki and L. S. King, *Amer. J. Anat.* **58**, 421 (1936).
3. G. W. Harris, *Neural Control of the Pituitary Gland* (Arnold, London, 1955).
4. W. Bargmann, *Histochemie* **34**, 610 (1949); E. Scharrer and B. Scharrer, *Recent Progr. Horm. Res.* **10**, 183 (1954).
5. J. E. Markee, C. H. Sawyer, W. H. Hollingshead, *Recent Progr. Horm. Res.* **2**, 117 (1948).
6. M. Saffran and A. V. Schally, *Can. J. Biochem.* **33**, 408 (1955); M. Saffran, A. V. Schally, B. G. Benfey, *Endocrinology* **57**, 439 (1955); A. V. Schally, M. Saffran, B. Zimmerman, *Biochem. J.* **70**, 97 (1958).
7. M. X. Zarrow, J. M. Yochim, J. L. McCarthy, *Experimental Endocrinology* (Academic Press, New York, 1964), p. 1.
8. A. V. Schally, A. Arimura, C. Y. Bowers, A. J. Kastin, S. Sawano, T. W. Reading, *Recent Progr. Horm. Res.* **24**, 497 (1968).
9. C. H. Sawyer, in *The Hypothalamus*, W. Haymaker, E. Anderson, W. H. J. Nauta, Eds. (Thomas, Springfield, Ill., 1969), p. 389; E. M. Bogdanove, in *Vitamins and Hormones* (Academic Press, New York, 1964), vol. 22, p. 206; C. A. Barraclough and E. W. Haller, *Endocrinology* **86**, 542 (1970); J. M. Davidson, in *Frontiers in Neuroendocrinology*, W. F. Ganong and L. Martini, Eds. (Oxford Univ. Press, New York, 1969), p. 343.
10. S. Reichlin, *Amer. J. Med.* **43**, 447 (1967); W. Locke and A. V. Schally, Eds. *The Hypothalamus and Pituitary in Health and Disease* (Thomas, Springfield, Ill., 1972); R. Burgus and R. Guillemin, *Annu. Rev. Biochem.* **39**, 444 (1970).
11. R. Guillemin, W. R. Hearn, W. R. Cheek,

D. E. Householder, *Endocrinology* **60**, 488 (1957).
12. E. Anderson, *Science* **152**, 379 (1966); G. A. Hedge, M. B. Yates, R. Marcus, F. E. Yates, *Endocrinology* **79**, 328 (1966); A. H. Mulder, J. J. Geuze, D. De Wied, *ibid.* **87**, 61 (1970); G. Seiden and A. Brodish, *ibid.* **90**, 1401 (1972).
13. Abbreviations: Ac, acetyl; Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Cys, cystine; Glu, glutamic acid; Gln, glutamine; Gly, glycine; His, histidine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Trp, tryptophan; Tyr, tyrosine; Val, valine; Ile, isoleucine.
14. A. V. Schally and C. Y. Bowers, *Metabolism* **13**, 1190 (1964).
15. W. Doepfner, E. Sturmer, B. Berde, *Endocrinology* **72**, 897 (1963).
16. A. V. Schally and C. Y. Bowers, in *Proceedings of the 6th Midwest Conference on Thyroid and Endocrinology*, A. D. Kenny and R. E. Anderson, Eds. (Univ. of Missouri Press, Columbia, 1970), p. 25.
17. M. A. Greer, T. Yamada, S. Iino, *Ann. N.Y. Acad. Sci.* **86**, 667 (1960); S. Reichlin, *N. Engl. J. Med.* **269**, 1182, 1246, 1296 (1963); K. Brown-Grant, G. W. Harris, S. Reichlin, *J. Physiol. London* **136**, 364 (1957).
18. V. Schreiber, M. Rybak, A. Eckertova, V. Jirgl, J. Koci, Z. Franc, V. Kmentova, *Experientia* **18**, 388 (1962); V. Schreiber, A. Eckertova, Z. Franc, J. Koci, M. Rybak, V. Kmentova, *ibid.* **17**, 264 (1961).
19. R. Guillemin, E. Sakiz, D. N. Ward, *Proc. Soc. Exp. Biol. Med.* **118**, 1132 (1965).
20. A. V. Schally, C. Y. Bowers, T. W. Redding, J. F. Barrett, *Biochem. Biophys. Res. Commun.* **25**, 165 (1966).
21. ———, *J. Biol. Chem.* **244**, 4077 (1969); K. Folkers, F. Enzmann, J. Boler, C. Y. Bowers, A. V. Schally, *Biochem. Biophys. Res. Commun.* **37**, 123 (1969); J. Boler, F. Enzmann, K. Folkers, C. Y. Bowers, A. V. Schally, *ibid.*, p. 705; R. M. G. Nair, J. F. Barrett, C. Y. Bowers, A. V. Schally, *Biochemistry* **9**, 1103 (1970); F. Enzmann, J. Boler, K. Folkers, C. Y. Bowers, A. V. Schally, *J. Med. Chem.* **14**, 469 (1971).
22. R. Burgus, T. F. Dunn, D. Desiderio, D. N. Ward, W. Vale, R. Guillemin, *Nature* **226**, 321 (1970); D. Gillessen, A. M. Felix, W. Lergier, R. O. Studer, *Helv. Chim. Acta* **53**, 63 (1970).
23. W. Vale, J. Rivier, R. Burgus, *Endocrinology* **89**, 1485 (1971); J. Rivier, W. Vale, M. Monahan, N. Ling, R. Burgus, *J. Med. Chem.* **15**, 479 (1972); K. Hoffman and C. Y. Bowers, *ibid.* **13**, 1099 (1970); C. Y. Bowers, A. Weil, J. K. Chang, H. Sievertsson, F. Enzmann, K. Folkers, *Biochem. Biophys. Res. Commun.* **40**, 683 (1970); J. K. Chang, H. Sievertsson, B. Currie, K. Folkers, C. Y. Bowers, *J. Med. Chem.* **14**, 484 (1971); D. Gillessen, F. Piva, H. Steiner, R. O. Studer, *Helv. Chim. Acta* **54**, 1335 (1971).
24. C. Y. Bowers, A. V. Schally, F. Enzmann, J. Boler, K. Folkers, *Endocrinology* **86**, 1143 (1970).
25. J. C. Porter, W. Vale, R. Burgus, R. S. Mical, R. Guillemin, *ibid.* **89**, 1054 (1971).
26. A. V. Schally and T. W. Redding, *Proc. Soc. Exp. Biol. Med.* **126**, 320 (1967); T. W. Redding, A. Arimura, A. V. Schally, *Gen. Comp. Endocrinol.* **14**, 598 (1970).
27. J. C. Mittler, T. W. Redding, A. V. Schally, *Proc. Soc. Exp. Biol. Med.* **130**, 406 (1969).
28. C. Von Euler and B. Holmgren, *J. Physiol. London* **131**, 125 (1956).
29. T. W. Redding and A. V. Schally, *Proc. Soc. Exp. Biol. Med.* **131**, 420 (1969).
30. J. Martin and S. Reichlin, *Science* **168**, 1366 (1970).
31. J. Wilber and J. C. Porter, *Endocrinology* **87**, 807 (1970).
32. M. A. Mitnick and S. Reichlin, *Science* **172**, 1241 (1971).
33. T. W. Redding and A. V. Schally, *Endocrinology* **89**, 1075 (1971); *Neuroendocrinology* **9**, 250 (1972).
34. T. W. Redding and A. V. Schally, *Proc. Soc. Exp. Biol. Med.* **131**, 415 (1969); R. M. G. Nair, T. W. Redding, A. V. Schally, *Biochemistry* **10**, 3621 (1971).
→ F. Labrie, N. Barden, G. Poirier, A. De Lean, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 283 (1972); G. Poirier, N. Barden, F. Labrie, P. Borgeat, A. De Lean, *Excerpta Med. Int. Congr. Ser. No. 256* (1972), p. 85.
36. C. Y. Bowers, A. V. Schally, W. D. Hawley, C. Gual, A. Parlow, *J. Clin. Endocrinol. Metab.* **28**, 978 (1968).
37. M. Anderson, C. Y. Bowers, A. J. Kastin, D. Schalch, A. V. Schally, P. Snyder, R. Utiger, J. F. Wilber, A. J. Wise, *N. Engl. J. Med.* **285**, 1279 (1971); C. Y. Bowers, A. V. Schally, D. S. Schalch, C. Gual, A. J. Kastin, K. Folkers, *Biochem. Biophys. Res. Commun.* **39**, 352 (1970); C. Y. Bowers, A. V. Schally, A. J. Kastin, A. Arimura, D. S. Schalch, C. Gual, E. Castineda, K. Folkers, *J. Med. Chem.* **14**, 477 (1971); B. Karlberg, S. Almqvist, S. Werner, *Acta Endocrinol.* **67**, 288 (1971); E. D. Haigler, Jr., J. A. Pittman, Jr., J. Hershman, C. M. Baugh, *J. Clin. Endocrinol. Metab.* **33**, 573 (1971); R. Hall, J. Amos, R. Garry, *Brit. Med. J.* **2**, 274 (1970); J. Hershman and J. Pittman, Jr., *J. Clin. Endocrinol. Metab.* **31**, 457 (1970); N. Fleischer, R. Burgus, W. Vale, T. Dunn, R. Guillemin, *ibid.*, p. 109; B. Costom, M. Grumbach, S. Kaplan, *J. Clin. Invest.* **50**, 2219 (1971); R. Hall, I. Werner, H. Holgate, Eds., *Frontiers in Hormone Research, Thyrotropin Releasing Hormone* (Karger, Basel, 1971), vol. 1.
38. R. Bassiri and R. D. Utiger, *Endocrinology* **90**, 722 (1972).
39. L. Jacobs, P. Snyder, J. Wilber, R. Utiger, W. Daughaday, *J. Clin. Endocrinol. Metab.* **33**, 996 (1971); A. Tashjian, N. Barowski, D. Jensen, *Biochem. Biophys. Res. Commun.* **43**, 516 (1971); L. Debeljuk, T. W. Redding, A. Arimura, A. V. Schally, in preparation; C. Y. Bowers, H. Friesen, P. Hwang, H. Guyda, K. Folkers, *Biochem. Biophys. Res. Commun.* **45**, 1033 (1971).
40. J. E. Markee, J. W. Everett, C. H. Sawyer, *Recent Progr. Horm. Res.* **7**, 139 (1952); J. W. Everett, *Physiol. Rev.* **44**, 373 (1964).
41. S. M. McCann, S. Taleisnik, H. M. Friedman, *Proc. Soc. Exp. Biol. Med.* **104**, 432 (1960); H. J. Campbell, G. Feuer, G. W. Harris, *J. Physiol. London* **170**, 474 (1964); A. V. Schally and C. Y. Bowers, *Endocrinology* **75**, 312 (1964); A. V. Schally, A. Arimura, C. Y. Bowers, I. Wakabayashi, A. J. Kastin, T. W. Redding, J. C. Mittler, R. M. G. Nair, P. Pizzolato, A. J. Segal, *J. Clin. Endocrinol. Metab.* **31**, 291 (1970); R. Guillemin, M. Jutisz, E. Sakiz, *C. R. H. Acad. Sci.* **256**, 504 (1963); E. Endroczi and J. Hilliard, *Endocrinology* **77**, 667 (1965).
42. M. Igarashi and S. M. McCann, *Endocrinology* **74**, 440 (1964); J. C. Mittler and J. Meites, *Proc. Soc. Exp. Biol. Med.* **117**, 309 (1964); A. Kuroshima, Y. Ishida, C. Y. Bowers, A. V. Schally, *Endocrinology* **76**, 614 (1965).
43. V. D. Ramirez and C. H. Sawyer, *Endocrinology* **76**, 282 (1965); I. Chowers and S. M. McCann, *ibid.*, p. 700.
44. V. D. Ramirez and C. H. Sawyer, *ibid.* **78**, 958 (1966); A. Corbin and E. L. Daniels, *Neuroendocrinology* **2**, 304 (1967).
45. H. Minaguchi and J. Meites, *Endocrinology* **81**, 826 (1967).
46. G. Fink, R. Nallar, W. C. Worthington, Jr., *J. Physiol. London* **191**, 407 (1967); G. W. Harris and K. B. Ruf, *ibid.* **208**, 243 (1970).
47. J. N. Malacara, L. E. Seyler, Jr., S. Reichlin, *J. Clin. Endocrinol. Metab.* **34**, 271 (1972).
48. A. Negro-Villar, E. Dickerman, J. Meites, *Endocrinology* **82**, 939 (1968); T. Saito, S. Sawano, A. Arimura, A. V. Schally, *ibid.* **81**, 1226 (1967).
49. I. A. Kamberi, H. P. G. Schneider, S. M. McCann, *ibid.* **86**, 278 (1970); H. P. G. Schneider and S. M. McCann, *ibid.* **85**, 121 (1969); I. A. Kamberi, R. S. Mical, J. C. Porter, *Science* **166**, 388 (1969).
50. I. A. Kamberi, R. S. Mical, J. C. Porter, *Endocrinology* **89**, 1042 (1971).
51. W. F. White, in *Mammalian Reproduction*, H. Gibian and E. J. Plotz, Eds. (Springer-Verlag, Berlin and New York, 1970), p. 84.
52. A. J. Kastin, A. V. Schally, C. Gual, A. R. Midgley, Jr., C. Y. Bowers, A. Diaz-Infante, Jr., *J. Clin. Endocrinol. Metab.* **29**, 1046 (1969); A. J. Kastin, A. V. Schally, C. Gual, A. R. Midgley, Jr., C. Y. Bowers, E. Gomez-Perez, *Amer. J. Obstet. Gynecol.* **108**, 177 (1970); A. J. Kastin, A. Zarate, A. R. Midgley, Jr., E. S. Canales, A. V. Schally, *J. Clin. Endocrinol. Metab.* **33**, 980 (1971).
→ A. V. Schally, A. Arimura, Y. Baba, R. M. G. Nair, H. Matsuo, T. W. Redding, L. Debeljuk, W. F. White, *Biochem. Biophys. Res. Commun.* **43**, 393 (1971); A. V. Schally, R. M. G. Nair, T. W. Redding, A. Arimura, *J. Biol. Chem.* **246**, 7230 (19→ H. Matsuo, R. M. G. Nair, A. Arimura, A. V. Schally, *Biochem. Biophys. Res. Commun.* **43**, 1334 (19→ Y. Baba, H. Matsuo, A. V. Schally, *ibid.* **44**, 459 (1971); A. V. Schally, A. Arimura, A. J. Kastin, H. Matsuo, Y. Baba, T. W. Redding, R. M. G. Nair, L. Debeljuk, *Science* **173**, 1036 (1971).
54. H. Matsuo, A. Arimura, R. M. G. Nair, A. V. Schally, *Biochem. Biophys. Res. Commun.* **45**, 822 (1971).
55. M. Monahan, J. Rivier, R. Burgus, M. Amoss, R. Blackwell, W. Vale, R. Guillemin, *C. R. H. Acad. Sci.* **272**, 508 (1971); H. Sievertsson, J. K. Chang, C. Bogentoft, B. L. Currie, K. Folkers, C. Y. Bowers, *Biochem. Biophys. Res. Commun.* **44**, 1566 (1971).
56. R. Geiger, W. Konig, H. Wissman, K. Geisen, F. Enzmann, *Biochem. Biophys. Res. Commun.* **45**, 767 (1971); G. Flouret, W. Arnold, J. W. Cole, R. Morgan, M. Hedland, W. F. White, *J. Med. Chem.*, in press; S. Brady, D. F. Veber, S. Varga, R. Strachan, R. F. Hirschmann, in preparation; H. Immer, V. Nelson, M. Gotz, in preparation; N. Yanaihara, M. Sakagami, T. Kaneko, S. Saito, K. Abe, N. Nagata, H. Oka, in *Proceedings of the 9th Symposium on Peptide Chemistry, Shizuoka, Japan*, 1971, N. Yanaihara, Ed. (Protein Research Foundation Press, Osaka, 1972), p. 96.
57. A. V. Schally, A. Arimura, W. H. Carter, T. W. Redding, R. Geiger, W. Konig, H. Wissman, G. Jaeger, J. Sandow, N. Yanaihara, C. Yanaihara, T. Hoshimoto, M. Sakagami, *Biochem. Biophys. Res. Commun.* **48**, 366 (1972).
58. A. V. Schally, A. J. Kastin, A. Arimura, *Amer. J. Obstet. Gynecol.* **114**, 423 (1972); A. V. Schally, T. W. Redding, H. Matsuo, A. Arimura, *Endocrinology* **90**, 1561 (1972); A. V. Schally, A. J. Kastin, A. Arimura, *Proceedings of the International Congress on Endocrinology*, Washington, D.C., 1972.
59. A. V. Schally, Y. Baba, T. W. Redding, H. Matsuo, A. Arimura, *Neuroendocrinology* **8**, 347 (1971); Y. Baba, A. Arimura, A. V. Schally, *J. Biol. Chem.* **246**, 7581 (1971).
60. J. J. Reeves, A. Arimura, A. V. Schally, C. Kragt, T. W. Beck, J. M. Casey, *J. Anim. Sci.* **35**, 84 (1972).
61. A. Arimura, H. G. Spies, A. V. Schally, *J. Clin. Endocrinol. Metab.*, in press; L. C. Krey, W. R. Butler, G. Weiss, R. F. Weick, D. J. Dierschke, E. Knobil, in *Hypothalamic Hypophysiotropic Hormones: Clinical and Physiological Studies*, C. Gual and E. Rosemberg, Eds. (Excerpta Medica, Amsterdam, in press).
62. A. J. Kastin, A. V. Schally, C. Gual, A. Arimura, *J. Clin. Endocrinol. Metab.* **34**, 753 (1972); S. S. C. Yen, R. Rebar, G. Vendenberg, F. Naftolin, Y. Ehara, S. Engblom, K. J. Ryan, K. Benirscjke, *ibid.*, p. 1108; K. Abe, N. Nagata, S. Saito, K. Tanaka, T. Kaneko, N. Shimizu, N. Yanaihara, *Endocrinol. Jap.* **19**, 77 (1972).
63. A. Zarate, E. Canales, A. V. Schally, L. Ayala-Valdes, A. J. Kastin, *Fert. Steril.* **23**, 672 (1972).
64. A. Arimura, H. Matsuo, Y. Baba, A. V. Schally, *Science* **174**, 511 (1971).
65. V. L. Gay, A. R. Midgley, Jr., G. D. Niswender, *Fed. Proc.* **29**, 1880 (1970).
66. A. Arimura, L. Debeljuk, A. V. Schally, *Endocrinology* **91**, 529 (1972).
67. J. Hilliard, C. H. Sawyer, A. V. Schally, *ibid.* **88**, 730 (1971).
68. M. Amoss, R. Blackwell, R. Guillemin, *J. Clin. Endocrinol. Metab.* **34**, 434 (1972).
69. A. V. Schally, A. Arimura, L. Debeljuk, T. W. Redding, J. J. Reeves, in *Hypothalamic Hypophysiotropic Hormones: Clinical and Physiological Studies*, C. Gual and E. Rosemberg, Eds. (Excerpta Medica, Amsterdam, in press).
70. M. Jutisz and B. Kerdelhue, in *ibid.*
71. A. V. Schally, A. F. Parlow, W. H. Carter, M. Saito, C. Y. Bowers, A. Arimura, *Endocrinology* **86**, 530 (1970).
72. A. Arimura and A. V. Schally, *ibid.* **87**, 653 (1970).
73. L. Debeljuk, A. Arimura, A. V. Schally, *Proc. Soc. Exp. Biol. Med.* **136**, 774 (1972).
74. ———, *Endocrinology* **90**, 1578 (1972).

75. A. Arimura and A. V. Schally, *Proc. Soc. Exp. Biol. Med.* **136**, 290 (1971).
76. J. J. Reeves, A. Arimura, A. V. Schally, *Biol. Reprod.* **4**, 88 (1971).
77. ———, *J. Anim. Sci.* **32**, 123 (1971).
78. A. Arimura, L. Debeljuk, A. V. Schally, *Proc. Soc. Exp. Biol. Med.* **140**, 609 (1972).
79. M. Saito, A. Arimura, T. Kumasaka, N. Nishi, K. Kato, Y. Yaoi, T. Koyama, in *Hypothalamic Hypophysiotropic Hormones: Clinical and Physiological Studies*, C. Gual and E. Rosenberg, Eds. (Excerpta Medica, Amsterdam, in press).
80. A. Arimura, H. Matsuo, Y. Baba, L. Debeljuk, J. Sandow, A. V. Schally, *Endocrinology* **90**, 163 (1972).
81. M. Amoss, J. Rivier, R. Guillemin, *J. Clin. Endocrinol. Metab.* **35**, 175 (1972).
82. T. W. Reeding, A. V. Schally, A. Arimura, H. Matsuo, *Endocrinology* **90**, 764 (1972).
83. L. Debeljuk, A. Arimura, M. Shiino, E. G. Rennels, A. V. Schally, *Endocrinology*, in press.
84. M. Shiino, A. Arimura, A. V. Schally, E. G. Rennels, *Histochemie* **128**, 152 (1972).
→ P. Borgeat, G. Chavancy, A. DuPont, F. Labrie, A. Arimura, A. V. Schally, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 2677 (1972).
86. A. Van Tienhoven and A. V. Schally, *Gen. Comp. Endocrinol.*, in press.
87. A. Arimura, L. Debeljuk, H. Matsuo, A. V. Schally, *Proc. Soc. Exp. Biol. Med.* **139**, 851 (1972).
88. P. K. Chakraborty, J. J. Reeves, A. Arimura, A. V. Schally, *Endocrinology*, in press.
89. T. D. Golter, J. J. Reeves, C. C. O'Mary, A. Arimura, A. V. Schally, *J. Anim. Sci.*, in press.
90. L. Debeljuk, A. Arimura, A. V. Schally, *J. Clin. Endocrinol. Metab.* **35**, 918 (1972); A. J. Kastin, D. Gonzalez-Barcena, H. Friesen, L. S. Jacobs, D. S. Schalch, A. Arimura, W. H. Daughaday, A. V. Schally, *ibid.*, in press.
91. A. Zarate, E. Canales, A. J. Kastin, A. V. Schally, in preparation; R. Mancini, C. Bergada, O. Villar, A. V. Schally, in preparation.
→ R. Burgus, M. Butcher, M. Amoss, N. Ling, M. Monahan, J. Rivier, R. Fellows, R. Blackwell, W. Vale, R. Guillemin, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 278 (1972).
93. D. Coy, E. Coy, A. V. Schally, *J. Med. Chem.*, in press; I. K. Chang, A. J. Humphries, R. H. Williams, H. Sievertsson, K. Folkers, C. Y. Bowers, *Biochem. Biophys. Res. Commun.* **47**, 1256 (1972); R. Geiger, W. Konig, H. Wissman, G. Jaeger, J. Sandow, A. V. Schally, *ibid.*, in press; M. Monahan, J. Rivier, W. Vale, R. Guillemin, R. Burgus, *ibid.* **47**, 551 (1972).
94. J. K. Chang, H. Sievertsson, C. Bogentoft, B. L. Currie, K. Folkers, C. Y. Bowers, *Biochem. Biophys. Res. Commun.* **44**, 409 (1971).
95. H. J. Tracy and R. A. Gregory, *Nature* **204**, 935 (1964).
96. W. Vale, G. Grant, J. Rivier, M. Monahan, M. Amoss, R. Blackwell, R. Burgus, R. Guillemin, *Science* **176**, 933 (1972).
97. D. C. Parker, J. Sassin, J. Mace, R. Gotlin, L. Rossman, *J. Clin. Endocrinol. Metab.* **29**, 871 (1969).
98. W. G. Blackard and S. A. Heidingsfelder, *J. Clin. Invest.* **47**, 1407 (1968).
99. L. A. Frohman and L. L. Bernardis, *Endocrinology* **82**, 1125 (1968).
100. J. Roth, S. Glick, R. Yalow, S. Berson, *Metabolism* **12**, 577 (1963); G. J. Antony, J. J. Van Wyk, F. S. French, R. P. Weaver, G. S. Dugger, R. L. Timmons, J. F. Newsome, *J. Clin. Endocrinol. Metab.* **29**, 1238 (1969).
101. R. Deuben and J. Meites, *Endocrinology* **74**, 408 (1964); A. V. Schally, E. Muller, S. Sawano, *ibid.* **82**, 271 (1968); E. Dickerman, A. Negro-Villar, J. Meites, *Neuroendocrinology* **4**, 75 (1969).
102. J. Wilber and J. Porter, *Endocrinology* **87**, 807 (1970).
103. E. Knobil, V. Meyer, A. V. Schally, in *Growth Hormone*, E. Muller and A. Pecile, Eds. (Excerpta Medica, Amsterdam, 1968), p. 226.
104. L. Machlin, Y. Takahashi, M. Horino, F. Hertelendy, R. Gorden, D. Kipnis, *ibid.*, p. 292.
105. J. M. Malacara and S. Reichlin, in *Growth and Growth Hormone*, E. Muller and A. Pecile, Eds. (Excerpta Medica, Amsterdam, 1972), p. 299.
106. L. Frohman, J. Maran, A. P. S. Dhariwal, *Endocrinology* **88**, 1483 (1971).
107. J. Sandow, A. Arimura, A. V. Schally, *ibid.* **90**, 1315 (1972).
108. F. Greenspan, C. H. Li, M. Simpson, H. Evans, *ibid.* **45**, 455 (1949).
109. A. Pecile, E. Muller, G. Falconi, L. Martini, *ibid.* **77**, 241 (1965).
110. A. V. Schally, S. Sawano, A. Arimura, J. Barrett, I. Wakabayashi, C. Y. Bowers, *ibid.* **84**, 1493 (1969).
111. A. V. Schally, Y. Baba, R. M. G. Nair, E. Bennett, *J. Biol. Chem.* **246**, 6647 (1971).
112. D. Veber, C. Bennett, J. Milkowski, G. Gal, R. Denkewalter, R. Hirschmann, *Biochem. Biophys. Res. Commun.* **45**, 235 (1971); R. M. G. Nair, A. V. Schally, in preparation.
113. A. V. Schally, A. Arimura, I. Wakabayashi, T. W. Redding, E. Dickerman, J. Meites, *Experientia* **28**, 205 (1972).
114. J. Mittler, S. Sawano, I. Wakabayashi, T. W. Redding, A. V. Schally, *Proc. Soc. Exp. Biol. Med.* **133**, 890 (1970).
115. E. Couch, A. Arimura, A. V. Schally, M. Satio, S. Sawano, *Endocrinology* **85**, 1085 (1969).
116. D. S. Schalch and S. Reichlin, *ibid.* **79**, 275 (1966).
117. E. Muller, G. Giustino, D. Miedico, D. Cocchi, in *Growth and Growth Hormone*, E. Muller and A. Pecile, Eds. (Excerpta Medica, Amsterdam, 1972), p. 283.
118. S. Sawano, A. Arimura, C. Y. Bowers, T. W. Redding, A. V. Schally, *Proc. Soc. Exp. Biol. Med.* **127**, 1010 (1968); E. Muller, A. V. Schally, D. Cocchi, *ibid.* **137**, 489 (1971).
119. T. Uehara, A. Arimura, A. V. Schally, unpublished data.
120. A. J. Kastin, A. V. Schally, C. Gual, S. Glick, A. Arimura, *J. Clin. Endocrinol. Metab.* **35**, 326 (1972).
121. J. Wilber, T. Nagel, W. F. White, *Endocrinology* **89**, 1419 (1971).
122. L. Krulich, A. P. S. Dhariwal, S. M. McCann, *ibid.* **83**, 783 (1968).
123. J. Meites and C. S. Nicoll, *Annu. Rev. Physiol.* **28**, 57 (1966).
124. C. A. Nicoll, R. Fiorindo, C. McKennee, J. Parsons, in *Hypophysiotropic Hormones of the Hypothalamus: Assay and Chemistry*, J. Meites, Ed. (Williams & Wilkins, Baltimore, 1970), p. 115.
125. G. Ehni and N. Eckles, *J. Neurosurg.* **16**, 628 (1959).
126. F. Sulman and H. Winnik, *Nature* **178**, 365 (1956); D. Kleinberg and A. Frantz, *J. Clin. Invest.* **50**, 1557 (1971).
127. J. Meites, C. Nicoll, P. Talwalker, in *Advances in Neuroendocrinology*, A. Nalbandov, Ed. (Univ. of Illinois Press, Urbana, 1963), p. 238; J. Everett and N. Nikitovitch-Winer, in *ibid.*, p. 289.
128. P. Talwalker, A. Ratner, J. Meites, *Amer. J. Physiol.* **205**, 213 (1963); J. Pasteels, *C. R. H. Acad. Sci.* **254**, 2664 (1962).
129. A. V. Schally, A. Kuroshima, Y. Ishida, T. W. Redding, C. Y. Bowers, *Proc. Soc. Exp. Biol. Med.* **118**, 350 (1965); A. Kuroshima, A. Arimura, C. Y. Bowers, A. V. Schally, *Endocrinology* **78**, 216 (1966); C. Grosvenor, S. McCann, R. Nallar, *ibid.* **76**, 883 (1965); C. Grosvenor, F. Mena, A. P. S. Dhariwal, S. McCann, *ibid.* **81**, 1021 (1967).
130. A. Ratner and J. Meites, *Endocrinology* **75**, 377 (1964); H. Minaguchi and J. Meites, *ibid.* **80**, 603 (1967); M. Sar and J. Meites, *Proc. Soc. Exp. Biol. Med.* **125**, 1018 (1967); A. Dannon and F. G. Sulman, *Neuroendocrinology* **6**, 295 (1970).
131. J. Neill and L. Reichert, *Endocrinology* **88**, 548 (1971); G. Niswender, C. Chen, A. R. Midgley, Jr., J. Meites, S. Ellis, *Proc. Soc. Exp. Biol. Med.* **130**, 793 (1969).
132. Y. Amenomori and J. Meites, *Proc. Soc. Exp. Biol. Med.* **143**, 492 (1970); J. Watson, L. Krulich, S. McCann, *Endocrinology* **89**, 1412 (1971); C. Chen, Y. Amenomori, K. Lu, J. Voogt, J. Meites, *Neuroendocrinology* **6**, 220 (1970); M. Ben-David, A. Danon, R. Benveniste, C. P. Weller, F. G. Sulman, *J. Endocrinol.* **50**, 599 (1971).
133. A. Arimura, J. Dunn, A. V. Schally, *Endocrinology* **90**, 378 (1972).
134. D. L. Kleinberg, G. L. Noel, A. G. Frantz, *J. Clin. Endocrinol. Metab.* **33**, 873 (1971).
135. C. Chen, E. Bixler, A. Weber, J. Meites, *Gen. Comp. Endocrinol.* **11**, 489 (1968).
136. C. Birge, L. Jacobs, C. Hammer, W. Daughaday, *Endocrinology* **86**, 120 (1970).
137. A. J. Kastin, S. Viosca, A. V. Schally, in *Handbook of Physiology*, E. Knobil and C. H. Sawyer, Eds. (American Physiological Society, Washington, D.C., in press).
138. W. Etkin, *Gen. Comp. Endocrinol.* **1** (Suppl.). 148 (1962).
139. A. J. Kastin and G. T. Ross, *Endocrinology* **77**, 45 (1965).
140. ———, *ibid.* **75**, 187 (1964).
141. A. J. Kastin, *Proc. Endocrine Soc. Meet.* **47**, 98 (1965); ——— and A. V. Schally, *Excerpta Med. Int. Congr. Ser. No. 238* (1971), p. 211; B. B. Bercu and H. J. Brinkley, *Endocrinology* **80**, 399 (19€ → C. L. Ralph and S. Sampath, *Gen. Comp. Endocrinol.* **7**, 370 (1966).
142. A. J. Kastin and A. V. Schally, *Gen. Comp. Endocrinol.* **7**, 452 (1966); A. J. Kastin and A. V. Schally, *ibid.* **8**, 344 (1967); A. J. Kastin, A. V. Schally, S. Viosca, M. C. Miller, *Endocrinology* **84**, 20 (1969).
143. A. J. Kastin and A. V. Schally, *Endocrinology* **79**, 1018 (1966); A. J. Kastin, T. W. Redding, A. V. Schally, *Proc. Soc. Exp. Biol. Med.* **124**, 1275 (1967); A. J. Kastin, A. V. Schally, S. Viosca, L. Barrett, T. W. Redding, *Neuroendocrinology* **2**, 257 (1967); A. J. Kastin, L. Barrett, S. Viosca, A. Arimura, A. V. Schally, *ibid.*, p. 200; A. J. Kastin and A. V. Schally, *Nature* **213**, 1238 (1967); A. J. Kastin, M. C. Miller, A. V. Schally, *Endocrinology* **88**, 137 (1968).
144. A. V. Schally and A. J. Kastin, *Endocrinology* **79**, 768 (1966); R. M. G. Nair, A. J. Kastin, A. V. Schally, *Biochem. Biophys. Res. Commun.* **43**, 1376 (1971); R. M. G. Nair, A. J. Kastin, A. V. Schally, *ibid.* **47**, 1420 (1972).
145. A. J. Kastin, A. V. Schally, S. Viosca, *Proc. Soc. Exp. Biol. Med.* **137**, 1437 (1971).
146. M. E. Celis, S. Taleisnik, I. L. Schwartz, R. Walter, *Biophys. Soc. Annu. Meet. Abstr.* **15**, 98a (197 → M. E. Celis, S. Taleisnik, R. Walter, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 1428 (1971).
147. Sr. A. Bower, M. E. Hadley, V. J. Hruby, *Biochem. Biophys. Res. Commun.* **45**, 1185 (1971); V. J. Hruby, C. W. Smith, A. Bower, M. E. Hadley, *Science* **176**, 1331 (1972).
148. A. J. Kastin, N. P. Plotnikoff, R. M. C. Nair, T. W. Redding, M. S. Anderson, *Excerpta Med. Int. Congr. Ser.*, in press.
149. S. Taleisnik and R. Orias, *Amer. J. Physiol.* **208**, 293 (1965); M. E. Celis, S. Taleisnik, R. Walter, *Biochem. Biophys. Res. Commun.* **45**, 564 (1971).
150. A. J. Kastin, L. H. Miller, R. Nockton, C. A. Sandman, A. V. Schally, L. O. Stratton, *Progr. Brain. Res.*, in press; D. De Wied, B. Bohus, H. M. Greven, in *Endocrinology and Human Behavior*, R. P. Michael, Ed. (Oxford Univ. Press, London, 1968), p. 188; A. J. Kastin, S. Kullander, N. E. Borglin, B. Dahlberg, K. Dyster-Aas, C. E. T. Krakau, D. H. Ingvar, M. C. Miller, C. Y. Bowers, A. V. Schally, *Lancet* **1968-I**, 1007 (1968); A. J. Kastin, L. H. Miller, D. Gonzalez-Barcena, W. D. Hawley, K. Dyster-Aas, A. V. Schally, M. L. V. D. Parra, M. Velasco, *Physiol. Behav.* **7**, 893 (1971).
151. N. P. Plotnikoff, A. J. Kastin, M. S. Anderson, A. V. Schally, *Neuroendocrinology*, in press; *Life Sci.* **10**, 1279 (1971); *Proc. Soc. Exp. Biol. Med.* **140**, 811 (1972).
152. M. B. Nikitovitch-Winer, A. H. Pribble, A. D. Winer, *Amer. J. Physiol.* **208**, 1286 (1965); A. Arimura, A. V. Schally, T. Saito, E. E. Muller, C. Y. Bowers, *Endocrinology* **80**, 515 (1967).
153. W. Hansel, *Int. J. Fert.* **6**, 241 (1961).

154. Some research described here was supported by grants from the Veterans Administration (A.V.S., A.J.K.); by PHS grants AM-07467 (A.V.S.), AM-09094 (A.A.), and NS-07664 (A.J.K.); and by a grant from Population Council, New York (A.V.S.). We thank Dr. W. Locke, Dr. L. Debeljuk, and Dr. E. Bresler for editorial suggestions.