ASPECTS OF HYPOTHALAMIC REGULATION OF THE PITUITARY GLAND WITH MAJOR EMPHASIS ON ITS IMPLICATIONS FOR THE CONTROL OF REPRODUCTIVE PROCESSES

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by
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I am profoundly grateful for the great honor which has been bestowed upon me in recognition of my research efforts. It is a privilege for me to give an account of my search for the hypothalamic regulatory hormones. Since my work on the hypothalamus has extended over 23 years, it will be necessary to give a somewhat simplified version of it and omit reference to studies which did not contribute directly to my main objective, that is, demonstration of hormonal activity in hypothalamic extracts and the purification, isolation, determination of the structures of hypothalamic hormones and their testing in biological and clinical settings. Also, in order to avoid significant overlapping with Dr. Guillemin's lecture, I will concentrate primarily on the LH-releasing hormone (LH-RH).

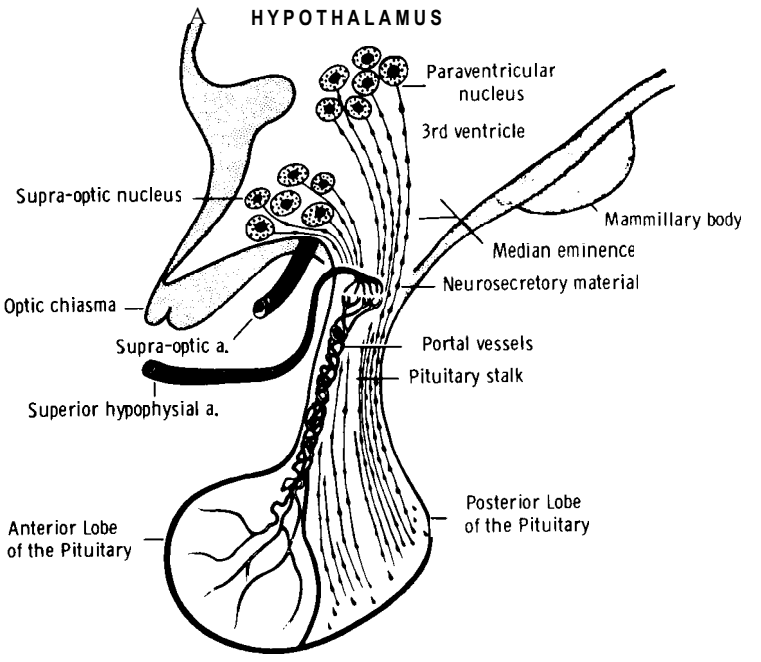


Figure 1. Simplified schematic reconstruction of the hypothalamic-hypophysial nerve tracts and blood supply to illustrate the principles of neurohumoral control of the anterior pituitary gland.

I was attracted to the hypothalamic endocrine field in 1954 while still an undergraduate student at McGill University in Montreal. A decisive stimulus was provided by the formulation by G. W. Harris and others (1) of hypotheses relating to the hypothalamic control of secretion of the anterior pituitary gland (Fig. 1).

Harris and others postulated that neurohumoral substances might originate in the median eminence of the tuber cinereum, reach the anterior lobe by way of the hypophysial portal system, and thus regulate pituitary secretion (1). About the same time Sawyer et al (2) demonstrated involvement of the central nervous system in the control of gonadotropin secretion. Without the brilliant work of these men my contributions would not have been forthcoming. It was clear that despite a strong circumstantial case favoring hypothalamic control of the pituitary, the proposition would remain speculative until direct evidence for the existence of specific hypothalamic chemotransmitters controlling release of pituitary hormones could be demonstrated.

In the beginning it was not possible to prove the existence of and isolate hypothalamic hormones because of a lack of specific methods for the detection of their activity. Working on the problem of control of ACTH secretion, M. Saffran and I reached the conclusion that the hypothalamic theory best explained most of the then existent experimental facts. We devised a test system for measuring the release of ACTH using isolated rat anterior pituitary fragments (Fig. 2) (3). This in vitro pituitary system was delightfully simple and consisted of exposure of symmetrical portions of the gland to test sub-

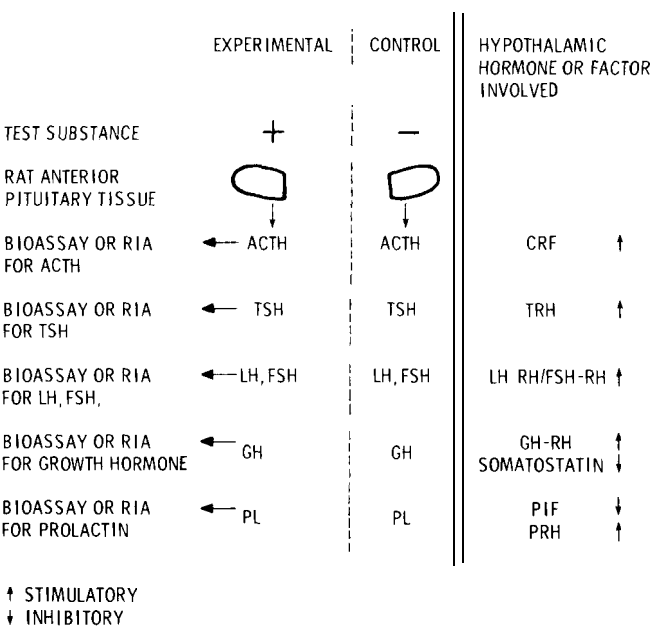


Figure 2. A diagrammatic representation of the *in vitro* test system for the detection of hypothalamic hormones and factors controlling the release of anterior pituitary hormones.

Table 1. CRF Activity of Pig Hypothalamic Preparations Measured by Stimulation of ACTH Release *in vitro* from Isolated Rat Pituitary Fragments.

PREPARATION	% ACTH RELEASED DOSE (μg)	RATIO EXPERIMENTAL TO CONTROL	95% LIMITS
Oxycel non-adsorbed	10	340	220—520
Oxycel non-adsorbed	10	240	130—470
Oxycel adsorbed	10	240	130—430
HOAc insoluble	10	240	90—660

A. V. Schally Ph. D. Thesis, McGill University, April, 1957.
Table 29, p. 91; Also, Schally et al, *Biochem. J.*, Vol. *70*, No. 1, p. 97—103, 1958.

stances. This permitted compensation for any possible indirect effect or contamination with trophic hormones and proved to be of decisive importance not only for demonstrating the existence of the corticotropin releasing factor but also hypothalamic hormones regulating the secretion of TSH, GH, LH, FSH, and prolactin. I still vividly recall the great sense of exaltation when we found that hypothalamic or neurohypophysial extracts added to the anterior pituitary tissue caused an unequivocal increase in the release of ACTH (Table 1). We "knew" then that we had done it, that the existence of a substance which stimulated the release of ACTH had been demonstrated experimentally for the first time (3-5). We named this substance corticotropin releasing factor (CRF). We still apply the term "factor" to those hypothalamic substances whose activity cannot be ascribed to a specific chemical structure. However, for those substances which have had their structures determined and which have been shown to be likely physiological regulators of secretion of respective anterior pituitary hormones, we employ the name "hormone" (Table 2).

In our early attempts to purify CRF we used mainly posterior pituitary powders, since large quantities of hypothalami were not readily available. We obtained evidence that CRF was a polypeptide (5), but despite seven years

Table 2. Hypothalamic hormones or factors controlling the release of pituitary hormones.

	Abbreviation
Corticotropin (ACTH)-releasing factor	CRF
Thyrotropin (TSH)-releasing hormone	TRH
Luteinizing hormone (LH)-releasing hormone/ Follicle-stimulating hormone (FSH)-releasing hormone	LH-RH/FSH-RH
Growth hormone (GH)-release inhibiting hormone	GH-RIH; somatostatin
Growth hormone (GH)-releasing factor	GH-RF
Prolactin release-inhibiting factor	PIF
Prolactin releasing factor	PRF
Melanocyte stimulating hormone (MSH)-release-inhibiting factor	MIF
Melanocyte-stimulating hormone (MSH)-releasing factor	MRF

of intensive effort, two with M. Saffran in Montreal and five with R. Guillemin in Houston, we were unable to isolate enough material for the determination of its structure. However, during that period (1955-1962) new *in vivo* assays for hypothalamic hormones and improved purification methods were developed. Techniques of gel filtration on Sephadex, which I learned at the Institute of Biochemistry in Uppsala with Dr. J. Porath, proved to be of particular value.

Arrangements were also made in 1962, after I moved to New Orleans, for the procurement of hundreds of thousands of hypothalami. Oscar Mayer & Co. generously donated about a million pig hypothalami. This enabled us to undertake a large-scale effort aimed at the purification of adequate amounts of material to permit chemical characterization. In addition to CRF, we systematically investigated purified fractions for the presence of TRH, LH-RH, FSH-RH, GH-RF, PIF and MIF (6-15), since the discovery of CRF opened the way to the demonstration of these other releasing factors.

THYROTROPIN-RELEASING HORMONE (TRH)

Our next great effort was devoted to the isolation and identification of TRH. We first demonstrated the presence of TRH in pig, beef, and human hypethalami using *in vitro* assays based on the release of TSH from rat pituitary glands and *in vivo* assays based on I^{131} release from thyroid glands of mice (6, 12, 13, 15). Then with the help of C. Y. Bowers and T. W. Redding I undertook the purification of bovine and porcine TRH. In 1966 we isolated 2.8 mg of TRH from 100,000 pig hypothalami (16) by Sephadex gel filtration, phenol extraction, CMC chromatography, CCD (Fig. 3), free flow electrophoresis (Fig. 4), and partition chromatography (Fig. 5). One ng of this homogeneous porcine TKH was active in our *in vivo* assay, and in vitro 0.01 ng stimulated TSH release (16). We also correctly reported that it had

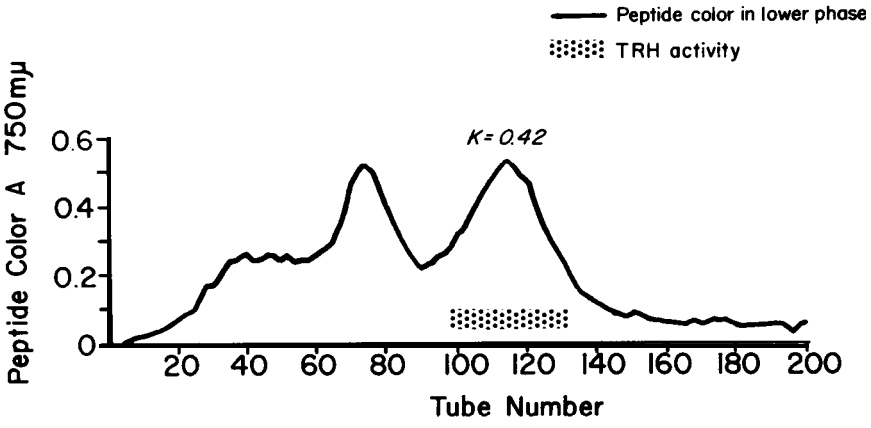


Figure 3. Countercurrent distribution of 1.0 g of porcine TRH from Cm-cellulose in a system of 0.1% acetic acid-l-butanol-pyridine, 11: 5: 3 (v/v). The number of transfers was 400. Peptide analyses were carried out on 50 μl aliquots lower phase. Based on Schally *et al*, *Biochem. Biophys. Res. Commun.* 25: 165, 1966; Schally *et al*, *J. Biol. Chem.* 244: 4077, 1969.

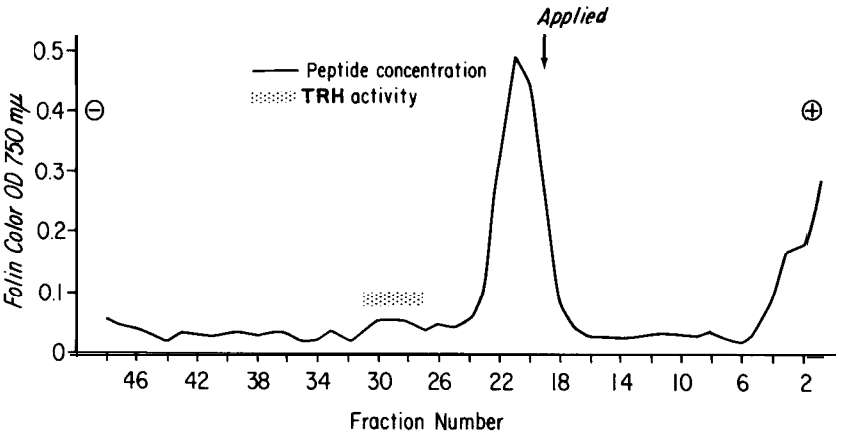


Figure 4. Free-flow electrophoresis of porcine TRH from CCD in pyridine acetate buffer pH 6.1. Conditions: 1800 V, 160 mA, 5°C, 7 hours. Peptide analyses carried out on 50 μl aliquots. Courtesy of Schally *et al*, *Rec. Prog. Hormone Res., Vol. 24, p.* 514, 1968.

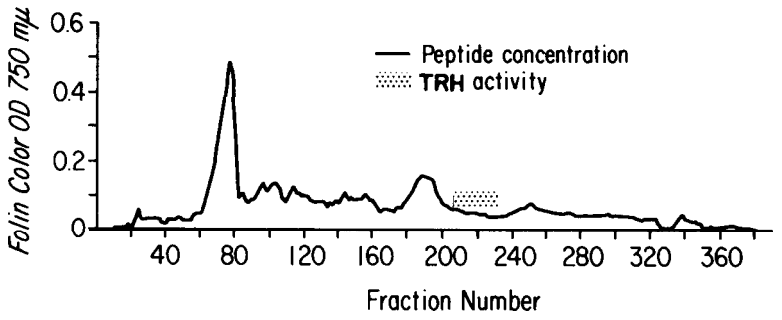


Figure 5. Partition chromatography of porcine TRH from FFE on a column of Sephadex G-25 0.9 x 76 cm. The solvent system consisted of upper phase of n-butanol: acetic acid: water = 4: 1: 5. Fraction size, 1 ml. H.U. volume = 20 ml. Courtesy of Schally *et al*, *Rec. Prog. Hormone Res. Vol. 24, p. 5* 14, 1968.

three amino acids, glutamic acid, histidine and proline in equimolar ratios (16), which established for the first time that TRH was a peptide. By mass spectra we detected a band due to the diketopiperazine of His-Pro and we also determined that an intact histidine was necessary for full biological activity of TRH, but unfortunately we did not take full advantage of these original early findings (13, 16).

Although the TRH problem could have been solved in 1966, three more years had to elapse for additional technological breakthroughs necessary to determine its precise structure. Since we lacked synthetic capabilities at that time, Merck, Sharp and Dohme Laboratories synthesized for us eight tripeptides containing histidine, proline and glutamic acid or glutamine, one of which was in the correct sequence, Glu-His-Pro. None of these, however, proved to have biological activity (13) and a complete series of possible analogs was not made. Somewhat discouraged by these negative results, I turned my attention to LH-RH, leaving the problem of the structure of TRH to my chemists with whom I was working. However when Burgus and Guillemin announced in

1969 that they found the same three amino acids in ovine TRH as I had three years earlier for porcine TRH (16), my enthusiasm for the program was rekindled and we intensified our efforts.

Fortunately, since I thought that the amount of TRH originally isolated would be insufficient to allow complete determination of structure, I took the precaution of obtaining about five additional milligrams of TRH from 250,000 pig hypothalami (17). Its structure then was systematically investigated by a series of degradation reactions (17, 18). First with R. M. G. Nair we established the correct amino acid sequence in New Orleans (17) and then in a parallel effort between my group and F. Enzmann and J. Baler working in K. Folkers laboratory in Austin, Texas, we were able to assign the correct structure to porcine TRH and synthesize it (18-20). The structure was based on: 1. the amino acid sequence of TRH established in my laboratory (17); 2. comparison of activity of synthetic analogs of Glu-His-Pro in assays carried out by C. Y. Bowers and, independently by T. W. Redding (19, 20) ; 3. mass spectra of natural and synthetic preparations (18), and 4. synthetic modification and physico-chemical comparisions of these synthetic analogs and natural TRH (19, 20). Thus, synthetic experiments were carried out on Glu-His-Pro to modify both the amino and the carboxyl ends in order to generate TRH activity. Treatment of the methyl ester of Glu-His-Pro with anhydrous ammonia led predominantly to formation of (pyro)Glu-His-Pro-amide, and

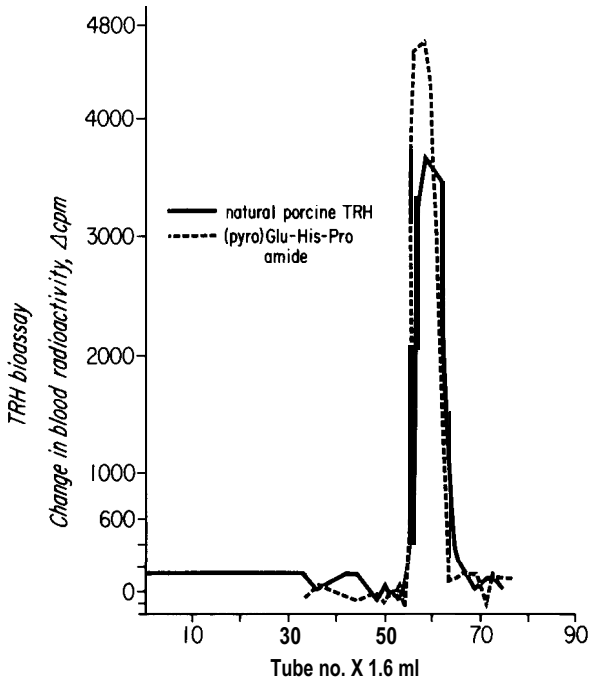
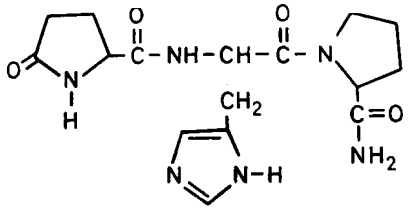


Figure 6. Gel filtration of natural porcine TRH (160 μg) and synthetic (pyro)Glu-His-Pro amide (200 μg) on Sephadex G-25. Column 1.1 x 123 cm. Solvent 0.2 M acetic acid. Fraction size 1.6 ml. The biological activity of effluents was followed by bioassay for TRH. From Schally *et al,* in *Proc. 6th Midwest Conf: on Thyroid and Endocrinology,* 1970, Univ. of Missouri-Columbia Press, p. 42.

to generation of TRH activity (19). Synthetic L-(pyro)Glu-L-His-L-Pro-amide gave R_f values identical to those of natural TRH in 17 chromatographic systems (20). Upon gel filtration on Sephadex G-25 columns in 0.2 M acetic acid, natural porcine TRH and synthetic (pyro)Glu-His-Pro-amide displayed identical migration rates (Fig. 6). The structure of TRH was thus (pyro) Glu-His-Pro-amide, or 2-pyrrolidone-5caboxylyl-His-Pro-amide (Fig. 7). The biological activity of synthetic TRH was the same as that of natural porcine TRH (21). It is somewhat ironic to realize that had Merck, Sharp and Dohme Laboratories furnished us with Glu(NH_2)-His-Pro-NH_2, it would have partially cyclized to the active (pyro)Glu-His-Pro-NH_2, form after the synthesis, and we would in all probability have solved the problem of TRH structure three years earlier.

The structural work of Burgus and Guillemin (22) on ovine TRH paralleled that of our group and they elucidated the structure of ovine TRH about the same time. Subsequent studies disclosed that bovine and human TRH probably have the same structure as the porcine and ovine hormone.



(Pyro) Glu — His — Pro-NH_2

Figure 7. Molecular structure of thyrotropin-releasing hormone (TRH).

We have conducted various physiological studies since 1962 with natural preparations of TRH (12, 13, 16) and confirmed and extended them by using synthetic TRH, demonstrating that the concentration of TSH in the plasma increased when TRH was administered intravenously, subcutaneously, intraperitoneally, or orally (21). Later we found that TRH also stimulates prolactin release in sheep (23). A direct action on the pituitary tissue *in vitro* in picogram doses was demonstrated in the pituitaries of rats, sheep, and goats (24). In pituitary tissue cultures, TRH was shown to stimulate the synthesis as well as the release of TSH. A dose-response relationship, both *in vivo* and *in vitro*, *was* also demonstrated; i.e., increasing doses of TRH caused a progressively greater release of TSH (21, 24). Thyroxine (T_4) and triiodothyronine (T_3) blocked the stimulatory effect of TRH on TSH release (13, 24). This occurred not only *in vivo* but also with pituitary fragments *in vitro* (Table 3), thus confirming that thyroid hormones must exert an action directly on the pituitary gland. We also suggested that among the physiological stimuli that may release TRH is exposure to mild cold (25). In pursuing the characterization of this hormone, we showed that it is rapidly inactivated in the blood stream and studied its excretion and metabolism

Table 3. Inhibition by Triiodothyroxine (T_3) of the in vitro Stimulation of TSH Release Induced by 0.5 Nanograms of TRH.

ADDITIONS	TSH ASSAY Change in blood ^{131}I (cpm) at 2 hours ± SE		MEAN Δ cpm	P
	Control, no TRH	Experimental, with TRH		
None	100 ± 55	818 ± 92	718	.01
None	60 ± 25	668 ± 117	608	.01
1 μg T_3	144 ± 27	184 ± 54	41	NS
1 μg T_3	162 ± 23	155 ± 38	−7	NS
1 μg T_3	106 ± 51	166 ± 36	60	NS
1 μg T_3	92 ± 17	118 ± 24	26	NS

NS: not significant ($p > 0.05$).
From: Schally and Redding (1967) (Ref. 25). Courtesy of the Proceedings of the Society for Experimental Biology and Medicine, and Academic Press.

(13, 25). Recent results suggest that TRH in addition to its effect on the pituitary might have central nervous system (CNS) effects, possibly as a neurotransmitter or modulator (26). These studies and subsequent ones by others helped establish the physiological importance of TRH.

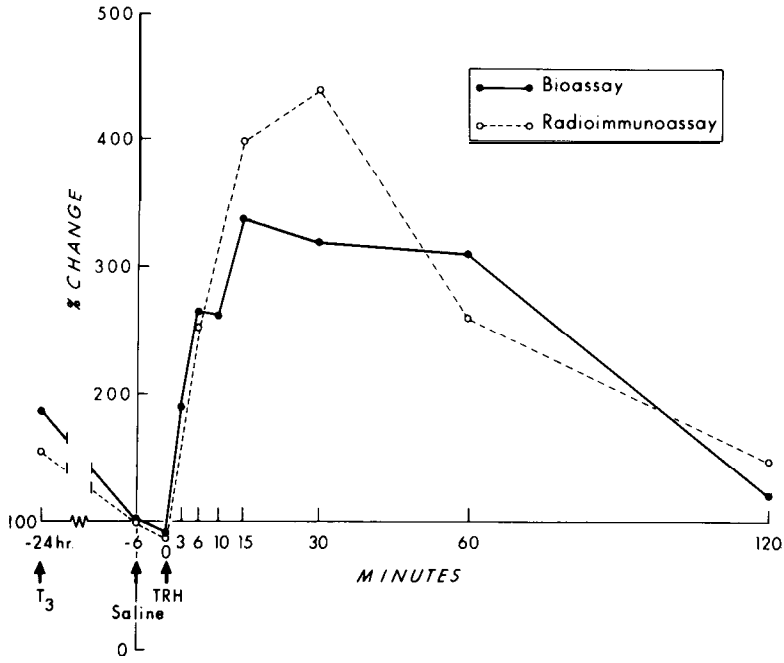


Figure 8. Changes in plasma TSH levels after the injection of TRH to cretins: at -24 h 25 μg T_3 was given orally and at 0 time 300 μg porcine TRH was given iv. Plasma samples were measured by both bioassay and radioimmunoassay. From Bowers, Schally, et *al*, *Endocrinology 86:* 1143, 1970.

The first clinical studies with TRH, carried out in 1967 with Dr. C. Bowers and Dr. C. Gual, showed that natural porcine TRH stimulated TSH release in humans (27) as measured by both bioassay and radioimmunoassay (Fig. 8). After synthetic TRH became available, these findings were confirmed and extended by us and others (28-31). It has been particularly gratifying to me that TRH is useful clinically for the differentiation between hypothalamic and pituitary hypothyroidism and for the diagnosis of mild hyper- and hypothyroidism, since one always enjoys seeing one's work bear fruit clinically.

After identification of TRH, we redoubled our efforts on the LH and FSH releasing hormone.

LUTEINIZING HORMONE- AND FOLLICLE STIMULATING HORMONE-RELEASING HORMONE (LH-RH/FSH-RH)

It has long been known that the reproductive activity of animals is influenced by seasonal and external environmental factors such as nutrition, light and temperature and that aberrations in the menstrual cycles of women can occur as a result of adverse environmental and psychological stimuli and emotional disturbances (31). In the late 1920's, after the involvement of the pituitary in the processes of reproduction was established, systematic investigations were initiated on the link between the hypothalamic region of the central nervous system (CNS) and the secretion of pituitary gonadotropins.

Based on experiments involving electrical stimulation of the hypothalamus, interruption of the blood vessels between the hypothalamus and the anterior pituitary by sectioning the hypophysial stalk, and the transplantation of the pituitary to various sites, Harris proposed the hypothesis of neurohumoral regulation of gonadotropin secretion (1). Sawyer et al (2) also adduced evidence for involvement of the CNS in control of the secretion of gonadotropins by demonstrating neuropharmacological stimulation and inhibition of ovulation with centrally acting stimulants and blocking agents.

The question that remained to be resolved was how information perceived in the CNS would be communicated to the pituitary. It was our aim to find that link between the hypothalamus and the pituitary insofar as the control of reproductive functions was concerned.

Although strong evidence for the existence of LH-RH and FSH-RH in hypothalamic extracts of rats and domestic animals was provided in the early 1960's (7, 8, 11, 32-40), it was thought that these activities were due to two different substances. We were able to demonstrate that materials with the properties of peptides purified from beef and pig hypothalami stimulated LH release not only *in vivo* but also *in vitro* (7, 8). The latter was the first demonstration that hypothalamic materials release LH by a direct action on the pituitary. With purified LH-RH at our disposal, we initiated work on how the interaction between LH-RH and sex steroids regulates gonadotropin secretion. At first we postulated that the inhibitory effect of contraceptive steroids on gonadotropin release was exerted mainly on the hypothalamus (41, 42), but subsequently with Drs. A. Arimura, C. H. Sawyer, and J. Hilliard (31,43,44) we were able to prove that estrogens, progestins and androgens also suppressed

in part the response to LH-RH at the pituitary level. Later, we confirmed by *in* vitro studies this inhibitory effect (negative feedback) of steroids on the pituitary (45). Several laboratories, including ours (31, 44, 46) obtained evidence that estrogens and progesterone can also exert a positive feedback at the pituitary and the hypothalamus, and augment the pituitary responsiveness to LH-RH. These results may be correlated with events in the human menstrual cycle and the estrous cycle of animals. Thus, an increase in estrogen concentration in plasma which precedes the ovulatory surge of LH in animals and women appears to augment the pituitary responsiveness to LH-RH. Conversely, the large amounts of estrogen and progesterone which are secreted

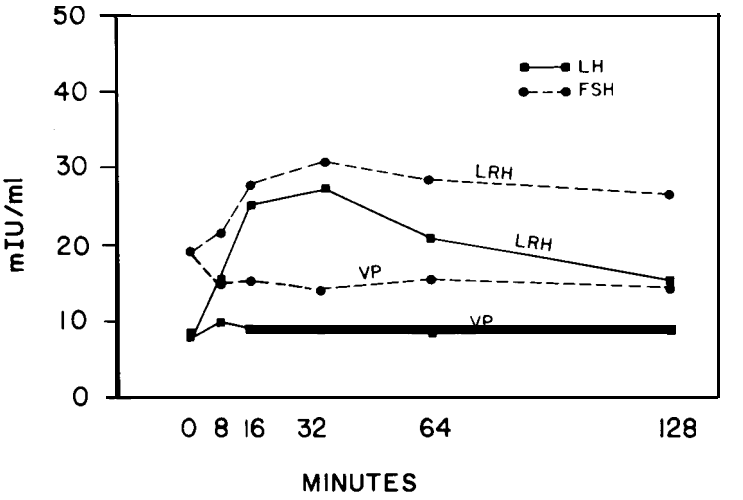


Figure 9. Effects of administration of 0.7 mg iv of porcine LH-RH on the levels of LH and FSH in a normal woman, on day 9 of the menstrual cycle. Based on Kastin, Schally *et al.*, *Amer. J. Obstet. Gynecol. 108*: 177, 1970.

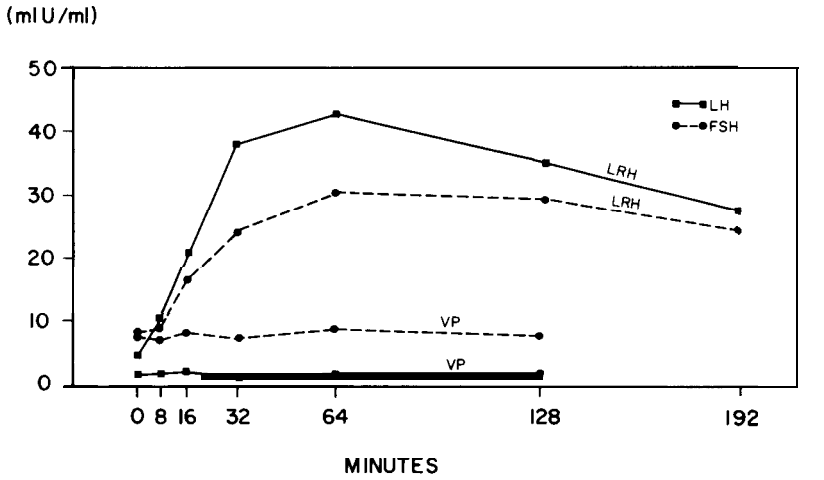


Figure 10. Effects of administration of .7 mg sc of porcine LH-RH on the levels of LH and FSH in a man pretreated with 1.5 mg ethinyl estradiol for 3 days. From Kastin, Schally, *et al*, *Amer. J. Obstet. Gynecol. 108*: 177, 1970.

after ovulation may lower pituitary responsiveness to LH-RH. With the aid of LH-RH, we also found that clomiphene exerts a central effect on the hypothalamus (47).

In view of the relative purity and apparent absence of visible toxicity of porcine LH-RH, I decided to test it in humans. These studies carried out in 1968 and 1969 in collaboration with Dr. A. J. Kastin and Dr. C. Gual in Mexico (48, 49) unequivocally established that highly purified LH-RH released LH and FSH in men and women under a variety of conditions (Figs. 9-10). Realizing that LH-RH might be useful clinically, we intensified our efforts to establish the structure of LH-RH. As in the case of TRH, tens of thousands of hypothalami had to be laboriously extracted, concentrated and purified to obtain enough material for a chemical characterization. The first isolation of 800 μg LH-RH/FSH-RH from ventral hypothalami of 165,000 pigs was achieved by twelve successive purification steps which included extraction with 2 N acetic acid, gel filtration on Sephadex, phenol extraction, chromatography and rechromatography on CM-cellulose, free-flow electrophoresis, countercurrent distribution (CCD), partition chromatography in two different solvent systems, and high voltage zone electrophoresis (Figure 11) (50). During the purification, the LH-RH

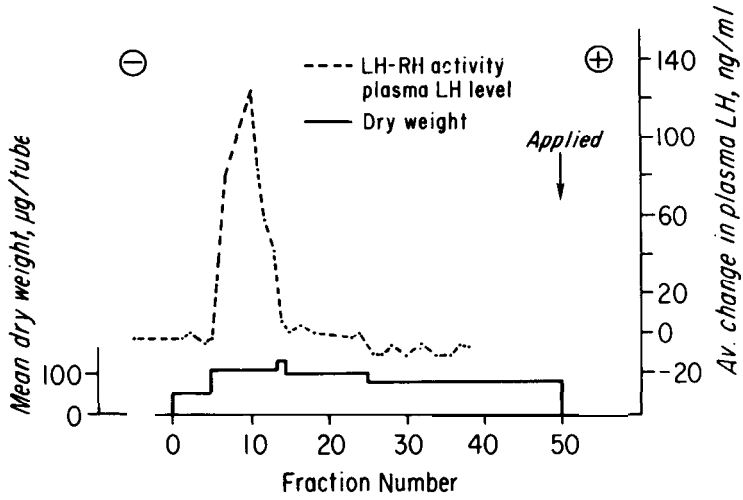


Figure 11. High voltage electrophoresis of 2.3 mg LH-RH in 0.18 M pyridine acetate buffer, pH 6.3. Vertical column 0.9 x 97.6 cm, with external cooling at 5°C, packed with cellulose powder. After the electrophoretic separation at 2570 V; 20 mA for 18 hrs, the column was eluted with buffer and 1.3 ml fractions were collected. From Schally et al, *Biochem. Biophys. Res. Commun. 43: 393,* **1971.**

and FSH-RH activities were followed by bioassay *in vitro* and *in vivo*. The LH and FSH released were determined by bioassays and later by radioimmunoassays. Subsequent isolation of 11 mg amounts of LH-RH/FSH-RH from 250,000 pig hypothalami was carried out mainly by the countercurrent distribution technique (Figs. 12-13) (51). In all the isolation steps, the LH-RH activity and FSH-RH activity were located in identical fractions.

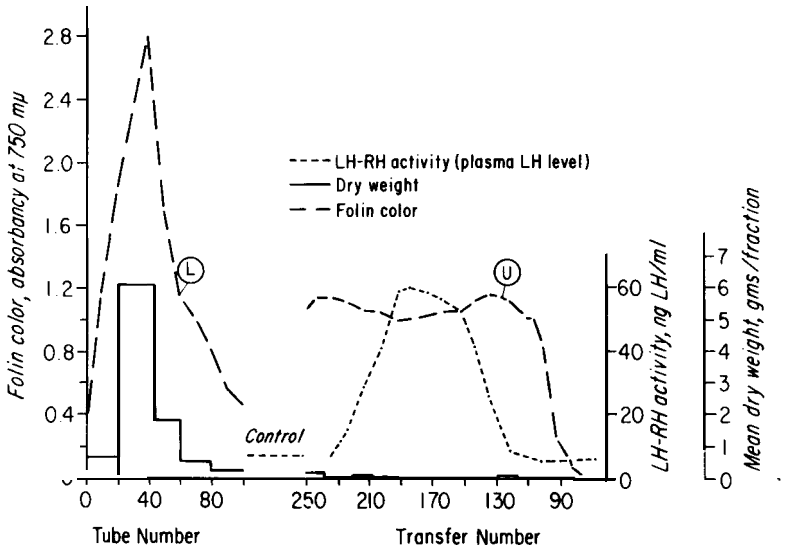


Figure 12. Preparative countercurrent distribution of LH-RH concentrate in a system of 0.1% acetic acid-1-butanol-pyridine, 11: 5: 3 by the single withdrawal method. Phenol extract (179.9 g) was extracted with the distribution solvent (1 liter of lower phase and 500 ml of upper phase) and 171.3 g of material which dissolved was loaded in tubes 0 to 19. One hundred cell train was filled with 50 ml of lower phase and 25 ml of upper phase. Two hundred fifty transfers were performed. Folin-Lowry analyses were carried out on 10 μl of lower phase (L) and 25 μl of upper phase (U). LH-RH activity was determined on 1-μl aliquots of upper phase, equivalent to approximately 0.8 μg dry weight. From Schally *et al*, *Anal. Chem. 43*: 1527, 1961.

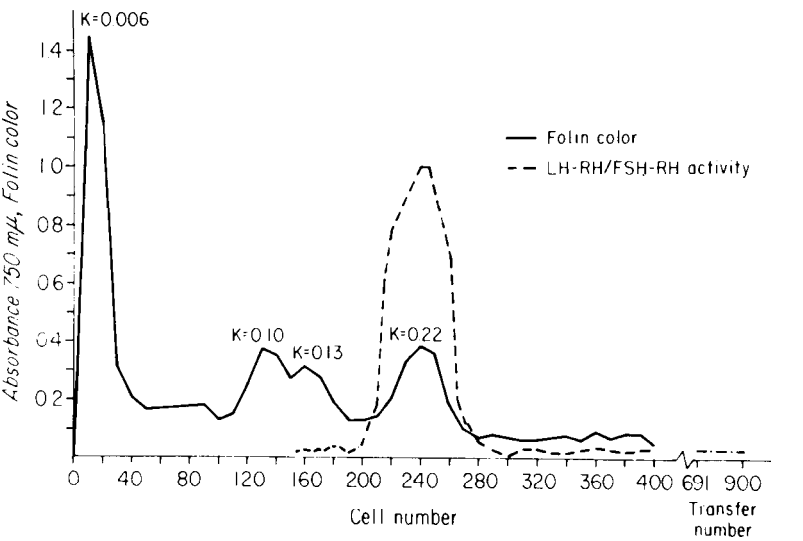


Figure 13. Countercurrent distribution (III) of 55.4 mg of LH-RH from countercurrent distribution (II) in a system of 1-butanol-acetic acid-water, 4: 1 : 5 (v/v). Lower phase was 3 ml and upper phase 5 ml. The number of transfers was 900. Folin-Lowry analyses were done on 100-μl aliquots of lower phase. Assays for location of LH-RH activity were carried out on 2 μl of lower phase per rat. From Schally *et al, J. Biol. Chem. 246: 7230,* 1971.

They could not be separated by additional partition chromatography in 10 different solvent systems (50, 51). The amino acid composition determined after hydrolysis with 6 N HCl at 110″ showed the presence of the following nine amino acids: His 1, Arg 1, Ser 1, Glu 1, Pro 1, Gly 2, Leu 1, and Tyr 1 (50). Since hydrolysis in 6 N HCl leads to destruction of Trp, the analysis for this amino acid was then carried out after acid hydrolysis in the presence of thioglycollic acid or by alkaline hydrolysis and showed the presence of one residue of Trp. Thus, the molecule of LH-RH/FSH-RH consisted of ten amino acids (52). Experiments with proteolytic enzymes showed that LH-RH/FSH-RH was a polypeptide (52). Both LH-RH and FSH-RH activities were simultaneously abolished by incubation with some endopeptidases (chymotropsin, papain, subtilisin, and thermolysin) but not by exopeptidases (leucine aminopeptidase, aminopeptidase M, and carboxypeptidase A and B) (52). Lack of inactivation by the Edman procedure and failure to detect any amino acid by the dansyl method indicated a blocked N-terminus. Inactivation by pyrrolidone carboxylyl peptidase suggested that the N-terminus was occupied by pyroglutamic acid. In the initial structural attack on LH-RH/FSH-RH with Dr. Matsuo and Dr. Baba we utilized the combined Edman-dansyl procedure coupled with the selective tritiation method for C-terminal analyses (53). These procedures were used directly on the digestion products of LH-RH with chymotrypsin and thermolysin without prior separation of fragments. Additional data were provided by high resolution mass spectral fragmentation of LH-RH/FSH-RH. On the basis of these results, we proposed the decapeptide sequence for LH-RH/FSH-RH seen in Fig. 14. The correctness of this structure was confirmed by additional conventional structural analyses involving the separation of chymotryptic fragments (54) after the cleavage of N-terminal pyroglutamyl residue in pyrrolidone carbox-

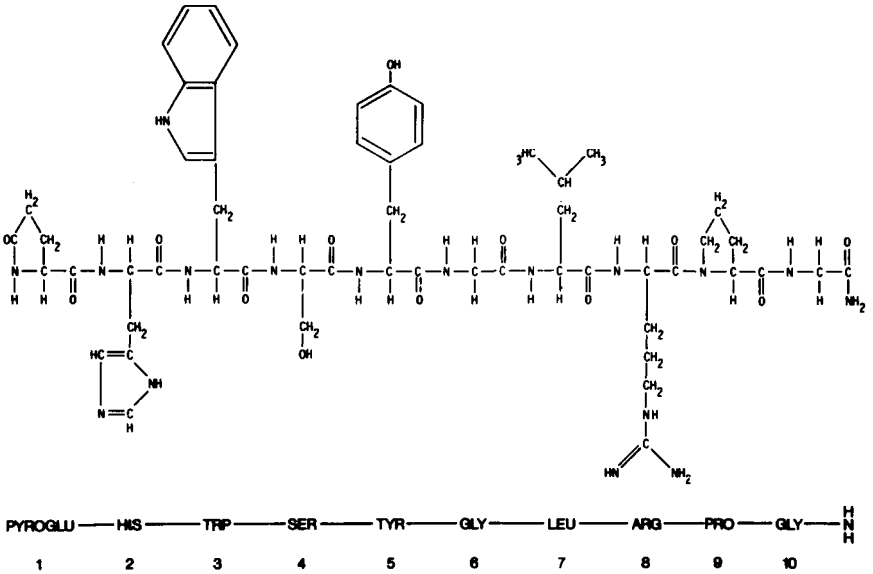


Figure 14. Molecular structure of LH- and FSH-releasing hormone (LH-RH/FSH-RH).

ylyl (PCA) peptidase as well as by synthesis of this material using the solid phase methods (55-57). Synthetic LH-RH/FSH-RH possessed the same properties as the natural material (56, 57). Thus, in rats it stimulated the release of LH and FSH *in vitro* and *in vivo* (58, 59) (Fig. 15). The time courses of LH and FSH release *in vitro* induced by natural or synthetic LH-RH

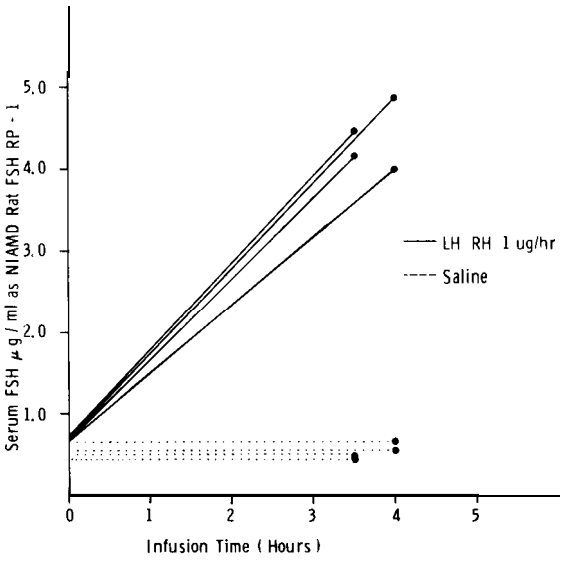


Figure 15. Serum FSH levels in immature male rats after prolonged iv infusion of synthetic LH-RH. Based on Arimura, Debeljuk, and Schally, *Endocrinology 91: 529,* **1972.**

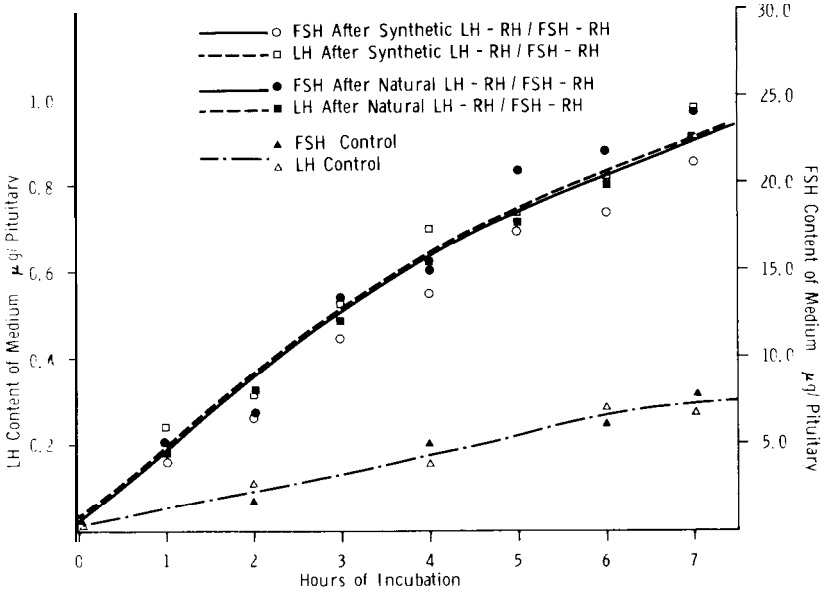


Figure 16. Release of LH and FSH from pituitaries of male rats (10 pituitary halves/beaker in 10 ml KRBG), containing 4 ng of natural or synthetic LH-RH/ml. The ordinates were adjusted to compensate for the content of LH and FSH. LH expressed as NIH-LH-S-17. FSH expressed as NIAMD-RAT-RP-I. From Schally *et al, Endocrinology 90:* **1561, 1972.**

were identical (Fig. 16) (58). Simultaneous studies demonstrated that in human beings synthetic LH-RH also raised plasma LH and FSH levels (56, 57, 60)(Figs. 17-18).

Because both natural LH-RH and the synthetic decapeptide corresponding to its structure possessed major FSH-RH as well as LH-RH activity, we took the bold step of proposing that one hypothalamic hormone, designated LH-RH/FSH-RH, could be responsible for this dual effect (56, 57). This

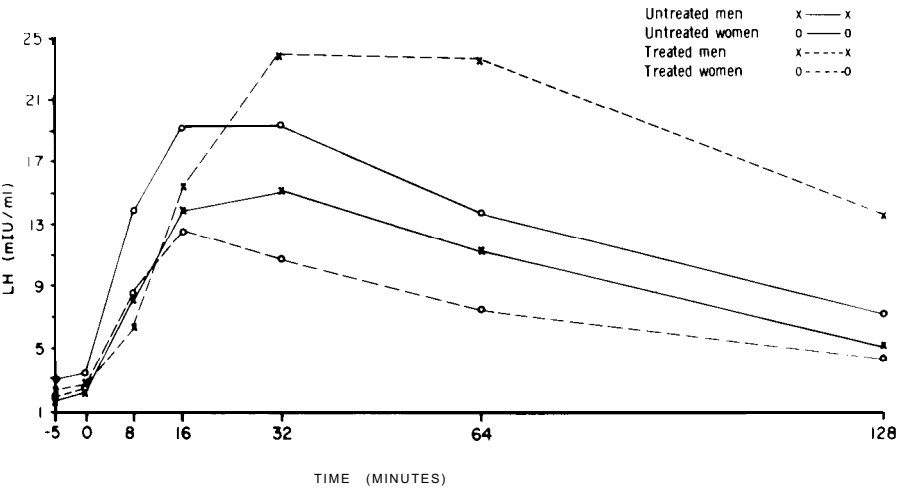


Figure 17. Mean plasma LH levels after iv administration of synthetic LH-RH equivalent to 38 μg to four groups of subjects: untreated men, untreated women, men pretreated with 1.0 mg ethinyl estradiol for 3 days; women pretreated with oral contraceptive Lyndiol for 1 week. From Kastin, Schally, et *al,* 3. *Clin. Endocrinol. Metab. 34: 753,* **1972.**

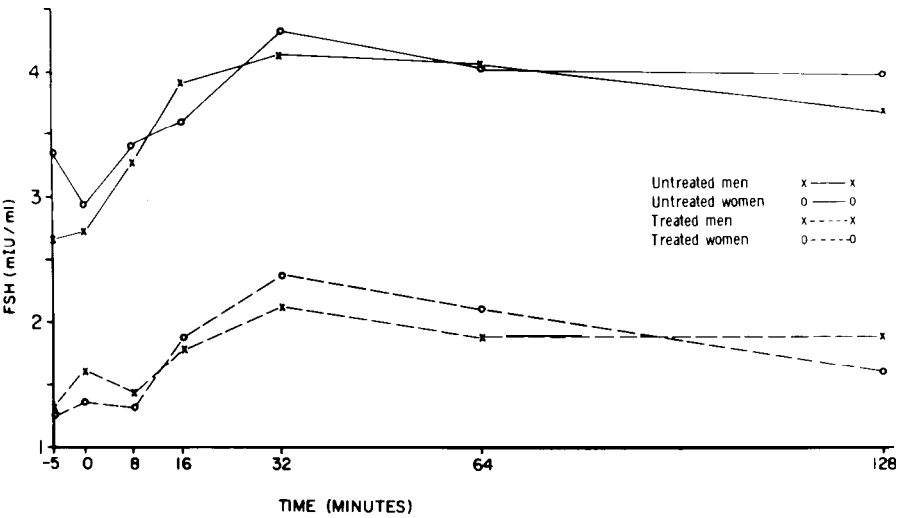


Figure 18. Mean plasma FSH levels after iv administration of synthetic LH-RH equivalent to 38 μg to four groups of subjects: untreated men, untreated women, men pretreated with 1.0 mg ethinyl estradiol for 3 days; women pretreated with oral contraceptive Lyndiol for 1 week. From Kastin, Schally, *et al,* 3. *Clin. Endocrinol. Metab. 34: 753,* **1972.**

concept is now supported by many physiological as well as immunological data. The LH-RH decapeptide represents the bulk of FSH-RH activity in the hypothalamus and it appears to be the principal FSH releasing hormone. Our subsequent studies in collaboration with Dr. J. Reeves and those of others established that in addition to rats and humans, LH-RH greatly enhances the release of LH and FSH in other mammals, including mice, nutria, rabbits, golden hamsters, mink, spotted skunk, impala, rock hyrax, sheep, cattle, pigs, horses and monkeys (Table 4) (31, 57, 61). In most of these species LH-RH can also induce ovulation. LH-RH was also found to be active in non-mammalian species such as chickens and pigeons, and even in some species of fishes such as brown trout and carp and in amphibia like newts and frogs (61). These studies in mammals, birds, fish, and amphibia indicate that species-specificity does not occur with LH-RH. We also obtained evidence that LH-RH can increase the synthesis of LH and FSH in addition to their release (62) and that prolonged treatment with LH-RH after hypophysectomy and transplantation of the pituitary stimulates spermatogenesis in male rats and follicular development in female rats (63-64). In another study with E. Rennels, we demonstrated that LH-RH increases the extrusion of secretory granules from LH gonadotrophs in rats with persistent estrus (Fig. 19) (65). Studies using synthetic ^{3}H-labelled LH-RH proved that LH-RH is rapidly degraded in blood by enzymatic cleavage of (pyro)Glu-His group and is excreted in the kidneys (66). Its, half-life is about four minutes in man.

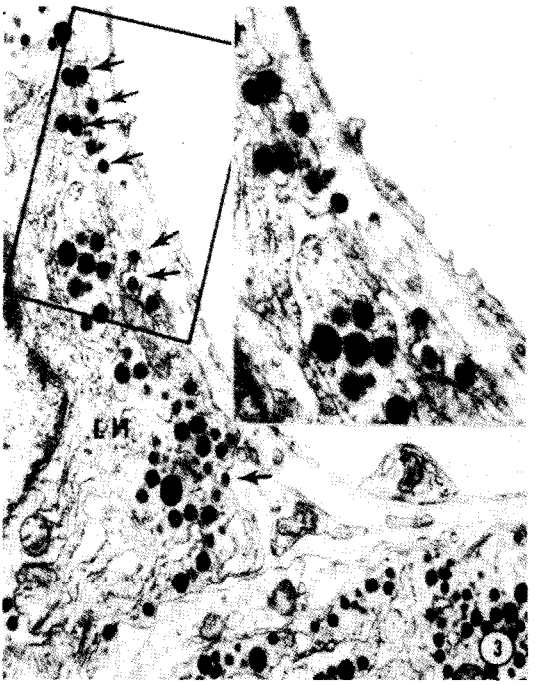


Figure 19. LH-gonadotroph of a persistent estrous rat sacrificed 15 minutes after injection of 200 ng synthetic LH-RH. Arrows indicate massive extrusion of secretory granules into pericapillary space (X 26300; insert X38000). From Shiino, Arimura, Schally, and Rennels, *Zeit. Zellforsch. Mikrosk. Amt. 128:* **152, 1972.**

Table 4. Effect of Natural and Synthetic LH-RH/FSH-RH. In Animals and Humans*

Species	Effects
Rat	Release of LH & FSH *in vivo* and *in vitro* (N.S.)
	Stimulation of synthesis of LH & FSH *in vitro* (N.S.)
	Stimulation of spermatogenesis (S)
	Stimulation of follicular maturation (S)
	Ovulation (N.S.)
	Can be given iv, ic, sc, orally, intravaginally, cutaneously (DMSO) and intraventricularly (3rd ventricle)
Mice	Release of LH, ovulation (S) (S.C.)
Golden Hamster	Release of LH *in vivo* (S)
	Ovulation (S)
Nutria	Release of LH *in vivo* (N) (I.V.)
Rock Hyrax	Release of LH *in vivo* (S)
Rabbit	Release of LH *in vivo* (N.S.)
	Ovulation (N.S.)
Mink	Ovulation
Spotted Skunk	Ovulation
Sheep	Release of LH & FSH *in vivo* (N.S.) (I.V., S.C., I.M.)
	Ovulation (S)
Pigs	Release of LH *in vivo* (S) (I.V., I.M.)
	Ovulation (S)
Cattle	Release of LH and FSH *in vivo* (S) (I.M., I.C., S.C.)
	Ovulation (S)
Impala	Release of LH *in vivo* (S)
Horses	FSH release > LH release, ovulation (S) (S.C.)
Monkeys	Release of LH (N.S.)
Humans	Release of LH and FSH (N.S.)
	Stimulation of spermatogenesis (S)
	Ovulation (N.S.)
Pigeons	
Chickens	Premature ovulation (S) and release of LH (I.C., I.V., I.M.)
Fish**	Release of Gonadotropins *in vivo* (N.S.) (I.V.)
Newts	Release of LH & FSH *in vivo* (S)
Frogs	Spermiation (S) (S.C.)

* N. Natural LH RH
S: Synthetic LH RH
** Brown Trout, Carp
Modified from: Schally and Arimura, in: *Clinical Neuroendocrinology* (L. Martini & G. M. Besser, eds) Academic Press

Immunological and Immunohistochemical Studies

Production of antisera to LH-RH by Arimura *et al* (67) and by others permitted the establishment of radioimmunoassays (RIA) and the performance of a variety of immunological studies (68-73). Male rabbits that were actively immunized with LH-RH and had generated its antibodies developed testicular atrophy associated with aspermatogenesis (67). Castrated rats actively immunized with LH-RH showed parallel decreases in serum LH and FSH levels associated with a rise in serum antibody titer to LH-RH. Administration of anti-LH-RH gamma-globulin to castrated rats prevented the rise in serum

LH and FSH levels normally seen after such operation, and the development of castration cells in the pituitary (68). Passive immunization of normal cycling rats or hamsters with LH-RH arrested follicular maturation, prevented the preovulatory surge of LH and FSH, blocked ovulation (Table 5) and reduced serum estradiol levels (69, 70). We also showed that hypothalamic LH-RH is necessary for normal implantation and maintenance of pregnancy since passive immunization with LH-RH in early pregnancy causes a delay in implantation of fertilized ova or termination of pregnancy in rats, depending on the time the antibody to LH-RH is injected (71, 72). Before and during the preovulatory surge of LH-RH release, Arimura et al (73) detected by RIA a peak of LH-RH levels in the peripheral plasma in women, and others found it in the blood of rats, sheep, rabbits and monkeys. This indicates that this decapeptide is the mediator responsible for the release of the ovulatory quota of LH. These studies and others clearly established that LH-RH is the main link between the brain and the pituitary gland insofar as reproductive function is concerned.

Table 5. Effect of iv Injection of 1 ml of Anti-LH-RH Serum (No. 742) on Serum LH and FSH Levels and Ovulation in Cycling Rats

TREATMENT	No. of Rats INJECTED	No. of Rats OVULATED	MEAN no. of ova, ± S.E.	MEAN serum LH level ± S.E. (ng/ml)*	MEAN serum FSH level ± S.E. (ng/ml)**
Normal rabbit serum	4	4	12 ± 1.3	58. ±13.2	720 ± 108
Anti-LH-RH	4	0	0	0.8 ± 0.13+	145 ± 21+

One ml of normal rabbit serum or rabbit anti-LH-RH serum was injected iv into cycling rats at 9 a.m. on the day of proestrus and blood was collected at 4:30 p.m. for assays of serum LH and FSH level.

* Expressed in terms of NIH-LH-S-17.

** Expressed in terms of NIAMD-Rat-FSH-RP-1.

+ $P < 0.01$ by Student's t test as compared with the corresponding LH and FSH levels of the sera from the rats which were injected with normal rabbit serum.

From: Arimura, Debeljuk, and Schally, *Endocrinology 95*: 323, 1974.

The availability of antisera to LH-RH made possible various studies on localization of LH-RH by RIA or immunohistochemical methods. The bulk of LH-RH was localized in the median eminence and in the arcuate nucleus, and small but significant amounts were found in the preoptic and suprachiasmatic areas. In studies with Drs. B. Flerko and G. Sétáló, we found that the pathway of LH-RH-containing nerve fibers in the median eminence of rats coincides with the course of the nerve fibers of the tubero-infundibular tract (74) and that LH-RH is produced in neuronal cell bodies, especially in the medial preoptic and the suprachiasmatic area. However, other studies

showed that extrahypothalamic brain areas also contain LH-RH and may be involved in its synthesis. This could suggest that, in addition to being the regulator of the release of LH and FSH, LH-RH might act as a central neuromodulator. LH-RH has indeed been shown to excite sexual behavior in rats (75). This is in agreement with CNS effect of hypothalamic peptides, which I helped A. J. Kastin to demonstrate in 1971 (76, 77).

Analogs of LH-RH

The interest in possible veterinary and medical applications of LH-RH stimulated us and others to synthesize many hundreds of LH-RH analogs. Between 1972 and 1977 our laboratory synthesized more than 300 analogs by the use of rapid solid-phase techniques (61, 78, 79). Our aims were: 1. to develop analogs with prolonged biological activity, so that they would be more useful therapeutically than LH-RH itself; 2. to obtain inhibitory (antagonistic) analogs which could form the basis of new birth control methods. The studies on these peptides have shed much light on the relationship between biological activity and structure. Early results showed that the 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 (61, 80). Thus, very active small fragments cannot be obtained from LH-RH. In general, amino acids in position 1 and from 4 to 10 appeared to be involved only in binding to the receptors and in exerting conformational effects. However, histidine and tryptophan probably exert a functional effect in addition to providing receptor-binding capacity, since simple substitutions or deletions in positions 2 or 3 greatly decrease or abolish LH-RH activity. Dr. D. Coy in our laboratory (81-83) and others (84-87) showed that some analogs substituted in position 6, 10 or both are 10--60 times more potent than LH-RH and also possess prolonged activity; of these, the most interesting were [D-Phe^{6}]-LH-RH, [D-trp^{6}]-LH-RH, [D-Ala^{6}, desGly^{10}]-LH-RH ethylamide (EA), [D-Leu^{6}, desGly^{10}]-LH-RH EA, and [D-Ser(Bu^{t})^{6}, desGly^{10}]-LH-RH EA. These superactive LH-RH analogs cause a prolonged release of LH and FSH (78-79, 81-86).

However, it has also been recently demonstrated that chronic treatment with pharmacologic doses of these analogs or with large amounts of LH-RH can cause temporary and reversible impairment of reproductive functions. Thus, chronic administration of l-10 µg of [D-Leu^{6}, desGly^{10}]-LH-RH EA to mature female rats caused cessation of cycling and atrophy of the ovaries and uterus (88). Prolonged administration of the same analog to male rats resulted in a reduction of testicular LH/HCG receptors and of testosterone levels (89). Pharmacologic doses of LH-RH or superactive analogs block implantation and terminate gestation when given daily postcoitally to rats (90, 91). These paradoxical antifertility effects of LH-RH and its analogs appear to be directly related to hypersecretion of LH and after nidation to functional luteolysis and/or inhibition of progesterone secretion and have caused us and others to initiate investigations on their possible application as pre-coital (male and female) and postcoital contraceptives.

Clinical Uses of LH-RH and Its Superactive Agonistic Analogs

LH-RH has been used diagnostically to determine pituitary LH and FSH reserve. It is not a complete diagnostic tool, but used alone, especially repeatedly, or in combination with the clomiphene test, it may be helpful in differentiating pituitary and hypothalamic causes of hypogonadism (61, 92). LH-RH alone or in combination with HMG or HCG has also been used therapeutically in Mexico, Chile, USA, Israel, Sweden, Japan and other countries (93-96) to induce ovulation in amenorrheic women. The use of LH-RH and its analogs can prevent superovulation and the resultant multiple births which are not uncommon after administration of HMG and/or HCG. LH-RH has also been used in Argentina and England to treat oligospermia and hypogonadotropic hypogonadism in men (97, 98). We participated in many of these studies. Recently, LH-RH given intranasally was successfully used for treatment of cryptorchidism (99). It was determined in collaborative studies carried out in Mexico, Brasil, Japan, England, Spain and Germany (100--105) that single administration of the superactive analogs [D-Ala", desGly-NH_2^{10}] --LH-RH EA, [D-Leu^6, desGly-NH_2^{10}]-LH-RH EA, [D-Ser-(Bu^t)^6, desGly-NH_2^{10})]-LH-RH EA, or [D-Trp^6]-LH-RH can induce pro-. tracted stimulation of the release of LH and FSH lasting as long as 24 hours (Fig. 20). Consequently these analogs should be more convenient and practical to use than LH-RH, which has to be given repeatedly each day (96). Moreover, these analogs are active not only after parenteral but also intranasal (Fig. 21), intravaginal, intrarectal and oral administration if suitable doses are given (61, 103-105). No significant untoward side effects of LH-RH and analogs have been observed. However, in spite of some positive

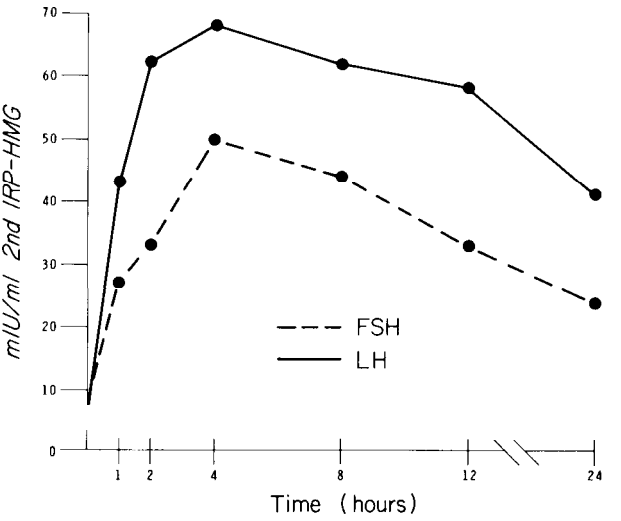


Figure 20. Plasma LH and FSH levels in 34-year-old woman with amenorrhea and galactorrhea after intramuscular administration of 250 μg of D-Leu-6-LH-RH ethylamide. From deMedeiros-Comaru, Rodrigues, Povoa, France, Dimetz, Coy, Kastin and Schally, *Internat. J. Fertil.* ***21:*** **239, 1976.**

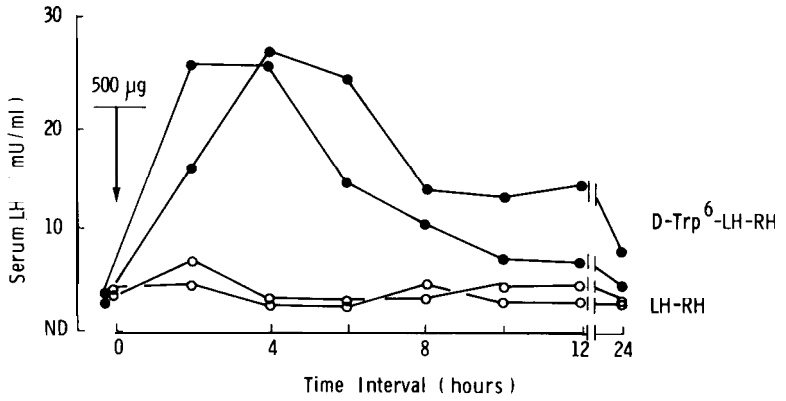


Figure 21. Effect of intranasal administration of 500 µg amounts of LH-RH or D-Trp[6]-LH-RH on serum LH levels in two men. Administration carried out in 1 ml saline using Pasteur pipette. From collaborative study with Prof. G. M. Besser and Prof. R. Hall.

results, our current knowledge about the use of these analogs for treatment of female and male infertility is inadequate and the therapeutic regimens are largely empirical. Moreover, in view of the paradoxical antifertility effects of large doses of LH-RH and longacting superactive analogs, caution must be exercised in devising clinical protocols. In order to fully exploit the potential of analogs of LH-RH for control of fertility at the level of the brain, we will need further work.

Inhibitory Analogs of LH-RH

The concept of antagonists of LH-RH proposed by us in 1971 (106) was based on the assumption that replacement or deletion of some amino acids in LH-RH might result in analogs possessing features requisite for binding, but lacking those which are necessary for a functional effect. Such analogs would be competitive inhibitors of LH-RH; that is, they would be devoid of LH-RH activity, but by competing for attachment to the receptor site with endogenous LH-RH would lead to a decrease of LH and FSH secretion. From the early inactivation studies on LH-RH (50-52, 57), we surmised the importance of His and Trp for the biological activity of LH-RH. However, the analogs based only on deletion of His or Trp were not very effective antagonists. [DesHis[2], desGlyl[10]]-LH-RH EA, made by Dr. D. Coy in our laboratory, was the first LH-RH inhibitor found to be active *in vivo* (107). Incorporation of a D-amino acid in the *6* position, in agreement with original report of Monahan *et al* (85), also improved the inhibitory activity (78, 79). It was then determined by Rees *et al* (108) that replacement of His in position 2 by D-Phe created more effective inhibitors than its deletion. Analogs such as [D-Phe[2], D-Phe[6]]-LH-RH and [D-Phe[2], D-Leu[6]]-LH-RH were synthesized (78, 79). The former was found to inhibit LH and FSH release for 6-8 hours after injection, and the latter to partially block ovulation in rats in doses of about 6 mg/kg (78, 79). [D-Phe[2], Phe[3], D-Phe[6]]-LH-RH was a still more potent inhibitor, since given at noon on the proestrous day it suppressed the preovulatory LH

(Fig. 22) and FSH surge, and completely blocked ovulation (Table 6) (109). The replacement of Trp by D-Trp in position 3 appeared to further increase the potency of inhibitory peptides (110). [D-Phe^2, D-Trp^3, D-Phe^6]-LH-RH (Fig. 23) is both longer-acting (nearly 10 hours in the rat) and more potent than [D-Phe^2, Phe^3, D-Phe^6]-LH-RH. Both these inhibitors also inhibit ovulation in hamsters and rabbits, and suppress LH release in monkeys (79, 111). We have observed that [D-Phe^2, D-Trp^3, D-Phe^6]-LH-RH and the superactive agonist [D-Trp^6]-LH-RH compete with LH-RH for its pituitary plasma membrane receptors, displacing the [^{125}I]-LH-RH more strongly than its parent hormone (Fig. 24) (112). Therefore, both stimulatory and inhibitory analogs of LH-RH may exert their action on the same pituitary plasma membrane receptors as those for LH-RH. Recently, in collaboration with Dr.

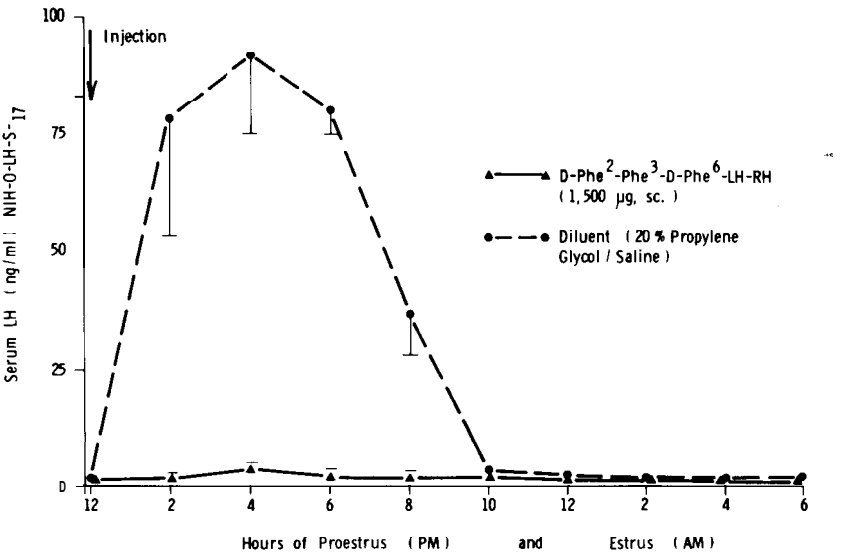


Figure 22. Effect of a single subcutaneous administration of [D-Phe^2, Phe^3, D-Phe^6]-LH-RH (1.5 mg) on the preovulatory surge of LH in proestrous rats. The differences in LH levels between animals treated with diluent (20 percent propylene glycol in saline) and analog were significant at 1400, 1600, 1800 and 2000 hours ($P<.01$). From de la Cruz, Coy, Vilchez-Martinez, Arimura, and Schally, *Science 191*: 195, 1976.

Table 6. Suppression of Ovulation in Rats by [D-Phe^2, Phe^3, D-Phe^6]-LH-RH

TREATMENT	DOSE (mg)	No. of Animals	No. of Animals Ovulating	No. of Ova (mean ± S.E.)	Suppression (%)	P
Diluent	(x3)	6	6	13.3 ± 0.8	———	———
[D-Phe^2, Phe^3, D-Phe^6]-LH-RH	1 (x3)	5	0	0.0 ± 0.0	100.0	.001

The rats had a 4-day estrus cycle and weighed 202.6 ± 1.6 g. Three subcutaneous injections were administered at 12:00, 14:30, and 17:00 hours (C.S.T.). The diluent was 20 percent propylene glycol in saline.

From: de la Cruz, Coy, Vilchez-Martinez, Arimura, and Schally, *Science 191:* 195, 1976.

p-GLU-D-PHE-D-TRP-SER-TYR-D-PHE-LEU-ARG-PRO-GLY-NH_2

1 2 3 4 5 6 7 8 9 10

D-PHE^2,D-TRP^3,D-PHE^6-LH-RH

Figure 23. Structure of the inhibitory analog [D-Phe², D-Trp³, D-Phe⁶]-LH-RH.

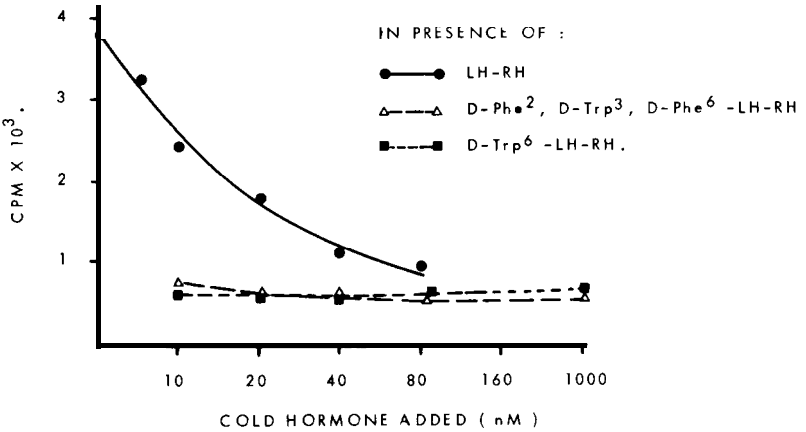


Figure 24. Effects of LH-RH and its analogs on the binding of ^{125}I-LH-RH by pituitary homogenates. The anterior pituitary was homogenized in Hepes buffer pH 7.2. The homogenate equivalent to 1 pituitary in 0.5 ml was incubated with 25 μl of a solution 4.5 nM of ^{125}I-LH-RH (300 μCi/μg) for 30 min and at 4°C in presence of LH-RH, [D-Phe², D-Trp³, D-Phe⁶]-LH-RH, or [D-Trp⁶]-LH-RH. Each point represents the average of triplicate experiments. From Pedroza, Vilchez-Martinez, Fishback, Arimura, and Schally, *Biochem. Biophys. Res. Commun.*, in press.

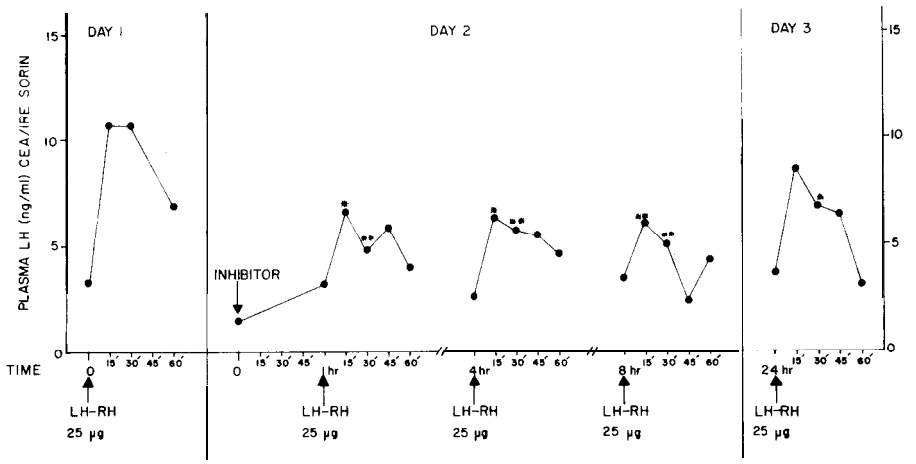


Figure 25. Mean response in serum LH levels of 4 men to 25 μg LH-RH before (control) and after im administration of 90 mg [D-Phe², D-Trp³, D-Phe⁶]-LH-RH. Asterisks indicate values significantly ($P<0.01$) different from the value at that time during the control period. From Gonzalez-Barcena, Kastin, Coy, Nikolics, and Schally, *Lancet ii*: 997, 1977.

D. Gonzalez-Barcena, we have determined that [D-Phe[2], D-Trp[3], D-Phe[6]]-LH-RH significantly suppressed the release of LH and FSH in response to LH-RH in normal men (Fig. 25) (113). We believe that the progress being made in this area may eventually lead to development of new, safer birth control methods.

GROWTH HORMONE-RELEASE INHIBITING HORMONE (GH-RIH, SOMATOSTATIN)

In 1973, Brazeau et al (114) isolated from sheep hypothalami and established the structure of a tetradecapeptide which they named somatostatin, or GH-RIH, which inhibited the release of GH in *vitro* and in *vivo* in rats. The presence of somatostatin in the hypothalamus was first observed by Krulich *et al* (115). Somatostatin was synthesized by several groups, including ours (116, 117). Subsequently, we isolated and determined the structure of porcine somatostatin, showed the primary structures of native porcine and ovine somatostatin to be identical (118), and thus confirmed the existence of this peptide (114) in another species. We also found larger and more basic forms of somatostatin in pig hypothalami (118). These materials are biologically and immunologically active, possess different physico-chemical properties from somatostatin, appear to have several amino acids including arginine attached to the N-terminus, and may represent precursors of somatostatin. We have also found high concentrations of somatostatin in extracts of pancreas, stomach and duodenum of the rat, as well as two types of immunoreactive somatostatin (119). In agreement with parallel studies by others, we found with Hall et al (92, 120) and Besser *et al* (12 1) that somatostatin inhibits the secretion of pituitary GH and TSH in human beings. A physiological role for somatostatin in the regulation of GH and TSH secretion is supported by our observations with Dr. A. Arimura that passive immunization with anti-somatostatin elevates basal GH levels, prevents the stress-induced decrease of GH in rats (122) and increases the TSH response to TRH (123). In collaborative clinical studies in England, also with Profs. Hall and Besser, parallel to those of others, we then determined that somatostatin suppresses the secretion of glucagon and insulin in humans (124). In joint investigations with Dr. S. Konturek we later established that somatostatin affects the exocrine pancreas as well, since it reduced the secretin-induced secretion of pancreatic fluid and bicarbonate (125). With Bloom et al (126), Gomez-Pan *et* al (127) and Konturek *et al (128)* we made the original observations that somatostatin decreases the circulating levels of gastrin in men and dogs, and that it also exerts a direct antisecretory effect on gastric parietal and peptic cells, since it inhibits pentagastrin-induced gastric acid and pepsin secretion in cats (Fig. 26) and dogs. These studies established for the first time that this hormone can exert exocrine, as well as endocrine, effects. In our work with Konturek *et al* (125), it was also determined that somatostatin inhibited the release of secretin and cholecystokinin/pancreozymin from the duodenal mucosa.

Dr. Arimura in our laboratory was the first to generate antisera to soma-

tostatin and to establish a RIA for this hormone (129). These antisera were used by us and Hakfelt *et al* (130) for the localization of immunoreactive somatostatin in the brain, including hypothalamus, D-cells of pancreas, the gastrointestinal mucosa and other tissues by immunocytochemical methods.

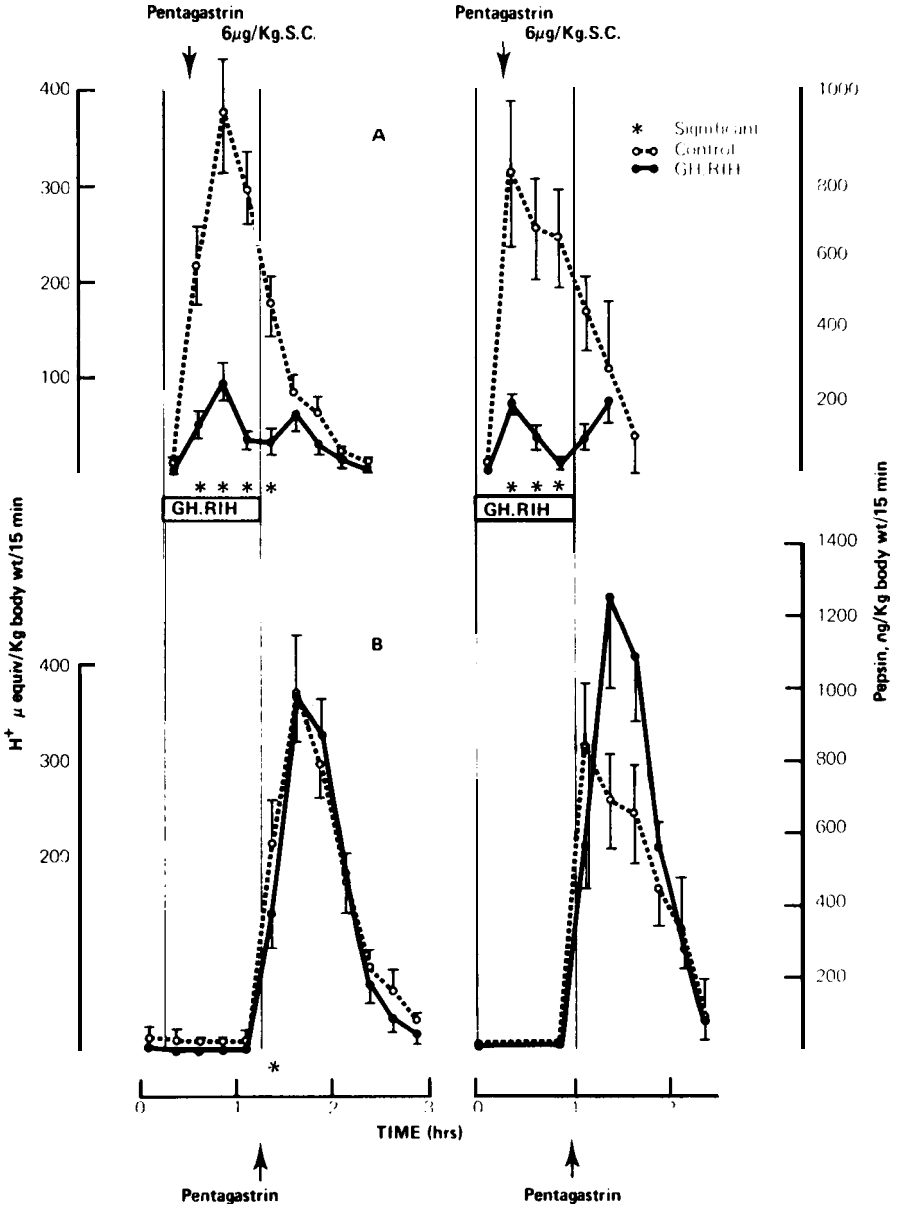


Figure 26. The mean acid and pepsin responses to pentagastrin of cats with gastric fistulae in control experiments (○— ——○) compared in (A) with those when pentagastrin was injected during the infusion of GH-RIH (10 μg/kg/hr) (●————●) and in (B) with those when the injection of pentagastrin was preceded by the infusion of GH-RIH (●————●). Responses are expressed as the mean ± S.E.M.. Asterisk indicates significant difference between the means at least at the 5% level. From Gomez-Pan, Reed, Albinus, Shaw, Hall, Besser, Coy, Kastin, and Schally, *Lancet i*: 888, 1975.

These studies support the view that somatostatin plays a role in the regulation not only of the pituitary but also of the pancreas, duodenum and stomach.

Somatostatin itself is of little therapeutic value because it has multiple actions and a short biological half-life. Attempts are therefore continuing by us and others to produce analogs of somatostatin with prolonged activity and the ability to inhibit the release of some or only one hormone. We showed that [D-Ala^{2}, D-Trp^{8}]-somatostatin (131) has a potency 20 times greater than somatostatin on inhibition of GH release, but is only three times as potent in inhibiting pentagastrin-induced gastric acid secretion (132). Meyers et al (133) in our laboratory, and others. (134), synthesized [D-Cys^{14}]-somatostatin and [D-Trp^{8}, D-Cys^{14}]-somatostatin which selectively inhibited GH and glucagon release more than insulin secretion. [D-Trp^{8}, D-Cys^{14}]-somatostatin has a ratio of 22:1 for the selective inhibition of glucagon over insulin, 100:1 for that of GH over insulin (133) and 3 : 1 for that of GH over gastric acid. Since potent analogs of somatostatin with selective activities can be prepared and promising results with long-acting analogs have already been realized in our laboratory, it is possible that future analogs may be useful in the treatment of such disorders as acromegaly, diabetic retinopathy, juvenile diabetes, peptic ulcers, and other diseases.

CONCLUSIONS AND PERSPECTIVES

At the inception of my scientific career, the concept of hypothalamic control of anterior pituitary function was in its formative stage. It was my good fortune to have arrived on the scene at such a crucial time and to have helped place it on the solid foundation on which it now rests. At present, the validity of this concept stands proven by the isolation, structural identification, and synthesis of three hypothalamic regulatory hormones. The presence of at least six other hypothalamic hormones which stimulate or inhibit the release of pituitary hormones from the pituitary gland is now reasonably well-established. It is likely that additional hypothalamic hormones will be found. Many clinical applications are now well-established and more will come. The information gathered from both animal and human studies with natural and synthetic TRH, LH-RH and somatostatin, has provided us with new understanding and even more importantly, I believe, opened vast new vistas for probing ever more deeply into these marvelously integrated systems of which the animate world is composed.

In any case, I hope that my work will be of practical use to humanity, and that I will be able to make new contributions in this field in the years to come.

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