Current Knowledge of Gonadotrophin Releasing Factor(s)*

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In discussing the subject of gonadotrophin releasing factor(s) I cannot help but feel that for once in my life I am really in tune with the times, since the same subject was recently given a lengthy and serious airing on a popular morning television program and has also been mentioned in the lay press. It seems safe to predict that practitioners of gynecological medicine will soon be asked, possibly even deluged with demands, to explain the significance of the releasing factor(s) to their patients. I am not sure that what I am going to say here will be exactly what these patients should be told, or what they will want to hear, but I think it should form the basis for a frank representation of the facts.

What I want to discuss are not the potential therapeutic or diagnostic uses of these newly available compounds, which are really very speculative, but certain features of the physiological control system in which these compounds are thought to play key roles. Any rational system of therapeutics or prophylaxis involving these releasing factors, or their derivatives, must be based on an adequate understanding of (1) the physiological roles of these compounds, (2) how these roles may be altered by disease, and (3) the effects specific disturbances of the control system(s) in which they operate can be expected to have on reproductive processes.

I will not review the background for my remarks in detail, since I have recently done that elsewhere (Bogdanove, 1972). The relevant literature is quite extensive but efforts to synthesize meaningful interpretations of it have not been lacking. There are several recent or imminent review articles,† and even an entire book (Meites, 1970), which would be particularly helpful as keys to this literature.

It is generally accepted today that many if not all of the secretory functions of the anterior pituitary gland are controlled, at least to some extent, by the central nervous system. The vascular link between the brain and the pituitary, the hypothalamic-pituitary portal system of veins, is viewed as the final common pathway of neural control. The idea that the neural influences are mediated by "neurohumors,"‡ transmitted to the gland by these portal veins, was first suggested by Friedgood (Friedgood, 1936) and later placed on a solid experimental footing by G. W. Harris and his associates (Harris, 1948; Harris, 1955; Harris and Campbell, 1966).

A large body of experimental evidence indicates that the pituitary gland secretes little or no LH and FSH if it is deprived of contact with the hypothalamus, but that the injection of hypothalamic extracts can cause these two hormones to be secreted. Similar partial or complete dependence upon hypothalamic "neurohumoral" support is characteristic of the secretion of TSH, growth hormone, and ACTH. However, the hypothalamic influence upon the secretion of prolactin has been asserted to be inhibitory, rather than stimulatory, since the isolated pituitary secretes a lot of prolactin and treatment with hypothalamic extracts can suppress this hypersecretion.

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† McCann and Porter, 1969; Burgus and Guillemin, 1970; Schally and Kastin, 1970; Gay, 1972; Saffran, 1972; Schally, Kastin, and Arimura, 1972.

‡ Although these compounds do originate in the nervous system, there is no evidence that they are secreted by neurons. The possibility that they may be secreted by glial elements (Knigge and Scott, 1970) cannot be overlooked.

The active component(s) of the crude hypothalamic extracts have been frustratingly elusive, so much so that some investigators (Schreiber, 1967) have occasionally wavered in their faith that they would eventually be isolated. Those who held the faith, however, (as well as the rest of us) have been rewarded by the recent isolation and identification of several of the releasing factors* and the consequent preparation of pure molecules by organic synthesis.

The two releasing factors which now appear to be important for reproduction are TRH and GnRH. It is the identifications of these two molecules which have been hailed (quite rightly) as achievements worthy of the attention of the news and propaganda media. It is on these two compounds, about which the lay public (ever ready to plumb the mysteries of sex) will soon be demanding information, that my remarks will focus.

The term "releasing factor" (RF) was coined by McCann (McCann, Taleisnik, and Friedman, 1960) to signify the effect such a compound could exert on the cells of the anterior pituitary gland—causing them to release a portion of their hormonal content. Over the past 10 years, this term—RF—has largely supplanted the earlier name for this group of neurohumoral factors—"hypophysiotrophins" or "hypophysiotrophic agents" (Guillemin and Rosenberg, 1955)—which had a broader denotation. The physiological process involved in hormone secretion can be considered to have two principal phases: *synthesis* (or production) of the hormone and *release* of the hormone into the circulation (Gay and Bogdanove, 1968). In the case of several of the hormones produced by the adenohypophysis, both phases of the secretory process depend upon the integrity of the hypothalamic-pituitary unit. If either the hypothalamus or the vascular connection between the hypothalamus and the anterior pituitary lobe is damaged, both release and synthesis of the pituitary hormones are impaired (Bogdanove, 1972). Under acute experimental conditions, it is much easier to determine whether something has stimulated release of a hormone than whether the processes of hormone synthesis have been influenced.

Figure 1 diagrams the relationship of the rates of synthesis and release to the amount of hormone stored in the gland (compartment I), as well as the relation between the rate of release from the pituitary and the levels of the hormone in the circulation. These levels, or titers, are the result of input and output rates. When hormone enters the blood faster than it leaves, titers increase; when the entry rate is less than the exit rate, titers decline. The concept is deceptively simple, however, since the size of compartment II, which represents the serum or plasma volume *plus* the summed volumes of a series of extravascular compartments in equilibrium with the blood, is not known. Consequently, it is not yet possible to establish the *amount* of hormone which has to be added or removed to produce a given change in serum hormone *concentration*. Conversely, it is not yet possible to quantitate, from measurement of changes in serum hormone concentrations, the causative inequality between the entry rate (rate of release of hormone from the pituitary into the blood) and the exit rate (the combined rates of destruction and excretion of circulating hormone) during the time that serum hormone titers were changing. One can merely infer that the inequality existed. However, since exit rates from the blood do not seem to vary as widely as rates of entry into the blood, major changes in serum hormone levels must reflect major changes in release rates.

Since the volume of compartment I can be measured by simply weighing the pituitary (at least in an experimental animal), it should be a very simple matter to relate quantitative changes in intrapituitary hormone stores to transient inequalities of synthesis (entry) and release (exit) rates. The catch lies in establishing changes in intrapituitary hormone stores, which poses a number of practical problems. The foremost is that sampling of intrapituitary hormone levels requires removal of the gland. This procedure, in contrast to blood sampling, cannot be repeated. However, in theory at least, any *change* in intrapituitary hormone content during a finite period of time would have to represent the product of that period of time and the algebraic sum of the synthesis (entry) and release (exit) rates.

Thus, *release* of a pituitary hormone can be detected, if not measured, solely by observing an increase in the concentration of the hormone in the circulation. To establish a concomitant change in hormone *synthesis* would be far more difficult. Since our conceptions tend to reflect our fields of vision, we tend to speak of "releasing factors" simply because effects on hormone release are more visible than effects on hormone synthesis.

In using the pituitary as a model for illustrating

* Nair, *et al,* 1970; Burgus, Dunn, *et al,* 1969; Baba, *et al,* 1971.

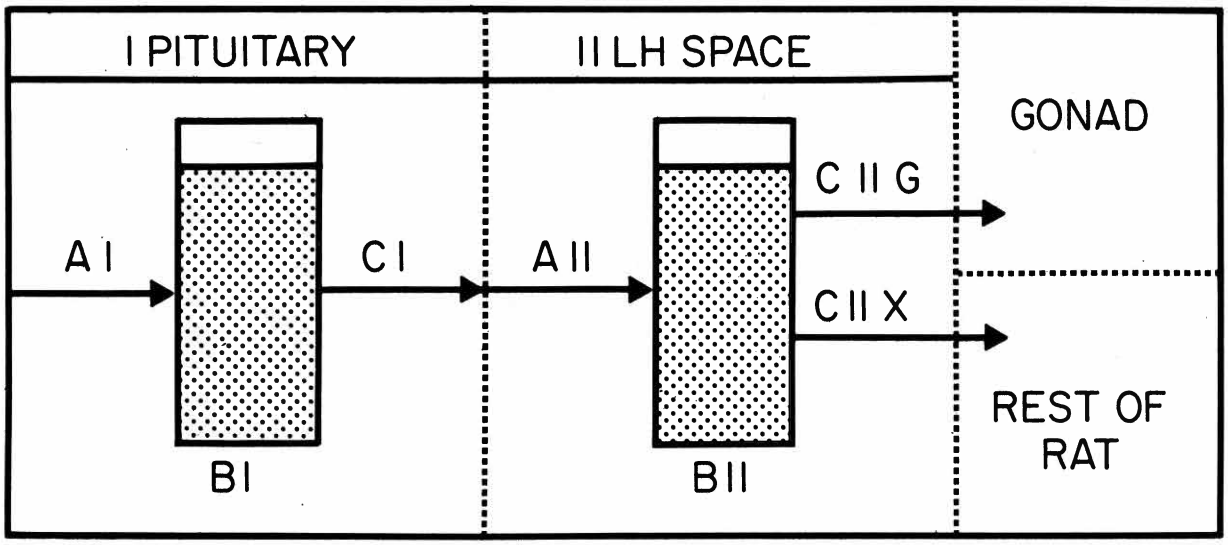

Fig. 1—Simplified, 2-compartment model illustrating LH secretory dynamics but convertible to any endocrine subsystem. Arrows labeled A (I or II) represent rates of entry into each of the 2 adjacent compartments. Arrows labeled C (I, II_G or II_X) represent total or fractional rates of exit. In compartment I, the pituitary (in the case of LH), AI is the *net* rate of LH *synthesis* and CI is the rate of LH *release*. Compartment II, the LH "space" (which seems to approximate the plasma volume) corresponds to the "inner pool" of Tait and Burstein. The rate of LH entry from the hypophysis into the LH space (AII) must, at all times, be commensurate with CI, the LH release rate. Rates CII_G, and CII_X, especially the latter, might better be drawn as $\rightleftarrows$ to indicate that they represent *net* flux between the LH space and the gonad (CII_G) and all other extrahypophysial spaces (CII_X). If entry of LH into the plasma from these extrahypophysial spaces (Tait and Burstein's "outer pool") were substantial, the apparent rate of exit (CII_X in our studies) would be slower than the true rate and the decay curve would not be described by the simple formula we have used.

If, in one of the compartments, a transient disequilibrium between A and C occurs, a change in B (stores or content) must result, according to the relationship $A - C = \Delta B$, where ΔB is the change in stores during the unit of time selected to express rate. BI can be measured (as concentration $\times$ weight-content) and BII can be calculated from the concentration of the hormone in the plasma, if the distribution volume is known (concentration $\times$ distribution volume = total circulating hormone). None of the rates (AI, CI, AII, CII_X, or CII_G) has ever been measured. However, in a relatively steady state, as in a rat castrated 4 or more weeks previously (where $CII_G = 0$), $\Delta BI \backsimeq \Delta BII \backsimeq 0$ and therefore $AI \backsimeq CI = AII \backsimeq CII_X$.

In the "stop-entry" experiment (acute removal of compartment I by hypophysectomy) AII instantaneously becomes zero but CII_X, the rate of exit from the plasma, slows gradually. Although CII_X immediately starts to decrease, seemingly as an exponentia function of BII, its instantaneous initial (*zero* time) value, which must about equal the steady state (pre-hypophysectomy) values of AI, CI and AII, can easily be calculated, as explained in the text. (Redrawn with permission from Gay and Bogdanove. *Endocrinology* 82:359, 1968.)

the input-output relationships involved in the processes of secretion, I have focused on the most meaningful accessible index of activity—changes in serum hormone concentration (ΔBII in Fig. 1). If we wish to focus on *hypothalamic* secretory activity, the problems become very much greater. Figure 2 illustrates a current concept of how the hypothalamus and pituitary are integrated with other organs involved in the control of reproductive activity. The evolution of this model has been reviewed elsewhere (Bogdanove, 1972). For our present purpose, a discussion of current knowledge of gonadotrophin releasing factor(s), this model is presented merely to locate the subject of discussion.

As shown in Fig. 2, arrow 3 represents the *secretion* of releasing factors, which act (either alone or in conjunction with other internal environmental influences) to induce secretion of pituitary gonadotrophic hormones (arrow 4). It is precisely this stimulus-response relationship which has provided the existing operational definitions of the so-called releasing factors. Thus, a factor which released FSH was called FSH-RF (Igarashi and McCann, 1964). One which released LH was called LH-RF, or LRF (McCann, Taleisnik, and Friedman, 1960). A factor thought to affect FSH synthesis (Corbin and Milmore, 1971) or prolactin synthesis (Nicoll and Fiorindo, 1969) was given still another name. One which decreased the rate of prolactin release was dubbed PIF or PRIF, for prolactin release inhibiting factor. In every case, the definition was *indefinite*—there was never any valid reason for believing that LRF and FSH-RF were separate entities (despite published conclusions to that effect which will not be cited here). As long as the changes in pituitary hormone release rates which were ob-

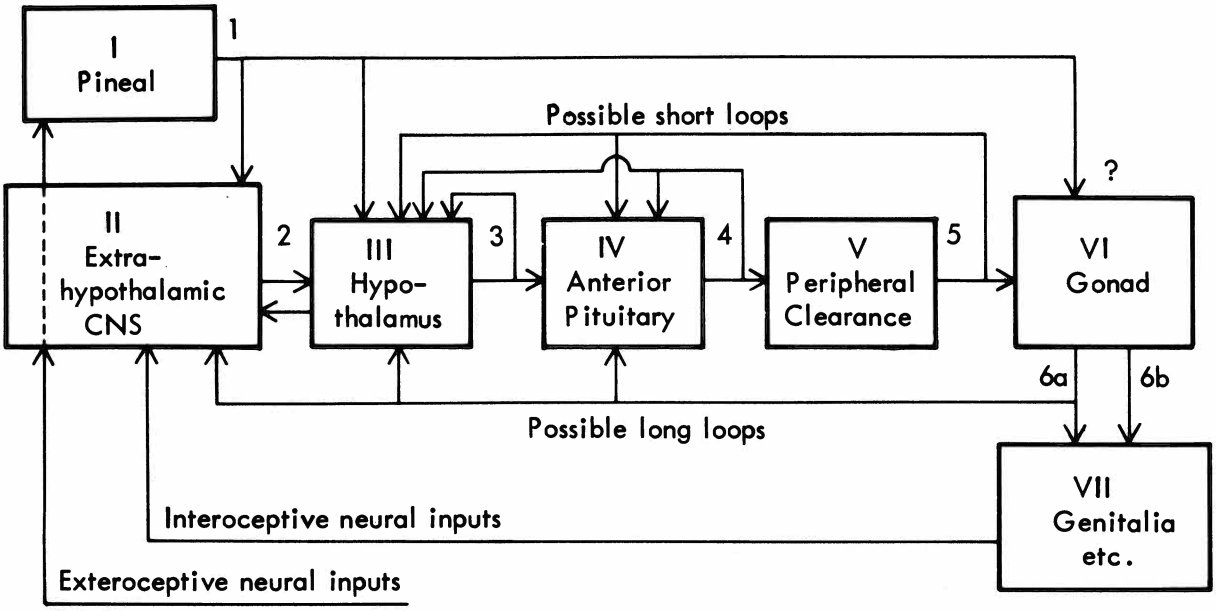

Fig. 2—Major components of brain-pituitary-gonadal control system. Boxes represent *loci* (generally organs) of physiological responses to stimuli. Arrows, which constitute responses as well as stimuli, represent signals (which may be either neural or humoral). To conserve space, some arrows have been numbered: 1) pineal secretion (melatonin?); 2) afferent and efferent neurons; 3) hypophysiotrophin secretion; 4) LH, FSH, etc. in portal venous blood; 5) LH, FSH, etc. in peripheral blood; 6a) gonadal steroid secretion; 6b) eggs or sperm.

served—plus any changes which may also have occurred without being detected—had been induced by administration of crude, or even partially purified, hypothalamic extracts, it was simply impossible to attribute the response(s) to specific components of the extracts. Thus, acid extracts of rat (or sheep, or pig, or steer) hypothalamus could release TSH, growth hormone, ACTH, LH, FSH, and, under some conditions, MSH (melanophore-stimulating hormone, or *intermedin*). At the same time, they could inhibit release of prolactin. The extent to which these, and other, effects could be attributed to the presence of specific hypophysiotrophic molecules in these extracts still remains to be determined. Until all such demonstrations have been reproduced, using "RFs" of unequivocal purity, our views of how the hypothalamus might exert its effects on pituitary secretory activity will have to remain indefinite.

This was the urgent reason for the intense and sustained efforts of the several laboratories which were engaged in the great releasing-factor hunt of the last decade. The task of collecting and extracting hundreds of thousands of hypothalamic fragments from sows and cows and ewes, in order to obtain, at the end of nearly 10 years, the smidgins of purified materials needed to define the chemical structures of the RFs, can truly be described as epic. The results of these Augean labors have finally begun to be visible. The structure of the thyrotrophin releasing hormone TRF (or TRH, using Schally's nomenclature†) was discovered barely a year before that of the single decapeptide molecule which appears able to release both LH and FSH (Baba, *et al,* 1971). The name of this molecule has not yet been settled. Schally has given it the quasi-acronym "LH-RH/FSH-RH," but the same molecule is being prepared synthetically, by Abbott Laboratories, under the name of GnRH (for gonadotrophin-releasing hormone).

Far more important than what this compound should be called is the question of what it can do;

† Schally has proposed (Schally, Arimura, *et al,* 1968) that the RFs be called RHs (for releasing hormones) on the basis that they ought to be recognized as full-fledged members of the community of hormones. Guillemin and others (Burgus and Guillemin, 1970; Bogdanove, 1972) have objected to Schally's terminology, for several reasons. Still other nomenclature has recently been proposed (Saffran, 1972). Debate about etymological propriety seems pointless since these compounds will be known best by the names under which they are distributed by the pharmaceutical companies which undertake to mass produce them. Therefore, despite my previous objections, I will refer to Schally's LH-RH/FSH-RH as GnRH.

first for the physiologist and subsequently for either the physician and his individual patient, or for the public health and agricultural scientists who are concerned with the control of human and animal fertility on a larger scale. I think the answers to these questions are of vital importance, but I do not think I or anyone can give them just yet.

We do not yet know whether GnRH is the only factor secreted by the hypothalamus which can influence LH and FSH secretion. As a matter of fact, we do not even know that GnRH is secreted by the hypothalamus, but only that it can be extracted from it. [The increased LH and FSH releasing *activity* which can be shown in portal venous blood after the hypothalamus has been stimulated (Kamberi, *et al,* 1971), may or may not be due to increased GnRH concentration in that blood.] If GnRH is, in fact, the *only* gonadotrophin-releasing factor, what is its physiological role? Is it involved in disease? How can we make use of it?

Much work lies ahead before these questions will be answered. If we pause to think for a moment about some of the major steps toward our present understanding of the pituitary hormones involved in reproduction, we may be able to glimpse some parallels in the problems which lie ahead in the study of hypothalamic hormones.

The demise of the Aristotelian notion of pituitary function came about when pioneer surgeons and physiologists (Crowe, *et al,* 1910; Smith, 1927) removed the gland from the living animal and discovered that the major resultant deficiencies were not in the production of nasal mucus, but in somatic growth (particularly at epiphyseal plates) and the activities of what we have now come to know as the pituitary target glands: thyroid, adrenal cortex, and gonads. (Similar deficiencies result from damage to the hypothalamus or isolation of the pituitary from hypothalamic influence.) The next steps were to show that injection of extracts of anterior pituitary tissue could remedy these various deficiencies and that specific fractions of such extracts could selectively restore specific functions. It is through this *substitutive* approach that the existence of seven distinct adenohypophyseal hormones was finally defined: LH, FSH, TSH, ACTH, prolactin, growth hormone, and MSH. It took a long time to accomplish this partial goal, primarily because it took so long to determine the actual structures of the macromolecules secreted by the anterior pituitary. (In this respect, the oligopeptidic hypothalamic hormones should present much less of an obstacle.)

As sufficiently pure pituitary hormones became available it was found that FSH alone could produce follicular growth, but not estrogen secretion, in hypophysectomized rats (Greep, 1968). If a little LH was administered with the FSH, steroid secretion also resulted. Ovulation and formation of a corpus luteum required a rapid surge of a large amount of LH, superimposed on this "priming" of the follicle by FSH and a trace of LH. Physical maintenance of the corpus luteum appeared to require nothing from the pituitary (corpora lutea survived for months in hypophysectomized rats) although *secretion* of luteal hormones did. In the rat and mouse, but not in other animals, prolactin was found to be luteotrophic, capable of activating the secretory machinery of the corpus luteum. In the male, FSH alone has been credited with a role in the production and maintenance of spermatogenesis (Steinberger, 1971), while LH is clearly capable of stimulating androgen production by the Leydig cells. (The androgen, in turn, stimulates spermatogenesis.) The male does not seem to use prolactin as a gonadotrophin, although he can use it as a lactogenic hormone under certain conditions.

We are now entering a comparable phase of *substitutive* investigation of the releasing factors. So far, only two or three facts have emerged which merit comment. One is that the response of the pituitary to GnRH can be influenced, at least quantitatively, by steroid feedback (long-loop arrow, probably to box IV, in Fig. 2), as well as by either genetic sex or some consequence thereof. The evidence for this is that when identical doses of natural porcine GnRH were injected into male and female castrated rats which had been pretreated with ovarian steroids or testosterone (four groups in all), the response in the spayed rats which had been pretreated with ovarian steroids greatly exceeded that in the similarly pretreated orchidectomized rats and in the testosterone-pretreated castrates of either sex (Rennels, *et al,* 1971). Evidence that steroids can act directly at the pituitary level to qualitatively alter pituitary activity, presumably by modifying the effects of either GnRH or some other hypophysiotrophic agent(s), has been presented recently (Kingsley and Bogdanove, 1971). These two facts suggest, but do not establish, that it may not be necessary to postulate neural participation in every change in LH or FSH secretory rate, or every shift in the LH:FSH ratio, which may occur under physiological or experimental conditions. Thus, although changes in LH and FSH secretion may result from changes in GnRH secretion, it is also pos-

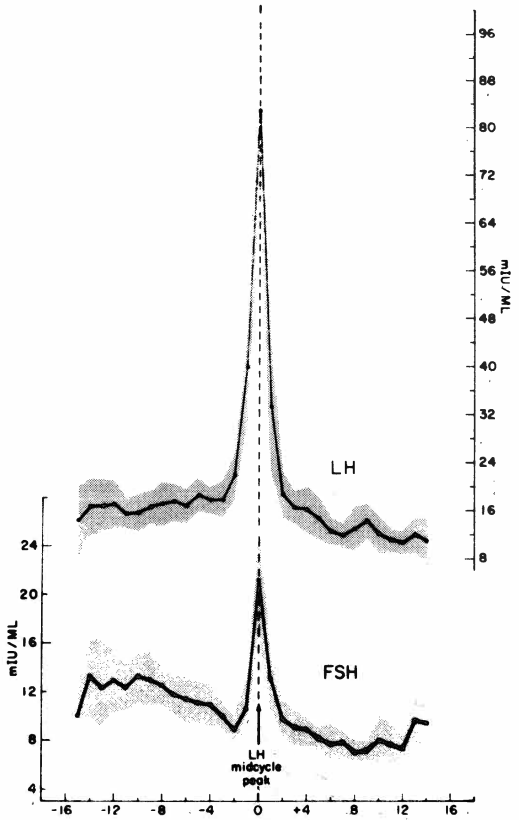

Fig. 3—Patterns of radioimmunoassayable serum LH (upper graph) and FSH (lower graph) in women during 16 presumptively ovulatory cycles. Shaded areas represent 95% confidence limits of means. (Reprinted with permission from Ross, et al. *Rec. Progr. Horm. Res.* 26:1, Academic Press, 1970.)

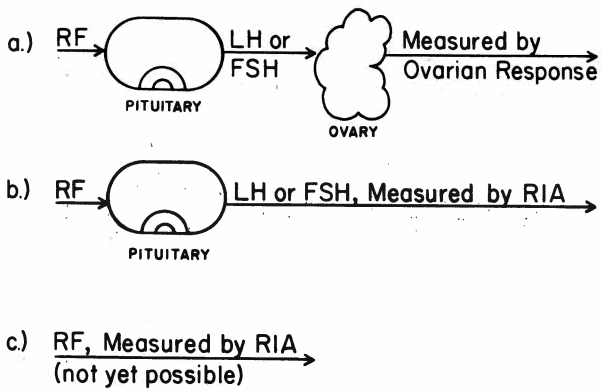

Fig. 4—Components of RF assays: a. "Double" bioassay, in which end-point is an ovarian response; b. "Single" bioassay, in which end-point is a pituitary response determined by radioimmunoassays (RIA) of serum; c. Direct assay, which does not involve any biological response. (This type of assay is not yet available.)

sible that changes in the pituitary output of these two hormones can occur without any precedent change in hypothalamic secretory activity. For this to happen, it would be necessary for pituitary responsiveness to GnRH to vary under the influence of one or several control agents other than GnRH. It remains to be determined whether sex steroid feedback, which apparently can influence pituitary responsiveness to releasing factor(s) under experimental conditions, can also do so under physiological conditions.

Another fact is noteworthy. Both human (Gual, *et al,* 1972) and rat (Tashjian, *et al,* 1971) pituitary cells can be stimulated to release prolactin by TRH! The mechanism of this unexpected finding remains to be established.

As additional synthetic releasing factors become available, and additional clever or lucky experiments are carried out, a considerable body of data will develop. From it, the physiologist will venture, about the secretion of endogenous releasing factors, opinions for which today there is not yet a sufficient foundation. The aim of substitutive research must be to supply the pituitary gland deprived of hypothalamic control with a sufficiently elaborate replacement for its natural releasing factor input (a sort of prosthetic hypothalamus), so that its behavior will mimic that seen when neural controls are allowed to operate. Even from such substitution studies, however, conclusions should be drawn with caution. The *caveat* I would stress is that substitution studies reveal only what a hormone *can* do, not necessarily what it *does*. As an example of the distinction, consider the impression—based on the demonstrable proportionality between the amounts of FSH injected and the resultant sizes of ovarian follicles—that the progressive growth of the follicle during the preovulatory phase of the cycle reflects a progressive increase in the rate of FSH secretion. This impression, derived from substitution studies, has not been borne out by direct observation. Figure 3 shows the patterns of LH and FSH in the serum during the menstrual cycle in the human. Note that, during the follicular phase of this cycle, serum FSH levels do *not* increase, but actually seem to *decline*. This finding would not have been anticipated on the basis of substitution experiments.

Ultimately, it is always necessary to follow the substitutive approach with an analytical one, aimed at characterizing patterns of secretion by direct observation. However, I think it will be some time before direct analysis of releasing factor secretion becomes a possibility. For most of the past 10 years,

measurements of releasing factor activity have required a "double bioassay" (Fig. 4) in which the effect of the factor on the pituitary (a biological response) could be assessed only by bioassay (involving a second biological response). The order of error in such an assay system was usually, perhaps always, sufficiently enormous that conclusions had to be based on intuitive selection among several possibilities. The advent of radioimmunoassay methods for measuring pituitary hormones very precisely has reduced error by eliminating the second, but not the first, biological response. I think at least some of what has been reported on the basis of double bioassays will not bear careful scrutiny using single bioassays.

Total elimination of a bioassay step, through development of radioimmunoassays for releasing factors, is of course desirable. However, some interesting calculations by Gay (Gay, 1972) are noteworthy. These calculations, based on substitution studies, suggest that the concentrations of releasing factor(s) in the hypothalamic-pituitary portal circulation would have to be 2 to 3 orders of magnitude below the limits of sensitivity of any known radioimmunoassay. In peripheral blood, they would be lower still. When this is considered together with the fact that there are no methods available for sampling portal venous blood in an unanesthetized animal, the chances for characterizing patterns of spontaneous releasing factor secretion by direct observation still seem very remote.

If I have drawn too dismal a picture, I am sorry. Schally's gift of GnRH is a great one, and a beautiful scientific achievement. To the physiologist, it is a find comparable to the Rosetta stone, without which the system depicted in Fig. 2 could never be understood. To the physician, it may prove to be a useful diagnostic tool and perhaps even, ultimately, to have some therapeutic value. But its primary value is that of the key to an unsolved puzzle. While the key may now be at hand, the solution(s) to the puzzle must still be worked out.

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