adrenal response to ACTH and suggested that zinc was required for pituitary-adrenal function.

We tested the relationship of stress, ACTH, corticosterone, and four elements in 18 adult, male Sprague-Dawley rats (300 to 425 g), divided into control, stress, and stress-recovery groups of six each. By a corticosterone-based bioassay in 18 additional rats (*2*), ACTH was determined in the pituitaries of the test animals; corticosterone was measured fluorometrically in the rat adrenals (*3*); and four elements (calcium, copper, magnesium, and zinc) in serum were measured by atomic absorption spectroscopy. All animals were anesthetized with pentobarbital sodium (3 mg/kg), and a catheter was inserted into the right carotid artery for withdrawal of blood. Oligemic hypotension, a maintained mean arterial pressure of 50 mm-Hg for 1 hour, was used as stress; the 2 hours after reinfusion was stress recovery. The rats were then decapitated, the pituitary and both adrenals were removed, and homogenates were prepared.

The severity of the oligemic stress episode was reflected in the elevated ACTH levels of approximately twice the control values, which quickly responded during the stress recovery by dropping to levels below control values. The activity of these changing ACTH concentrations was monitored by adrenal corticosterone changes. The ACTH activity was evident in the rise and fall of corticosterone levels during stress and stress recovery (see Fig. 1).

Blood serum zinc was the only one of the mineral elements determined that responded with a significant increase during stress. The zinc levels increased sharply during the oligemic stress episode, whereas control levels of calcium, copper, and magnesium either remained the same or decreased during stress. Figure 1 illustrates the changes in ACTH and elemental levels during stress and stress recovery. Unlike calcium, copper, and magnesium, which varied little during stress recovery, zinc again followed the pattern of ACTH and decreased during recovery.

A Pearson r correlation was computed on the basis of 18 levels for each of the four elements, each level being correlated with the corresponding rat ACTH. The correlations were made to determine if a relationship existed between one or more of the elements commonly associated with stressful states. Zinc had the greatest correlation of the four elements ($r = 0.85$), whereas calcium ($r = -0.22$), copper ($r = 0.14$), and magnesium ($r = 0.16$) correlated with ACTH to a far lesser extent.

The action of ACTH on the adrenal cortex during a traumatic experience has long been established, but little has been said about the involvement of calcium, copper, magnesium, and zinc in stress. The delicate balance of ACTH release is well demonstrated by the relationship of corticosterone levels to ACTH activity. During the stress-recovery period, the corticosterone levels remained elevated over control values, which suppressed the mechanism for ACTH production. The relationship of zinc to ACTH as illustrated in Fig. 1 strongly suggests some type of physiological or coupling action on the part of zinc during stress. The control values for zinc of 150 μg/100 ml were within the normal range for rat serum, as given by Luecke *et al.*, of approximately 147 μg/100 ml (*4*). The remaining three trace elements, calcium, copper, and magnesium, showed little relationship to ACTH responses in stress.

The discussion by Sandstead *et al.* of the Egyptian dwarfs did not clearly define any role for zinc in ACTH (*1*). Zinc, as $Zn_3(PO_4)_2$ or $Zn(OH)_2$, has been found, however, to increase and prolong the physiological action of ACTH (*5*). In the Trace Element Center, a relatively high zinc level was found in two commercial ACTH preparations (*6*). Whether the role is functional or passive, zinc does appear to be linked with the rise of ACTH, which strongly correlates with a stress situation. None of the other three elements determined (calcium, copper, or magnesium) related to the changes in the adenohypophyseal-adrenal cortex function and stress. Further studies are needed to unravel this correlation and zinc's association with ACTH.

ARTHUR FLYNN
WALTER J. PORIES
WILLIAM H. STRAIN
ORVILLE A. HILL, JR.
Trace Element Center, Cleveland Metropolitan General Hospital, Case Western Reserve University School of Medicine, Cleveland, Ohio 44109

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6. Commercial ACTH preparations tested for zinc levels: (i) corticotropin (Nutritional Biochemicals; injectable; bovine; 40 unit/ml; zinc, 396 parts per million); (ii) Acthar [40 units (lyophilized); mixed 40 unit/ml in double-distilled, deionized H_2O; zinc, 112 parts per million].
7. Work supported in part by a grant from the Cleveland Foundation.

3 May 1971

Gonadotropin-Releasing Hormone: One Polypeptide Regulates Secretion of Luteinizing and Follicle-Stimulating Hormones

Abstract. *A polypeptide isolated from porcine hypothalami stimulates the release of both luteinizing hormone and follicle-stimulating hormone from the pituitaries of several species. This polypeptide has been structurally identified as (pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 and synthesized. The natural and synthetic materials share biological properties. It appears that this peptide represents the hypothalamic hormone regulating the secretion of both luteinizing hormone and follicle-stimulating hormone.*

The hypothalamus controls the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (*1*). The work of various investigators clearly demonstrated that there are, in hypothalamic extracts of animals, including man, substances, or one substance, capable of stimulating release of LH and FSH from the pituitary (*1, 2*). Much evidence also exists that sex steroids are involved in this regulation. Initially, it was thought that two different substances designated luteinizing hormone-releasing hormone (LH-RH) and follicle-stimulating hormone-releasing hormone (FSH-RH) were responsible for stimulating release of LH and FSH, respectively (*1*). However, it became necessary to question this belief when porcine LH-RH, obtained in a

high state of purity, stimulated release of both LH and FSH in rats, chimpanzees, and human beings (*3–5*). Furthermore, after the addition of purified porcine LH-RH to an incubation system in vitro in which pituitaries of male rats were used, LH and FSH were released simultaneously with superimposable time courses (*3*). Stimulation of LH release by this material was also unequivocally established in sheep (*6*) and rabbits (*7*), but because of the unavailability of specific radioimmunoassays, it was difficult to test for the effect on FSH secretion in these two species.

It was not clear at first whether this FSH-releasing activity of porcine LH-RH was an intrinsic property of its molecule or whether it was due to a contamination with FSH-RH. Chemical or enzymatic inactivation of LH-RH was always accompanied by loss of FSH-releasing activity (*3, 4*). Moreover, during the fractionation of pig hypothalamic extracts, the location of FSH-RH activity always coincided with that of LH-RH (*8*). After porcine LH-RH was isolated in an essentially homogeneous state by a combination of 12 different purification steps or by countercurrent distribution, it was found that it too stimulated the release of FSH in vivo and in vitro in doses smaller than 1 ng (*8*). Further fractionation by partition chromatography in ten different solvent systems (*8*) did not result in separation of the LH-RH activity from the FSH-RH activity. In addition to stimulating the release of both LH and FSH, the pure natural LH-RH/FSH-RH significantly augmented the synthesis of these two pituitary hormones in tissue cultures (*9*), an effect that had been obtained with LH-RH of lesser purity (*10*).

As a result of the foregoing studies, we postulated that the isolated polypeptide represents the hypothalamic hormone that controls the secretion of both LH and FSH from the pituitary (*8*). However, we could not exclude the possibility, even by the use of most advanced fractionation methods, that two peptides with an identical amino acid composition and molecular weight but with a different amino acid sequence were present in this material, which we had assumed to be homogeneous. This final doubt was removed as a result of the recent determination of the structure of the hypothalamic polypeptide with both LH-RH and FSH-RH activities, but only one amino acid sequence: (pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (*11, 12*). This structure was confirmed by synthesis (*12*). When the synthetic LH-RH/FSH-RH was tested for its effect on the release of LH and FSH in vivo and in vitro, it showed the same spectrum of biological activities as the pure natural material. Thus, synthetic LH-RH/FSH-RH raised the concentration of LH in the plasma in ovariectomized rats that had received prior treatment with estrogen and progesterone (*8, 12*). Synthetic LH-RH/FSH-RH increased plasma FSH levels, as well as LH, in castrated male rats pretreated with testosterone propionate. A direct effect on the anterior pituitary gland was proven by stimulation of FSH and LH release in vitro from the pituitaries of male rats (*8, 12*). An example of these results is shown in Table 1. The FSH released was measured by the Steelman-Pohley assay (*13*). This system is considered at present the most specific for measuring stimulation of FSH release by hypothalamic materials (*14*). Furthermore, synthetic LH-RH/FSH-RH stimulated both the release and synthesis of FSH, as well as LH, in tissue cultures of rat pituitaries (*9, 10*). Preliminary results indicate that synthetic decapeptide also increased the plasma LH and FSH in humans (*15*). The response of one of these subjects can be seen in Fig. 1.

Table 1. The effect of natural and synthetic LH- and FSH-releasing hormones (LH-RH/FSH-RH) on the stimulation of release of FSH from rat pituitaries. Ten pituitary halves of male rats were incubated in 10 ml of Krebs-Ringer bicarbonate medium for 6 hours (*14*). The FSH released was measured by bioassay (*13*), and it is expressed in terms of NIH FSH-S-4 units.

Group	Addition	Dose (ng/ml)	Ovarian weight (mg ± S.E.)	P*	FSH (ng/ml)
1	Control		45.0 ± 2.6		19.7
2	Natural LH-RH/FSH-RH	2	77.9 ± 6.9	.005	29.7
3	Synthetic LH-RH/FSH-RH	2	71.4 ± 10.2	.05	27.2
4	Natural LH-RH/FSH-RH	8	93.6 ± 11.4	.005	35.9
5	Synthetic LH-RH/FSH-RH	8	86.8 ± 9.4	.005	33.4

* Student t-test.

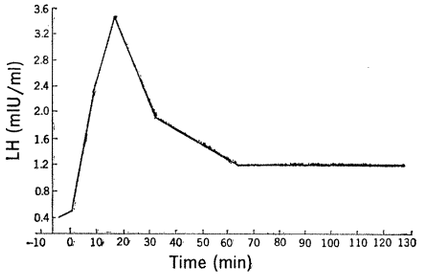

Fig. 1. Effect of an intravenous administration of an equivalent of 1.5 μg of synthetic LH-RH/FSH-RH in plasma LH concentrations in a woman who had been treated for 15 days with the oral contraceptive preparation Lyndiol. Plasma LH concentrations were measured by radioimmunoassay (*16*) and are plotted as milli-international units (*mIU*) of the 2nd IRP-HMP (second international research preparation of human menopausal gonadotropin) per milliliter of serum.

In summary then, it appears that the natural material purified from porcine hypothalami and the synthetic substance with the same structure represent a polypeptide that exerts a specific and profound effect on the pituitary secretion of hormones regulating the reproductive cycle. The overall control of FSH and LH secretion and the preferential release of one or the other of these gonadotropins may be mediated by the interplay of the hypothalamic polypeptide and sex steroids.

The availability of this hypothalamic hormone and its analogs should prove useful for studies on the enhancement or inhibition of fertility.

A. V. Schally, A. Arimura
A. J. Kastin, H. Matsuo, Y. Baba
T. W. Redding, R. M. G. Nair
L. Debeljuk
Veterans Administration Hospital and
Tulane University School of Medicine, New Orleans, Louisiana 70140
W. F. White
Abbott Laboratories, North Chicago, Illinois

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Marihuana and Memory: Acquisition or Retrieval?

Abstract. *Two experiments were conducted to determine the means by which marihuana affects human memory. The results of these studies indicated that marihuana did not affect retrieval of information in memory when the method of free recall was used, but did affect recognition processes such that subjects were less able to discriminate between items that had been presented previously and items that had not appeared a short time before. With respect to initial learning, marihuana was shown to affect acquisition processes involved in the storage of information.*

Marihuana has been shown to have deleterious effects on human memory (*1*). The actual process by which this occurs, however, has not as yet been determined. For instance, marihuana may interfere with either acquisition of information, or storage of acquired information, or retrieval of stored material, or any combination of these processes. An earlier study (*2*) failed to detect any effect of marihuana on retrieval, thereby suggesting that acquisition or storage processes were being affected. The following studies were designed to investigate this possibility.

The first of the present studies constitutes a replication and extension of work by Abel (*2*) wherein marihuana was found to have no significant effect on the retrieval of information already present in memory. Forty-nine adult males and females served as either marihuana, placebo, or control subjects. Assignment of subjects was similar to that previously described (*2*), the only provision being that subjects that had not used marihuana previously were placed in either the control or the placebo condition. Subjects that were familiar with the effects of marihuana were allocated to any of the three test conditions.

The design of the study was similar to that used by Cohen (*3*). Eighteen ten-item lists of words were read aloud at a rate of approximately 1.5 seconds per word. One minute was allowed for spoken free recall of a list immediately after its presentation. After completion of the initial free recall of the last list, the subject was presented with a list containing 60 words, 30 of which had appeared on the first three lists of the prior test, along with 30 new items, or "lures." The subject was asked to circle all those he thought were on the prior lists, and for each item circled he was to indicate how confident he felt about the accuracy of his response, using a 5-point scale similar to that used by Murdock (*4*). This task lasted approximately 8 to 10 minutes, and will be referred to as the immediate recognition test.

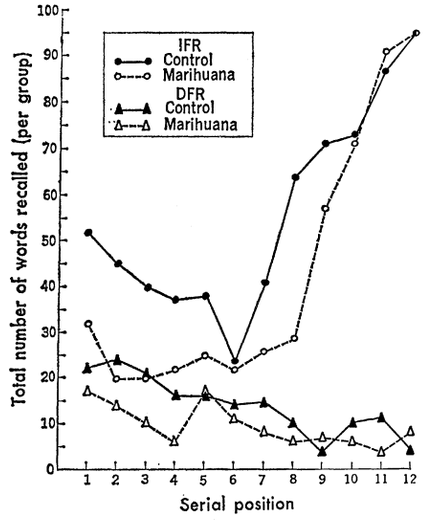

Fig. 1. Free recall as a function of serial position; *IFR*, initial free recall; *DFR*, delayed free recall.

Upon completing the task, subjects in the marihuana group were allowed to smoke one marihuana cigarette, the tetrahydrocannabinol content of which had not been determined. Subjects in the placebo group were given a cigarette containing ordinary tobacco, but were told that it had been dipped in tetrahydrocannabinol, the active ingredient in marihuana, and that as a result it would "taste and smell like tobacco, but the psychological effect will be like that of smoking marihuana." The smoking period lasted approximately 5 to 10 minutes. Control subjects were left undisturbed during this period.

Immediately after the smoking period, the experimenter made a pretense of testing "concept formation" and administered the Block Design Test and the Picture Arrangement Test —two subtests of the Wechsler-Bellevue Intelligence Test (*5*). These tests were conducted for purposes of occupying the subjects with some distracting task in the time period between the end of the initial free recall test and the start of the next phase of the study. Twenty-five minutes after the initial free recall test, subjects in the marihuana and placebo conditions were given a 10-point rating scale and were asked to rate how "high" they felt at that moment (1, not high at all; 3, slightly high; 5, moderately high; 8, very high; 10, extremely high). The subjects were then given 5 minutes to write out as many words as they could remember from the prior lists (this task being delayed free recall). After this, a second recognition task was administered (delayed recognition). The test lists in this second recognition test contained 300 items, 150 items from the last 15 lists and 150 lures, none of which had been used in the first recognition test.

Only marihuana subjects who rated themselves at 5 or more were included in the analysis of the data. The marihuana subjects were then matched by inspection with an equal number of subjects in the control and placebo groups on the basis of their scores on the initial free recall test ($N = 13$ for each group). The words used in the initial free recall and recognition tests were selected from the lists in Thorndike and Lorge (*6*). One-third were high-frequency words (A and AA), one-third were of low frequency (five or fewer occurrences per million), and