Stimulation of Human Prolactin Secretion by Intravenous Infusion of L-Tryptophan

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ABSTRACT Previous studies have demonstrated that the secretion of human prolactin is regulated primarily by factors that influence catecholamines of the hypothalamus. In an effort to identify other factors that may regulate prolactin secretion, the amino acid L-tryptophan, a precursor in the synthesis of serotonin, was infused into normal human volunteers. Intravenous infusion of L-tryptophan, 5–10 g over a 20 min period, but not equivalent amounts of 17 other amino acids, induced marked increases in serum prolactin concentrations in eight normal human volunteers. Increases of 20–200 ng/ml above the control level were observed with peak values at 20–45 min after initiation of the infusion. In addition, infusion of L-tryptophan was associated with decreases in serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyrotropin in those subjects in whom the base-line serum hormone concentration was above the lower limits of assay detectability. No consistent change was observed in serum concentrations of growth hormone, cortisol, or glucose. Four subjects with juvenile diabetes demonstrated increases in serum prolactin values comparable with those observed in healthy individuals in response to infusions of L-tryptophan. Serum prolactin values in patients with surgically induced hypopituitarism were undetectable or deficient after infusion of 10 g of L-tryptophan. In this respect, infusion of L-tryptophan was equally effective in these subjects as the standard chlorpromazine stimulation test in identifying patients with hypopituitarism, indicating that the infusion of L-tryptophan may serve as a sensitive and reliable clinical test of prolactin secretory reserve. Further studies relating to the possible mechanism of action of L-tryptophan indicated that infusion of 5-hydroxytryptophan represents a much more potent stimulus for the secretion of prolactin and that premedication with the serotonin antagonist, methysergide maleate, serves to blunt the effect of L-tryptophan on prolactin secretion. These results support the concept that the effect of L-tryptophan on the secretion of human prolactin is mediated through its conversion to serotonin and are consistent with reported experimental observations that serotonin may participate in the reciprocal regulation of prolactin and gonadotropins.

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INTRODUCTION

Recent studies on human prolactin have indicated the primary importance of hypothalamic mechanisms in the regulation of secretion of this hormone by the pituitary (1–3). A large body of experimental observations in animals and of pharmacological studies in the human has indicated the presence of catecholaminergic mechanisms in the hypothalamic regulation of prolactin secretion. In these studies we report an additional substance that can reciprocally regulate the secretion of prolactin and gonadotropins, the amino acid L-tryptophan. Effects observed with the intravenous infusion of this amino acid appear to relate to the presence of a serotonergic pathway mediating neuroendocrine regulation of prolactin secretion (4, 5) and provide an additional means of probing the human hypothalamic-pituitary axis.

METHODS

Materials. Reagent grade amino acids were purchased from Sigma Chemical Co., St. Louis, Mo. The amino acids were prepared for intravenous administration by dissolving 5–30 g in 0.15 M sodium chloride (50–150 ml) by titration with sodium hydroxide to a final pH of 8.5–9.5. The solutions were sterilized by passage through a 0.45 μm millipore filter and were administered shortly after preparation by intravenous drip over a 20 min period. These intravenous solutions were not tested for pyrogenicity.

Methods. Serum prolactin concentrations were measured by an in vitro bioassay technique, as previously described (6). This technique measures the concentration of prolactin activity above the control activity represented by pooled serum derived at midmorning from normal males under resting conditions. All sera were examined at a single concentration, 50% in the culture medium. The sensitivity of these assays was 2 ng/ml.

Intraassay variability in these studies was somewhat greater than in previous studies and was represented by standard deviations of 20–25% over the range of activity reported. The results are reported as activities equivalent to an ovine prolactin standard (NIH Endocrinology Study Section, ovine prolactin. PS-9, 30.3 IU/mg). Other pituitary hormones were measured by standard radioimmunoassay techniques (7–10). Serum cortisol was measured by a competitive binding technique (11). Serum glucose was measured by a micromethod employing glucose oxidase.

Subjects. All patients were studied on the Clinical Research Unit. Patients were admitted to the unit at least one night before the testing, were kept fasting after midnight, and were tested only after obtaining their informed consent in writing. Infusion of amino acids or other pituitary tests were begun at 9:00 a.m., 1–2 h after the patients had awakened. Normal subjects were age 18–40 yr. All diabetic subjects had long-standing, juvenile diabetes. Hypopituitary patients were diabetic subjects who had been subjected several months previously to surgical hypophysectomy for rapidly advancing diabetic retinopathy. Growth hormone was undetectable in the sera of such subjects during insulin-induced hypoglycemia; they experienced severe adrenal insufficiency upon withdrawal of cortisone acetate medication or exhibited no rise in urinary 17-hydroxysteroid excretion during metyrapone testing. Creatinine clearance in all diabetic subjects was greater than 50 ml/min. None of the patients had ingested tranquilizers or antihypertensive drugs for at least 3 wk before study, although several of the normal female subjects were taking birth control pills. Venous blood was collected through an indwelling needle, and serum was rapidly separated for immediate assay or was stored at −30°C for 1–2 wk before assay.

RESULTS

The intravenous infusion of solutions of L-tryptophan (5–10 g) was generally well tolerated in all subjects. Occasional subjective feelings of light-headedness, flushing, or slight pain at the site of infusion were transient and often controlled by decreasing the rate of flow of the intravenous solution. The effect of intravenous infusion of L-tryptophan on serum prolactin concentrations is shown in the results depicted in Fig. 1. Serum concentrations of prolactin rose after an initial and variable lag period, reaching maximal values between 20 and 40 min after the beginning of infusion. All normal subjects tested consistently demonstrated elevations in serum prolactin concentrations after the infusion of 10 g of L-tryptophan. The majority of these subjects also responded to the infusion of as little as 5 g of L-tryptophan, whereas infusion of 4 g of L-tryptophan or less failed to produce consistent stimulations of prolactin secretion. The specificity of the observed effect of L-tryptophan stimulation was further tested by monitoring serum concentrations of other pituitary hormones. Fig. 2 shows the effect of infusion of L-tryptophan on serum concentrations of growth hormone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). No consistent effect upon serum levels of growth hormone was noted.

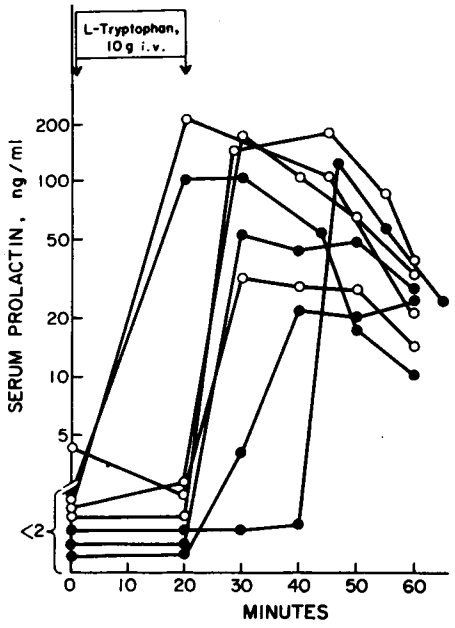

FIGURE 1 Effect of intravenous infusion of a solution containing L-tryptophan, 10 g, on the serum prolactin concentrations in eight normal subjects. Open symbols, females; closed symbols, males.

In a majority of those subjects in whom the serum concentrations of LH and FSH were above the lower limits of the assays (5 mIU/ml), a significant decrease in the levels of these hormones was observed after infusion of

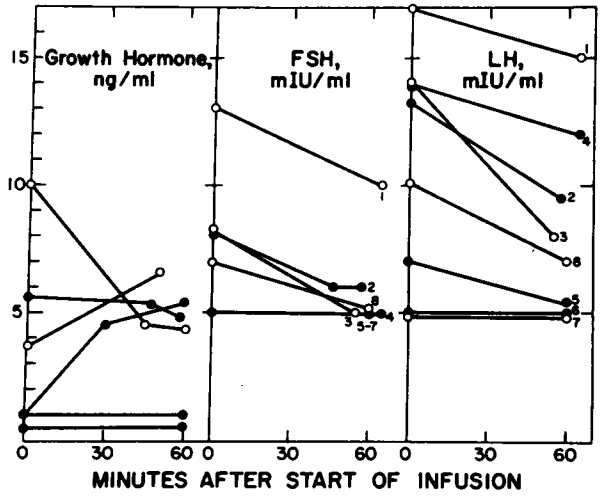

FIGURE 2 Effect of intravenous infusion of L-tryptophan, 10 g intravenously, on the serum values for growth hormone, FSH, and LH. Open symbols, females; closed symbols, males. Numbers for the values of FSH designate a specific patient and correspond to the numbered values for the same patient in the chart of LH values.

TABLE I

Effect of Intravenous Infusion of L-Tryptophan (10 g) on the Serum Concentration of TSH in Eight Normal Subjects

Subject	Sex	TSH, μU/ml	
		Control	Prolactin peak
R. S.	F	7.7	<2
N. F.	F	3.3	<2
C. H.	F	2.3	<2
P. W.	F	4.7	<2
T. S.	M	<2	<2
J. F.	M	<2	<2
W. B.	M	<2	<2
T. P.	M	<2	<2

L-tryptophan. The effect of L-tryptophan infusion upon serum thyroid-stimulating hormone (TSH) levels is shown in the results in Table I. During the peak effect on prolactin secretion there was no stimulation of TSH secretion. Furthermore, in all subjects in whom the serum concentration of TSH was measurable at the beginning of the experiment, infusion of L-tryptophan depressed serum TSH concentrations to below detectable levels. Monitoring of serum cortisol during the first 60 min period after L-tryptophan infusion revealed no consistent change in serum cortisol concentrations. Serum glucose concentrations were also monitored during each experiment in order to determine whether increases in serum prolactin concentrations could be secondary to changes in availability of glucose. As shown in Fig. 3, no significant changes in serum glucose concentrations were observed in any patient. In order to determine the specificity of L-tryptophan for stimulation of prolactin secretion, 5, 10, or 30 g of 17 other amino acids including the D-stereoisomer of tryptophan were infused into normal subjects. Table II shows the results of screening the activities of these amino acids alone or in combinations. No increases in serum prolactin concentrations were noted after the infusion of any of these substances, and these individual amino acids were not studied further.

It has been reported previously that a deficient growth hormone secretory response is observed in certain diabetic subjects after infusion of the amino acid, arginine (12). In order to determine the effect of intravenous infusion of L-tryptophan in the presence of diabetes, 10 g of L-tryptophan was administered intravenously to four diabetic subjects. As shown in Fig. 4, all diabetic patients exhibited increases in prolactin concentrations which paralleled those found in normal subjects under the same conditions.

The effect of an infusion of L-tryptophan upon prolactin secretion in patients who had been subjected to surgical hypophysectomy was also studied. The prolactin secretory responses to an infusion of L-tryptophan and responses in the same patients after treatment with chlorpromazine are depicted in Fig. 5. Similar prolactin secretory responses were noted with each testing procedure, with three of the four patients with surgically induced hypopituitarism showing no response to either test and the fourth showing a deficient but detectable rise in prolactin secretion.

In order to determine whether the stimulatory effect of L-tryptophan on prolactin secretion could be mediated by a metabolic conversion product of this amino acid, the prolactin secretory responses to L-tryptophan and to 5-hydroxy-L-tryptophan were compared in a single subject, as shown in Fig. 6. Although the amount of 5-hydroxy-L-tryptophan infused was only 1/20th that of the L-tryptophan load, this amount of the 5-hydroxy metabolite induced an equal or somewhat greater secretion of prolactin. Since 4 g of L-tryptophan did not significantly increase the serum prolactin secretion in other subjects, this result indicates the greater potency of 5-hydroxy-L-tryptophan on prolactin secretion. The infusion of this latter material was terminated after 5 min, however, since it subsequently induced an episode of shaking chills followed by a psychomotor seizure after 40 min and postictal loss of memory for several hours. Because of this reaction, therefore, the response in prolactin secretion may represent a more widespread effect on central nervous system activities than occurred with L-tryptophan. The prolactin activity induced by both agents was neutralized specifically by guinea pig antiovine prolactin antiserum (6). Since L-tryptophan may be converted to serotonin in the central nervous system, the response of prolactin secretion to an L-tryptophan load was compared before and after medication of subjects with the competitive antagonist of serotonin, methysergide maleate (Fig. 6). Before treatment with this drug,

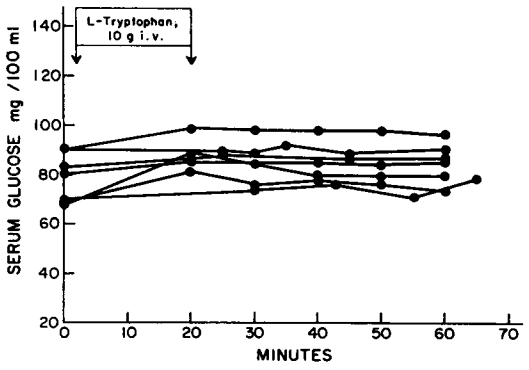

FIGURE 3 Values of the serum glucose concentration during the standard L-tryptophan infusion test in normal subjects.

TABLE II

Serum Prolactin Levels in Normal Subjects after Intravenous Infusion of Various Amino Acids

Subject	Amino acid	Amount	Serum prolactin Min after start of infusion 0	20	30	40	50	60
		g	*ng/ml above control*					
1	L-Tryptophan	10	<2	<2	50	48	50	30
	D-Tryptophan	10	<2	<2	<2	<2	<2	<2
2	L-Tryptophan	10	<2	<2	125	100	60	40
	D-Tryptophan	10	<2	—	8	<2	<2	<2
3	L-Tryptophan	5	<2	<2	40	38	38	12
	L-Aspartic acid	5						
	L-Glutamic acid	5						
	L-Methionine	5	<2	<2	<2	<2	<2	<2
	L-Serine	5						
	L-Proline	5						
4	L-Tryptophan	5	<2	<2	4	—	26	38
	L-Valine	5						
	L-Alanine	5	<2	6	<2	<2	<2	<2
	L-Glycine	5						
	L-Threonine	5						
5	L-Tryptophan	5	<2	110	160	100	60	44
	L-Leucine	5						
	L-Phenylalanine	5						
	L-Tyrosine	5	<2	<2	<2	<2	<2	<2
	L-Isoleucine	5						
	L-Histidine	5						
	L-Lysine	5						
6	L-Arginine	30	<2	<2	<2	<2	—	<2
7	L-Arginine	30	<2	<2	<2	<2	—	<2

Amino acids were prepared and administered individually or in the indicated mixtures over a 20 min period as described in "Materials." Sera tested after infusion of L-arginine were treated with guinea pig anti-human growth hormone antiserum before assay (6).

infusion of L-tryptophan induced a maximal rate of prolactin secretion after 20 min from the start of the infusion. After pretreatment with methysergide, the infusion of L-tryptophan caused a secretion of prolactin which was delayed in onset and which slowly rose to a maximal value at 60 min or later. Similar results were obtained in a second subject.

DISCUSSION

The results of these studies indicate that intravenous infusion of L-tryptophan exerts a highly specific stimulatory effect upon the secretion of human prolactin. In addition, infusion of this amino acid was associated with a mild inhibitory effect upon the normal serum concentrations of FSH, LH, and TSH. This effect could not be mediated by a reported inhibitory effect of L-tryptophan on gluconeogenesis (13), since the serum concentrations of glucose were unaltered during the period of increased prolactin secretion. It is unlikely that they occurred secondary to an increased secretion of insulin, since an equivalent response was consistently induced in juvenile diabetics. For the amounts of amino acid infused, the effect of L-tryptophan also appeared to be relatively limited to this amino acid, since the infusion of 17 other amino acids in equivalent concentration failed to elicit increased rates of prolactin secretion. Recently, it has been reported that infusion of much larger amounts of arginine, leucine, and phenylalanine (0.2–0.5 g/kg) into the carotid artery of the ewe induced significant increases in prolactin secretion (14), and it is possible that much larger amounts of other amino acids could cause significant increases in prolactin secretion in the human. The possibility should also be recognized that the activity of an individual amino acid may have been ob-

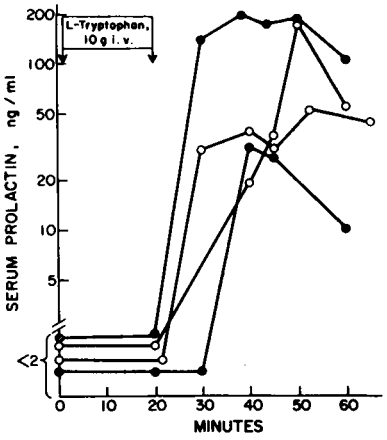

FIGURE 4 Effect of the infusion of L-tryptophan, 10 g intravenously, upon the serum prolactin concentrations of four juvenile diabetic patients. Open symbols, females; closed symbols, males.

scured by other amino acids in the infused mixtures, and that further analysis may reveal intrinsic prolactin-releasing activity, although of lesser degree than that of L-tryptophan, in other amino acids.

The stimulatory effect of intravenous infusion of L-tryptophan has been observed consistently in normal subjects, and the results of this study indicate that it may represent a highly reliable test for pituitary secretory reserve. In four subjects shown in this study, deficient pituitary reserve was identified by a standard treatment with L-tryptophan as well as by a standard test with chlorpromazine (15, 16). Infusion of 5-hydroxy-L-tryptophan represented a more potent stimulus to prolactin secretion in a single subject in these studies. Recently Lu and Meites (5) have observed that tryptophan and 5-hydroxy-L-tryptophan represent potent stimuli for prolactin secretion in the rat and that 5-hydroxy-L-tryptophan represents a much more potent stimulus. However, it is the opinion of the authors that the single adverse reaction to 5-hydroxy-L-tryptophan observed in the present studies makes it inadvisable to use this substance in the dosage employed here in further clinical testing. Although the range of responses characteristic of normal subjects and of patients with hypopituitarism appear to define values for normal pituitary function, it should be realized that these responses have been standardized in this laboratory with respect to a specific bioassay technique, and the application of this test to evaluate pituitary function by radioimmunoassay or in other laboratories may require standardizing the range of normal response observed with other techniques.

The specific biochemical mechanisms by which L-tryptophan induces the secretion of prolactin and suppresses the secretion of FSH, LH, and TSH in certain patients remains to be elucidated. However, a scheme of biochemical pathways through which this effect may be exerted may be tentatively proposed based upon the observations in these studies. This scheme is shown in Fig. 7. It is likely that the effect of L-tryptophan is mediated through its conversion to its metabolic product, 5-hydroxy-L-tryptophan. This substance was a much more potent influence on prolactin secretion in the rat

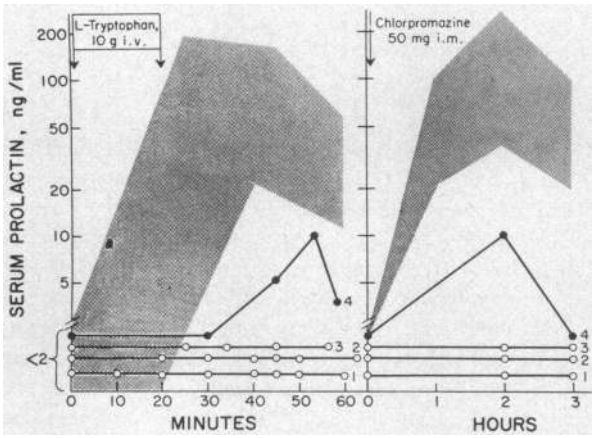

FIGURE 5 Effect of the intravenous infusion of L-tryptophan or intramuscular injection of chlorpromazine on the serum prolactin concentrations of four patients with hypopituitarism after surgical hypophysectomy. Open symbols, females; closed symbols, males. The chlorpromazine test was performed three days after treatment with L-tryptophan. The shaded areas represent ranges of response observed in eight normal subjects tested under similar conditions.

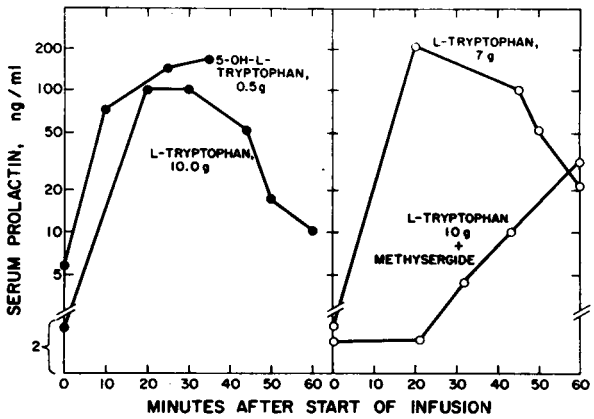

FIGURE 6 (Left) Comparison of the effects of infusion of L-tryptophan, 10 g intravenously over a 20 min period, or 5-hydroxy-L-tryptophan, 0.5 g intravenously over a 5 min period, in a single male patient. (Right) Comparison of the effect of intravenous infusion of L-tryptophan in a single female patient before and after 5 days of medication with methysergide maleate, 4 mg orally every 6 h. The final dose of methysergide was administered 2 h before the infusion.

(5) as well as in one human subject. Furthermore, it is generally recognized that the rate of serotonin formation in the central nervous system depends upon the degree of substrate saturation of the rate-limiting enzyme, tryptophan hydroxylase (17). It is likely that the effect of 5-hydroxy-L-tryptophan is primarily mediated by its subsequent conversion to serotonin. Kamberi, Mical, and Porter have demonstrated that the intraventricular instillation of serotonin in the rat induces increased concentrations of prolactin in the peripheral plasma and a decrease in the plasma concentrations of gonadotropins (4). Infusion of serotonin into the pituitary portal veseles did not release prolactin, indicating that this substance acts within the hypothalamus rather than on the pituitary (4). The observations in these studies, that methysergide, a competitive antagonist of serotonin, can inhibit the effect of L-tryptophan is consistent with the role of serotonin proposed in Fig. 7. However, it is likely that methysergide may have additional actions and that its effect in the central nervous system has not been completely characterized. Although the thyrotrophin-releasing factor of the hypothalamus has been shown to release prolactin from the human pituitary (18, 19), it is unlikely that this factor can be implicated in the mode of action of L-tryptophan, since a parallel increase in TSH secretion was not observed in these studies. It is thus proposed that the only other known regulator of prolactin secretion, the prolactin-inhibitory factor as identified by physiological studies, may be involved in this regulation. However, it should be noted that isolation of this material from the human hypothalamus has not yet been reported. The present results also cannot exclude the possible participation of other unidentified factors, such as an active prolactin-releasing factor, in the effect of L-tryptophan. It is likely that the concomitant fall in gonadotropin secretion observed after infusion of L-tryptophan results from a decrease in the release of the FSH and LH-releasing hormones from the hypothalamus (20, 21).

It has been proposed by Kamberi et al. (4) that the secretion of prolactin in the rat may be regulated by a balance between two competitive pathways: an inhibitory, catecholaminergic pathway; and a stimulatory, serotonergic pathway. These workers also hypothesized that an imbalance in these two hypothalamic pathways could result in "functional" hyperprolactinemia and hypogonadotropinemia associated with galactorrhea and amenorrhea in the human. The results of the present studies suggest the presence of a serotonergic pathway in the human hypothalamus for the reciprocal regulation of secretion of prolactin and gonadotropins, and thus lend further support to this proposal. It has been shown in studies in the rat that dietary amounts of L-tryptophan may alter brain serotonin content (22) and that the rate

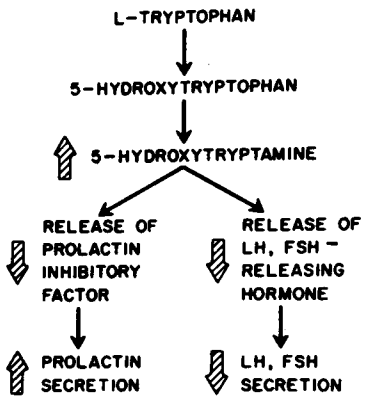

FIGURE 7 Proposed metabolic pathway through which intravenously administered L-tryptophan may affect the secretion of prolactin, LH, and FSH.

of uptake of tryptophan of dietary origin may be further modified by certain neutral amino acids (23). Since the present studies report only the effects of intravenously administered L-tryptophan, it remains to be determined whether dietary amounts of L-tryptophan or administration of L-tryptophan by other routes may alter pituitary secretion of prolactin.

ACKNOWLEDGMENTS

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