Elevation of Plasma Tryptophan by Insulin in Rat

By J. D. Fernstrom and R. J. Wurtman

The concentration of tryptophan in rat plasma increases by 30–40% 2 hr after fasting animals receive insulin (2 U/kg) or consume a carbohydrate meal. These treatments decrease the concentrations of all other amino acids examined: Insulin administration also lowers plasma glucose levels. Large doses of glucagon (1 mg/kg) reduce plasma tryptophan concentrations. Lower doses are without a significant effect. The sources of plasma tryptophan responsible for its increase after insulin administration have not been identified.

INSULIN ADMINISTRATION[1,2] or the secretion of endogenous insulin[3,4] depresses the plasma concentrations of most amino acids in mammals. This effect is rapid and is associated with increased amino acid uptake into muscle and accelerated incorporation into protein.[5] Relatively little information is available on the effects of insulin on plasma tryptophan. A few reports have appeared showing that plasma trypotopan concentrations decrease in human subjects responding to a glucose load[6] and in dogs receiving insulin injections,[2] and increase in alligators after insulin injection.[7]

The factors influencing tryptophan distribution and metabolism are of special significance considering this amino acid's scarcity in most foods[8] and its unique role in several important physiologic functions. Tryptophan is a precursor of the cofactor NAD,[9] the plasma concentration of tryptophan appears to influence the level of serotonin, a putative neurotransmitter, in the brain,[10] and the delivery of dietary tryptophan to the liver via the hepatic portal vein determines the extent to which hepatic polysomes are aggregated[11] and the rate at which certain hepatic proteins are synthesized.[12]

We have utilized a sensitive fluorimetric assay for typtophan[13] to examine the effects of insulin on the concentration of this amino acid in rat plasma.[14] Tryptophan differs from all other amino acids studied to date in that its concentration in rat blood rises after injection of insulin or ingestion of a carbohydrate load.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass.) weighing 150–200 g were housed four per cage and exposed to light (40–60 μw/s cm; Vita-Lite, Duro-Test Corp., North Bergen, N.J.) between 9:00 a.m. and 9:00 p.m. daily. All rats

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were acclimated to our animal quarters for at least 10 days prior to an experiment. During this time food (Big Red Laboratory Animal Food, Agway, Inc., Syracuse, N.Y.) and water were available ad libitum.

At 9:00 p.m. on the evening before an experiment, the rats were placed in clean cages and deprived of food. Between noon and 3:00 p.m. the next day, groups of six to ten animals received i.p. injections of 0.1–0.3 ml of crystalline insulin (Iletin, 40 U/ml; Eli Lilly & Co., Indianapolis, Ind.) diluted in water. Other groups of animals were injected i.p. with an aqueous solution of crystalline glucagon (provided by Dr. Ray W. Fuller, Eli Lilly Research Laboratories, Indianapolis, Ind.). The rats were decapitated at intervals after injection and blood from the cervical wound was collected in heparinized tubes and centrifuged. The plasma was separated and frozen until assay. Tryptophan was measured by the fluorimetric method of Denckla and Dewey[13] and blood glucose by a modified colorimetric method of Nelson.[15] The concentrations of other plasma amino acids were estimated using a Beckman model 120C amino acid autoanalyzer.

^{14}C-Cycloleucine (1-aminocyclopentane-1-carboxylic acid-carboxyl-^{14}C; New England Nuclear Corp., Boston, Mass.; specific activity, 5.74 MCi/mM) was administered i.p. in a dose of 8.5 μM/kg 24 hr before insulin injection. Plasma samples were deproteinized in 10% TCA and centrifuged, and the radioactivity in a 0.65-ml aliquot of the supernatant fluid was counted with a liquid scintillation spectrophotometer.[16] Data are presented as the mean ± SEM and analyzed statistically using Student's t test.

RESULTS

In initial experiments, rats were killed at intervals after receiving 2 U/kg of insulin i.p., and the concentrations of plasma tryptophan and glucose were measured. Within 1 hr of insulin administration, plasma tryptophan concentrations were elevated by 23%. By 2 hr they had attained levels 40% above those present in control animals ($p < 0.01$) (Fig. 1). Four hours after insulin injection, plasma tryptophan levels had returned to control values. Blood from rats killed 2 hr after reciving 1 U/kg of insulin also contained significantly elevated (28% $p < 0.01$) plasma tryptophan levels. Lower doses of insulin

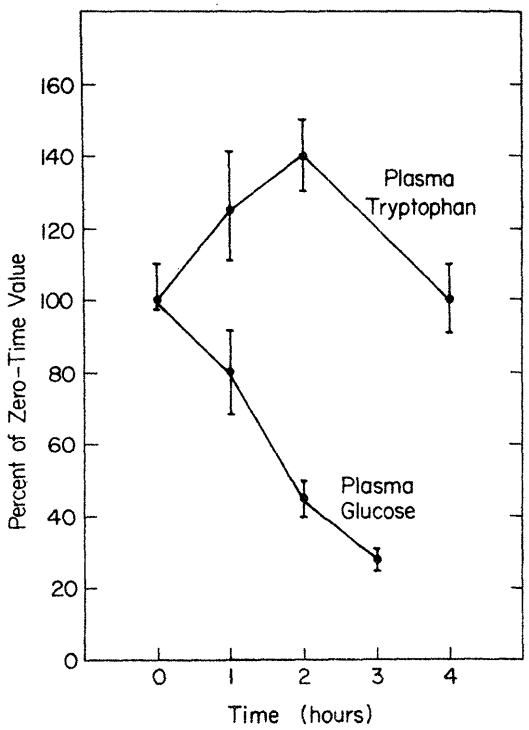

Fig. 1. Effect of insulin on concentrations of tryptophan and glucose in rat plasma. Groups of seven fasting male rats were killed at intervals after insulin injection (2 U/kg, i.p.). Plasma tryptophan concentration at zero time was 11.5 ± 1.5 μg/ml. Plasma glucose concentration was 96.0 ± 2.5 mg/100 ml. Vertical bars indicate SEM.

Table 1. Effect of Insulin on Concentrations of Tryptophan, Other Amino Acids, and Glucose in Rat Plasma*

Amino Acid	Concentration		Change (%)
	Control	Insulin	
L-Tryptophan	11.1 ± 0.6	16.6 ± 0.9	+50†
	(μg/ml)		
L-Tyrosine	11.4 ± 0.8	9.4 ± 1.0	−18
L-Phenylalanine	11.8 ± 0.3	10.8 ± 0.9	− 9
L-Serine	24.6 ± 1.2	17.0 ± 1.3	−31†
L-Glycine	29.6 ± 1.4	18.3 ± 2.0	−38†
L-Alanine	25.3 ± 0.9	11.7 ± 0.5	−54†
L-Valine	21.5 ± 1.3	17.3 ± 1.8	−20
L-Isoleucine	13.7 ± 0.8	7.1 ± 0.4	−48†
L-Leucine	21.3 ± 1.2	16.6 ± 2.1	−22
	(cpm/ml)		
^{14}C-Cycloleucine	162,000 ± 9500	122,000 ± 5200	−24†
	(mg/100 ml)		
Glucose	96 ± 2.5	43 ± 4.8	−55†

*Groups of five to ten fasting 150–200-g rats were killed 2 hr after receiving insulin (2 U/kg, i.p.).
†$p<0.01$.

only occasionally produced this effect. Doses of insulin that increased plasma tryptophan concentrations also elicited the expected decrease in plasma glucose levels (Fig. 1), as well as in the concentrations of all other plasma amino acids examined (Table 1). These doses also depressed plasma concentrations of the nonutilizable synthetic amino acid ^{14}C-cycloleucine (Table 1).

Insulin's effect on the tryptophan concentration of rat plasma was unrelated to its route of administration. Significant increases were observed 2 hr after animals received the hormone i.v., s.c., or i.p. (Table 2).

To determine whether the release of endogenous insulin similarly elevated plasma tryptophan while depressing other amino acid concentrations, tryptophan and tyrosine levels were measured in the plasmas of rats maintained on a 15-hr fast and for 1, 2, or 3 hr given free access to an agar-based carbohydrate diet consisting of 207 g dextrose, 220 g sucrose, 207 g dextrin, 150 g Mazola

Table 2. Relation Between Route of Administration and Plasma Tryptophan Response to Injected Insulin*

Route of Administration	Plasma Tryptophan (μg/ml)	Change (%)
Control	12.3 ± 0.97	
Intravenous	14.7 ± 0.45	+20†
Control	10.6 ± 0.37	
Subcutaneous	15.9 ± 0.89	+50‡
Intraperitoneal	15.6 ± 0.47	+48‡

*Groups of five to ten fasting 150–200-g rats were killed 2 hr after receiving insulin (2 U/kg).
†$p<0.05$ differs from control group.
‡$p<0.001$ differs from control group.

oil, 40 g Harper's salt,[24] 40 g agar, 4 ml choline (50% w/v), 10 g vitamin mix,[12] and 1000 g H_2O. The animals consumed an average of 5 g during the first hour and 2 g during each of the second and third hours. Plasma tryptophan concentrations rose significantly ($p < 0.02$) within 1 hr after food presentation and reached a peak 34% above control levels ($p < 0.001$) by the end of the second hour (Fig. 2). During this interval, plasma tyrosine concentrations fell by 33% ($p < 0.001$). The ratio of tryptophan to tyrosine increased more than twofold. This experiment also demonstrated that the increase in plasma tryptophan after insulin administration is not a result of the hypoglycemia caused by the insulin.

To examine the possibility that the changes in plasma tryptophan after insulin administration or carbohydrate ingestion are mediated by the release of glucagon from the pancreas, tryptophan levels were measured in fasting rats killed 1, 2, or 3 hr after receiving a single i.p. dose of glucagon (0.5 or 1.0 mg/kg). In contrast to the effect of insulin, glucagon treatment caused a 16% fall ($p < 0.05$; 1.0 mg/kg; animals killed after 3 hr) or no significant change (all other times and doses studied) in plasma tryptophan concentrations.

DISCUSSION

These data show tryptophan concentrations in rat plasma to rise after the injection of insulin or the ingestion of a carbohydrate meal. This phenomenon occurs concurrently with decreases in the plasma concentrations of glucose and most other amino acids and is independent of glucagon secretion.

The insulin-induced fall in the concentrations of most plasma amino acids

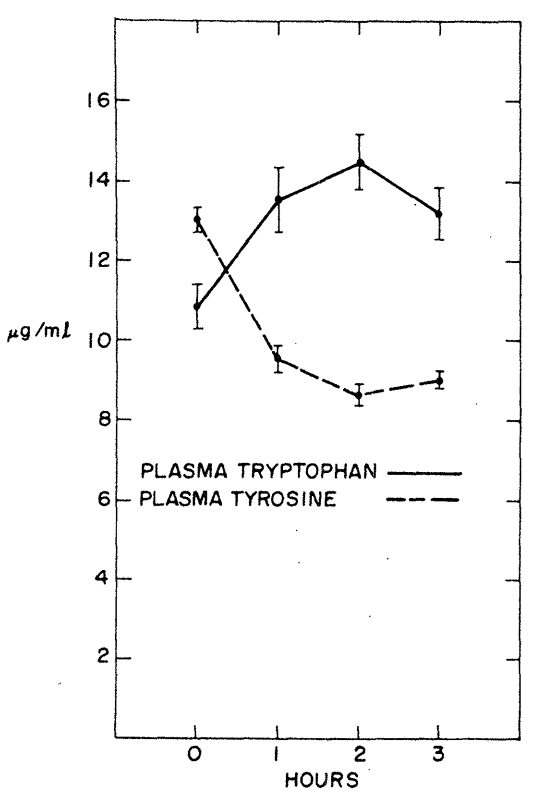

Fig. 2. Effect of carbohydrate ingestion on concentrations of tryptophan and tyrosine in rat plasma. Groups of ten fasting rats were given free access to carbohydrate diet and killed 1, 2, or 3 hr thereafter. Vertical bars indicate SEM.

has been attributed to their increased uptake into skeletal muscle and their incorporation into muscle protein.[5] Inasmuch as tryptophan is the scarcest amino acid in the body[17] and limits protein synthesis in the liver[18] and perhaps other tissues, it seems unlikely that the plasma tryptophan elevation produced by insulin reflects amino acid release from muscle. Secreted insulin must first pass through the liver and could act to release tryptophan from this organ. Since the rat's usual diet includes both carbohydrates and tryptophan-containing proteins, the normal postprandial secretion of insulin would not necessarily lower hepatic tryptophan levels. Relatively large amounts of dietary tryptophan would be entering the liver from the portal circulation at the same time that insulin was causing its release into the systemic circulation. We are attempting to identify the source of the increment in plasma tryptophan.

A major fraction of plasma tryptophan is normally bound to albumin[19] and thus probably not available for uptake into the tissues. Our assay method for tryptophan measures both free and albumin-bound amino acid.[19] If insulin also changes the ratio of bound to free plasma tryptophan, its net effect on the amount of plasma tryptophan available for tissue uptake might be considerably greater or less than the effect of a change in total plasma tryptophan. In preliminary studies we have observed that carbohydrate ingestion causes a considerably greater elevation of brain tryptophan than of total plasma tryptophan. This difference could reflect a selective elevation by insulin of the "free" plasma tryptophan pool or an effect of insulin (or of another hormone secreted in response to its presence) on the uptake of tryptophan by the brain.

We have recently shown that small, physiologic increases in the concentration of plasma tryptophan are quickly followed by increases in the level of its product, serotonin, in rat brain.[10] Since almost any food that the rat might consume should elicit insulin secretion and thus raise plasma tryptophan levels, eating per se might affect the levels of serotonin, a putative neurotransmitter, in the brains of rats and thereby influence mood, body temperature regulation, sleep, and other physiologic functions associated with serotonin-containing neurons.[20-23] Studies are in progress to test his hypothesis.

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