Hypoglycemic Action of Tryptophan

H. G. McDaniel, M.D., B. R. Boshell, M.D., and W. J. Reddy, Sc.D.,
Birmingham, Alabama

SUMMARY

These studies were designed to define the mechanism by which the tryptophan metabolite, quinolinic acid, blocks gluconeogenesis and to determine the role it plays in influencing this process under physiologic and pathologic conditions. An alteration in the level of gluconeogenic intermediates occurred at a quinolinic acid content of 43 μg. per gram of liver. At a content of 10 μg. per gram of liver there was no significant change in the level of these intermediates. The concentration of quinolinic acid (3.7 x 10^{-4} M) which was associated with inhibition of P-enolpyruvate carboxykinase in vivo correlated well with K_i (1 x 10^{-4} M) derived for ferrous quinolinate in vitro. This level of quinolinic acid was reached by the intraperitoneal administration of approximately one fourth and the intragastric administration of approximately one half the daily dietary intake of L-tryptophan. The content of quinolinic acid in the liver of fasted animals was twenty-five-fold lower than the level required to inhibit the carboxykinase reaction and there was no increase in the quinolinic acid content of the liver on a diet containing the usual amount of tryptophan. The effects of various parameters on the conversion of tryptophan to quinolinic acid were also investigated and the clinical implications of these findings are discussed. DIABETES *22*:713-18, September, 1973.

Tryptophan was initially shown to produce hypoglycemia in fasting rats by Mirsky.[1] Ray et al. subsequently showed that the fall in blood sugar was due to inhibition of gluconeogenesis in the liver.[2] They found that this inhibition of gluconeogenesis was characterized by a decrease in phosphenolypyruvate (PEP) and a rise in oxalacetate and its precursors in the liver, indicating that PEP carboxykinase was blocked.[2] Metabolites of tryptophan in the nicotinic acid pathway through quinolinic acid were found to be capable of blocking the formation of glucose from lactate, pyruvate and alanine by the liver, suggesting that the inhibition was due to quinolinic acid.[3] Lardy showed that ferrous quinolinate would inhibit PEP carboxykinase and postulated that this compound was responsible for the effect of tryptophan.[4] He also postulated that in addition to its direct effect quinolinic acid inhibited this enzyme by chelating ferrous iron which prevented the stimulation of PEP carboxykinase activity that occurred at a ferrous iron concentration of 5 x 10^{-5} M.[4] We have shown that ferrous quinolinate is a competitive inhibitor of PEP carboxykinase with an apparent K_i of 1 x 10^{-4} M.[5] Because of these studies indicating that a quinolinic acid concentration of 10^{-4} M and possible 10^{-5} M in the liver would have a regulatory influence on the activity of PEP carboxykinase (a rate-limiting enzyme in gluconeogenesis), and the fact that the conversion of tryptophan to quinolinic acid is decreased in diabetes mellitus,[6] we have examined the effect of varying amounts of L-tryptophan on the conversion of oxalacetate to PEP and the level of quinolinic acid achieved in order to define the mechanism by which this reaction is blocked in vivo. The level of quinolinic acid required to influence this reaction has been compared with those occurring in the fasted state and after a diet containing the normal amount of tryptophan so that the possible physiologic role of this compound may be more readily assessed. The effect of various metabolic alterations on the conversion of tryptophan to quinolinic acid has also been studied so as to assess its role in certain pathologic situations.

METHODS

Male Sprague-Dawley rats weighing 150 to 200 gm. were fasted for twenty-four hours prior to the experimental procedures. They were given L-tryptophan intraperitoneally as a suspension in normal saline. The control animals received only saline. The quinolinic acid content of the liver was measured according to the procedure of McDaniel et al.[7] The various metabolic intermediates were measured in livers that were either

From the Department of Medicine, Division of Endocrinology and Metabolism, University of Alabama School of Medicine and the Diabetes Research Laboratory, Veterans Administration Hospital, Birmingham, Alabama 35233.

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quick frozen with dry ice and acetone after the animals were stunned by a blow to the head or freeze clamped with aluminum tongs cooled in dry ice and acetone (the results obtained with these two methods of freezing were the same). The frozen livers were weighed in the cold and extracted with 5 vol. of 6 per cent perchloric acid and then 3 vol. of 3 per cent perchloric acid. After neutralization with potassium hydroxide to pH 4, the perchlorate ions were removed by centrifugation. Malate, aspartate, oxalacetate, lactate, pyruvate, PEP, and ATP were assayed by standard enzymatic methods.[8] Citrate was assayed chemically.[9] The blood sugar levels were measured with blood from the tail vein using the glucose 6-phosphate dehydrogenase and hexokinase assay.[10] Alloxan-diabetic animals were prepared by the method of Kass.[11] Only those animals with blood sugar levels greater than 250 mg./100 ml. two weeks after the alloxan were classified as diabetic. The cortisol-treated animals were given 5 mg. of cortisol twice a day for four days before receiving tryptophan. The glucagon-treated animals were given 50 μg. of glucagon twice daily for three days and then 50 μg. at the time the tryptophan was given. The standard laboratory diet used was Wayne Lab blox, Allied Mills, Chicago. Glucose disappearance was measured by injecting 14-C(U) glucose into the tail vein of 25 gm. male Sprague-Dawley mice which had been fasted overnight. Blood (50λ) was removed from the tail vein of these animals beginning at ninety minutes and continuing up to 300 minutes. After precipitation of the protein the sample was run through a mixed bed resin column (Dowex-1 and 50) and counted in a liquid scintillation counter.[12]

RESULTS

The content of certain metabolites involved in gluconeogenesis was examined in the liver three hours after the intraperitoneal injection of varying amounts of tryptophan. Ray et al. showed that this was the time of the maximum increase in the level of oxalacetate and its precursors.[2] There was no statistically significant increase in the three immediate precursors of oxalacetate (malate, aspartate and citrate) after the 2.5 and 5 mg. doses of tryptophan. Ten milligrams of L-tryptophan per 100 gm. of body weight resulted in approximately a fourfold increase in the level of these three precursors (table 1). The content of malate, citrate, aspartate and lactate increased further as the amount of tryptophan given was increased. There was no fall in the over-all level of PEP with 10 mg. of tryptophan but after 25 mg. there was a slight drop in the average value. Following 75 mg. of tryptophan the level of PEP fell to one-third of the normal fasting value. The lactate to pyruvate ratio was increased at the 25 mg. dose of tryptophan.

In order to define better the means by which tryptophan was blocking the PEP carboxykinase step and its significance, we measured the level of quinolinic acid in the liver after varying doses of tryptophan. Five milligrams of tryptophan per 100 gm. of body weight resulted in a value of 9.7 μg. per gram of liver wet weight. Ten milligrams of tryptophan per 100 gm. of body weight produced a value of 43.0 μg. per gram of liver (table 2). These values are the peak levels of quinolinic acid occurring in the liver after the intraperitoneal injection of tryptophan. This peak occurs two and one-half hours after the administration of tryptophan.

These changes in metabolite levels were correlated with the ability of the liver to produce glucose by measuring the blood sugar response to varying amounts of

TABLE 1

The effect of varying amounts of tryptophan on the level of certain gluconeogenic intermediates in the liver

Metabolite (nm./gm. liver wet weight)	Amount of L-tryptophan given intraperitoneally (mg./100 gm. body weight) 0	2.5	5	10	25	75
Malate	328 ± 74(3)	354 ± 167(6)	456 ± 220(6)	1,458 ± 651(3)*	5,225 ± 650(3)†	—
Aspartate	490 ± 95(3)	375 ± 13(6)	616 ± 387(3)	1,674 ± 322(3)†	2,490 ± 745(3)†	—
Citrate	343 ± 89(3)	215 ± 23(3)	311 ± 142(3)	1,348 ± 699(3)	1,910 ± 245(3)†	—
Lactate	544 ± 186(3)	554 ± 60(3)	—	820 ± 358(3)	1,473 ± 176(3)†	—
Pyruvate	29 ± 9(3)	34 ± 4(3)	—	38 ± 25(3)	27 ± 7(3)	—
Oxalacetate	3 ± 1(3)	—	—	24 ± 6(3)†	17 ± 1(3)†	—
PEP	155 ± 11(2)	—	—	154 ± 23(3)	127 ± 19(6)	53 ± 5(3)†

The mean values and their standard deviations are shown in the table. The number in parentheses represents the number of different determinations done. The values significantly different from the control values are marked in the table.
*$P < 0.05$
†$P < 0.01$

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TABLE 2

Quinolinic acid values in the liver following tryptophan

Route of administration	L-tryptophan (mg./100 gm. body weight)	Quinolinic acid (μg./gm. liver wet weight)
Intraperitoneal	5	9.7 ± 4(4)
	10	43.0 ± 8(5)
	25	168.0 ± 75(3)
Nasogastric tube	10	17.6 ± 1(2)
	20	62.2 ± 16(2)

The livers were removed and extracted for quinolinic acid two and one-half hours after the intraperitoneal or intragastric delivery of the tryptophan.

tryptophan. The blood sugar was measured both before and three hours after the intraperitoneal administration of tryptophan. Three hours was chosen because Mirsky showed this to be the time of the nadir of the blood sugar response to tryptophan administered in this manner.[1] Those animals given only saline had a slight fall in the blood sugar level after three hours (table 3). Those receiving 5 mg. of tryptophan per 100 gm. of body weight had an increase in the blood sugar value three hours later. With 10 mg. of tryptophan there was a 21 per cent decrease and with 25 mg. there was a 30 per cent decrease from the fasting value. The 30 per cent drop was significant at the $P<0.05$ level but the others were not. We measured the effect of tryptophan on glucose disappearance in an effort to be sure tryptophan was not lowering the blood sugar by affecting glucose disappearance in addition to inhibiting gluconeogenesis. As shown in table 3 the half-life of 14-C(U) glucose was slightly longer in the animals given tryptophan than in the control group.

Tryptophan does not alter metabolite levels in the liver of diabetic animals as it does in normal fasting rats. To determine if this lack of tryptophan effect is the result of a decrease in the formation of quinolinic acid from tryptophan or the result of some peculiarity of the gluconeogenic process in diabetic animals, we measured the level of quinolinic acid produced by 10 mg. of L-tryptophan per 100 gm. of body weight in alloxan-diabetic animals. This dose of tryptophan resulted in a quinolinic acid level of 0.28 μg. per gram of liver in these animals (table 4). This value is over a hundredfold less than the level produced in normal fasting animals following an equivalent amount of tryptophan. The administration of cortisol also markedly decrease the level of quinolinic acid found after 10 mg. of tryptophan, but glucagon administration did not significantly alter the level produced when compared with the response of normal animals (table 4).

The normal dietary intake of a rat over twenty-four hours contains approximately 40 mg. of tryptophan per

TABLE 3

The effect of varying amounts of L-tryptophan on the blood glucose of fasting animals and 14-C(U) glucose disappearance

Tryptophan (mg./100 gm. body weight)	Blood glucose (mg./100 ml.) Before	After	Per cent change	$T_{1/2}$ 14-C(U) glucose in blood (min.)
0	74 ± 5(5)	68 ± 6(5)	− 8	56 ± 4(3)
5	71 ± 12(4)	84 ± 39(4)	+18	
10	76 ± 31(4)	60 ± 14(4)	−21	
25	73 ± 14(4)	51 ± 11(4)	−30*	63 ± 7(3)

The blood (0.2 ml.) from the tail vein of the animal was lysed in distilled water. After precipitation of the protein with barium hydroxide and zinc sulfate, the glucose was measured as described under *Methods*. The 14-C(U) glucose concentration in the blood from ninety to 300 minutes after injection into the tail vein gave a linear plot on semilog paper from which the $T_{1/2}$ of glucose was determined for each animal.

*$P < 0.05$

TABLE 4

The effect of diabetes mellitus and other states on the formation of quinolinic acid from tryptophan

Condition of animal	Amount of quinolinic acid in liver (μg./gm. liver wet weight)
Diabetes mellitus	0.28
Hydrocortisone	0.31
Glucagon	36.00

The livers from four animals were combined into one determination for each measurement of quinolinic acid in the diabetic and cortisol-treated animals. The value for normal animals is 43 μg. per gram of liver.

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100 gm. of body weight.[2] However, this is not received into the portal circulation as a single bolus such as occurs when it is administered intraperitoneally. When the tryptophan was given by gastric tube the 10 and 20 mg. doses of tryptophan produced levels of quinolinic acid in the liver which were approximately one-half those achieved by the intraperitoneal injection of the tryptophan (table 2). Quinolinic acid levels were also measured in the fasting and fed states and after a meal containing the usual amount of tryptophan. No increase in the content of quinolinic acid was found after the animals ate the normal laboratory diet containing 0.31 per cent tryptophan (table 5). The amount of quinolinic acid in the liver was at the lower limits of detectability so that this probably obscured the increase that may have occurred with feeding.

DISCUSSION

If a water content of 70 per cent is assumed for liver then the concentration of quinolinic acid resulting from 5 mg. per 100 gm. of tryptophan intraperitoneally would be 8 x 10^{-5} M. The 10 mg. dose produced an average value of 3.7 x 10^{-4} M and the 25 mg. dose resulted in a value of 1 x 10^{-3} M. These calculations do not take into consideration the amount of quinolinic acid chelated to metals, bound to proteins or compartmentalized (quinolinic acid does not appear to enter mitochondria);[13] therefore, these figures must be considered at best only estimates of the effective concentration of quinolinate. With this point in mind, however, there is a close correlation between the K_i derived for ferrous quinolinate in vitro (1 x 10^{-4} M)[5] and the concentration of quinolinic acid found to be associated with an increase in the level of oxalacetate and its precursors in vivo (3.7 x 10^{-4} M). These studies show a poor correlation between the effect of tryptophan on PEP levels in the liver and its hypoglycemic action. The PEP measurements are a reflection of the total intra- and extramitochondrial content of PEP. Because quinolinic acid does not readily penetrate the mitochondria[13] it would not be expected to block the intramitochondrial PEP carboxykinase. Therefore, the rise in oxalacetate due to inhibition of cytoplasmic PEP carboxykinase activity would secondarily increase the intramitochondrial level of PEP and thereby decrease the over-all fall in PEP. The cytoplasmic level of PEP is the key factor in gluconeogenesis because enolase is located in the cytoplasm.

If quinolinic acid were preventing the activation of PEP carboxykinase by ferrous iron as has been proposed,[4] one would expect to have an increase in the level of oxalacetate and its precursors at a quinolinic acid concentration of 8 x 10^{-5} M which did not occur. Tryptophan has been shown to stimulate the release of insulin from the pancreas but its effect in this regard is not very marked in comparison to that of certain other amino acids[14] and this would certainly not in any way account for the metabolite changes in the liver after tryptophan. Another aspect of the effect of tryptophan on the ability of the liver to form glucose from amino acids and other precursors is its effect on energy metabolism. A precursor of quinolinic acid in the tryptophan to nicotinic acid pathway, 3-hydroxyanthranilic acid, will inhibit oxidative phosphorylation at a concentration of 0.25 to 1 mM.[15] Those animals given 25 mg. of tryptophan per 100 gm. of weight had a drop in the ATP concentration at three hours of one-third, so that interference with the energy metabolism of the liver may also contribute to the hypoglycemic action of tryptophan.[16]

The concentration of quinolinic acid required to block gluconeogenesis by inhibiting PEP carboxykinase appears to be at least twenty-five-fold greater than the value we found in the fasted state. Henderson and Ramasarma, using a microbiological assay for quinolinic acid, reported a value of 15 to 20 μg. per gram of liver wet weight[17] (a concentration which would be expected to influence the PEP carboxykinase step). The difference in our findings and theirs might be due to some biological variation in the animals used, dietary differences (i.e. higher tryptophan content in the diet) or to differences in the assay methods. Their microbiological assay may not have had as fine a specificity as our method in which quinolinic acid is first isolated and then measured. Although the content of quinolinic acid in the liver did not increase above the fasting value five hours after a diet containing the usual amount of tryptophan, with a different dietary composition quinolinic acid might

TABLE 5

The effect of feeding and fasting on quinolinic acid levels in the liver

State of the animal	Content of quinolinic acid (μg./gm. liver wet weight)
Fasting for 48 hr.	0.45
3½ hr. after eating	0.36
5 hr. after eating	0.45
Fed	0.51

The livers from four animals were combined into one determination at the times shown above. After fasting for forty-eight hours the animals ate 2½ gm. of food containing approximately 8 mg. of L-tryptophan. The fed animals were assayed in the morning after feeding overnight.

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reach a range which would influence the PEP carboxykinase step. Increased concentrations of tryptophan and its metabolites have been found in the blood and urine in association with nonpancreatic tumors which produced hypoglycemia.[18] Studies of some of these patients have shown they have multiple etiologies for their hypoglycemia, one of which is a decrease in gluconeogenesis.[19] Whether or not quinolinic acid reaches the level required to block gluconeogenesis in these patients remains conjectural at this point. Ray et al. inhibited the PEP carboxykinase step in adrenalectomized rats with one-half the amount of tryptophan required to block it in normal fasting rats.[2] This is probably a reflection of the relative increase in the conversion of tryptophan to quinolinic acid in adrenalectomized animals. Along with the decrease in the level of certain gluconeogenic enzymes and a decrease in the availability of amino acid substrates, quinolinic acid may play some role in the susceptibility to hypoglycemia in adrenal insufficiency. Although we thought that nephrectomized rats would convert more tryptophan to quinolinic acid because the kidney contains picolinic carboxylase,[20] which diverts the precursor of quinolinic acid into another pathway (figure 1), there was no increase in the formation of quinolinic acid from tryptophan in these animals, indicating that this compound does not play a role in the increased susceptibility of uremic individuals to hypoglycemia.

The alloxan-diabetic animals and those given cortisol had a marked decrease in the conversion of tryptophan to quinolinic acid. This is due to a marked increase in picolinic carboxylase activity in the diabetic animals[20] so that more of the immediate precursor of quinolinic acid is converted instead to picolinic acid (figure 1). In the cortisol-treated animals, this decrease is probably due to more of the tryptophan being shunted along other pathways (figure 1). Both of these pathologic states would at least double the needs of the individual for nicotinic acid, because normally approximately 60 per cent of the nicotinic acid is supplied[21] by conversion of tryptophan through the quinolinic acid pathway. Therefore, on marginal diets they would be much more susceptible to this particular vitamin deficiency just as patients with Hartnup disease are predisposed to the development of pellagra.

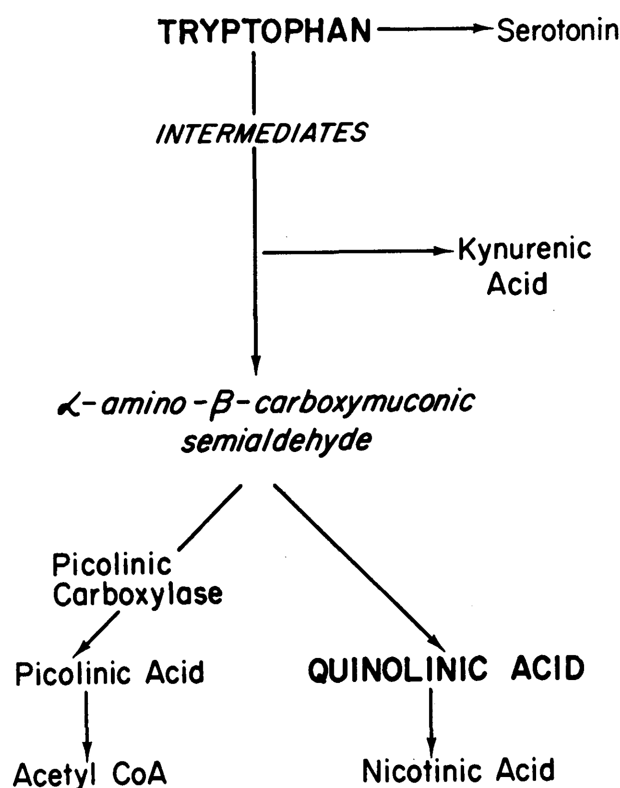

FIG. 1. Tryptophan may be converted to nicotinic acid via quinolinic acid or it may be metabolized by the other pathways shown.

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