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Intragastrically administered tryptophan blocks gluconeogenesis in 48-hr starved rats

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Tryptophan (TRP) has been reported to block the conversion of pyruvate (PYR) to phosphoenolpyruvate (PEP). The purpose of the present experiment was to determine the time course of the changes in phosphoenolpyruvate carboxykinase (PEPCK) activity and PEP production in response to intragastric intubation of 750 mg/kg BW TRP in 48-hr starved rats. Controls were intubated with a comparable volume of agar vehicle. Fifty-one, 6-week-old male Sprague-Dawley rats weighing between 132 and 155 g were used. Three experimental groups (n = 10/group) and four control groups (n = 5 or 6/group) were killed 0, 1, 2, or 6 hr after intubation. The rats were decapitated and quickly exsanguinated. Livers were dissected, weighed, then frozen using freeze clamps in methanol/dry ice and stored in a biological freezer for subsequent analyses. PEP production was initially increased by TRP treatment, though PEPCK activity of TRP treated rats increased only 2 hr and 6 hr after TRP, suggesting that TRP blocked PEP production, at least at the end of the 6-hr monitoring period. Curiously, at 6 hr after TRP circulating serum glucose was restored to 0-hr controls, suggesting that gluconeogenesis was being supported by an alternate substrate. © Elsevier Science Inc. 1996 (J. Nutr. Biochem. 7:567–570, 1996.)

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Introduction

Over the course of the past several years, different methods of blocking hepatic gluconeogenesis have been developed. Of these, some of the most successful have been the use of tryptophan (TRP) and one of its derivatives, mercaptopicolinic acid (MPA).[1–8] Both TRP and MPA block the conversion of oxaloacetate to phosphoenolpyruvate (PEP). Ray et al.[5] have reported that TRP administration promotes an increase in phosphoenolpyruvate carboxykinase (PEPCK) activity, though PEP production is blocked possibly by a metal cofactor.[7]

The purpose of the present experiment was to determine the time course of the changes in PEPCK activity and PEP production in response to an intragastric intubation of 750 mg/kg body weight (BW) TRP in 48-hr starved rats. A 48-hr starvation period is sufficient to promote complete dependence on gluconeogenesis for the maintenance of euglycemia. We have previously speculated that the glucocorticoids play an integral role in the control of food intake and BW gain via several mechanisms. Once such glucocorticoid endpoint is the control of hepatic gluconeogenesis. The present studies have been performed so as to assist in the development of a method for blocking hepatic glucose production without directly removing circulating glucocorticoids (adrenalectomy) or blocking their receptors. It was predicted that PEPCK activity would increase following TRP intubation, though hepatic PEP and serum glucose would not be affected appreciably. Further, plasma pyruvate (PYR) concentrations were predicted to increase, reflecting a block of this important gluconeogenic pathway.

Methods and materials

Fifty-one, 6-week-old male Sprague-Dawley rats (Charles River Laboratory, Raleigh, NC, USA) weighing between 132 to 155 g were used. On arrival, the rats were individually housed in hanging metal cages in a temperature and humidity-controlled room and maintained on a 12/12-hr day/night cycle. All rats were given ad libitum access to Purina 5001 Rat Chow and water for 5 days.

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After that time, AIN-76 A semipurified diet was substituted for the chow for 6 additional days. Animals were handled and weighed daily.

On day 12, the AIN diet was taken away and the rats were starved for 48 hr. Based on day 12 BW, the animals were divided into seven groups (four control and three experimental groups). Rats were killed, 0, 1, 2, or 6 hr after intragastric intubation on sacrifice day (day 14). Each control group consisted of five rats, with the exception of the 0 hr control group ($n = 6$) that also served as the reference control for evaluating changes over time subsequent to TRP intubation. Each experimental group was made up of 10 rats.

At 0 hr on day 14, all experimental rats were administered 750 mg/kg BW L-TRP (Sigma Chemical Co., St. Louis, MO, USA), using a 2% (w/v) agar vehicle. Rats were intragastrically intubated using a 3-inch 16 GA feeding tube (Bio-Medical Needles, Poper and Sons Inc., New Hyde Park, NY, USA) at light onset (10:00 a.m.). All of the rats assigned to the control groups received a comparable volume of an agar vehicle. Sacrifice was by decapitation. The animals were quickly exsanguinated. One 3-mL blood sample was deproteinized by adding 4 mL of 7% perchloric acid and vortexed immediately. All blood samples were stored in ice and centrifuged at 3,000 rpm (Beckman Model TJ-6R centrifuge, Columbia, MD, USA). The supernatant of each sample was collected and stored in a biological freezer (−70°C) for subsequent analyses. Livers were quickly dissected and weighed then frozen using freeze clamps in methanol/dry ice. Liver samples were also stored in a biological freezer for subsequent analyses.

Serum glucose concentrations were determined using a YSI blood glucose analyzer (Model 27, Yellow Springs International Co., Inc., Yellow Springs, OH, USA) using the glucose oxidase method. Plasma PYR concentrations were assayed using a Sigma lactate dehydrogenase method (Sigma Pyruvate Kit, Kit #726-UV, 1990). Linearity of this assay was established using standards that ranged in concentration from 0.5 to 3.0 mg/dL ($r = 0.99$, $P < 0.01$). Defrosted liver samples were homogenized (1:20 or 1:40 dilutions) for biochemical assays of PEP and PEPCK.

The PEPCK assay was performed by homogenizing a 0.5 g liver sample in 9.5 mL cold buffer to yield a 1:20 dilution. After centrifugation at 15,000 rpm, 0.1 mL of the enzyme is added to a cuvette containing the following: 1.5 mL Tris (0.1 M, pH 7.4), 0.1 mL $MnCl_2$ (0.03 M), 0.1 mL fresh PEP (0.03 M), 0.1 mL $NaHCO_3$ (0.5 M, pH < 8), 0.05 mL fresh NADH (8 mg/mL in Tris, pH 7.4), 1.0 mL H_2O, and 0.1 mL of sample. The contents were mixed and allowed to run endogenously while readings were taken using a Gilford Spectrophotometer (Model 240) at a wavelength of 340 nM in conjunction with a Gilford Recorder, Model 6051, Gifford International Laboratories, Inc., Oberlin, OH, USA. Upon observing a baseline, 0.15 mL IDP (0.03 M in H_2O) was added and changes in absorbance were recorded. Units of activity were defined as μmol of substrate (IDP) used/minute.

The PEP assay was developed in part by Sigma. This spectrophotometric technique used the following reagents:

Reagent A: 100 mM KH_2PO_4 buffer, pH 7.6 at 37°C (Fisher Scientific, Inc., Pittsburgh, PA Stock No. 285)
Reagent B: 0.06 mg/mL in dH_2O PEP (Sigma, Stock No. P7252)
Reagent C: Reduced β-NADH solution (Sigma, Stock No. N8129)
Reagent D: 1000 mM KCl solution (Baker Analyzed Reagent, J. T. Baker, Inc., Phillipsburg, NJ, USA Product No. 3040–01)
Reagent E: 50 mM $MgSO_4$ (Sigma, Stock No. M-1880)
Reagent F: 40 mM ADP (Sigma, Stock No. A-4386)
Reagent G: Pyruvate kinase/Lactate dehydrogenase mixed enzymes (Sigma, Stock No. 40-7)

Reagents A–F were mixed by inversion in a cuvette and equilibrated for 5 min at 37°C. After equilibration the initial absorbance was measured by a Gilford spectrophotometer at a wavelength of 340 nM. 0.05 mL of reagent G was added and absorbance readings were read until they minimized (Final Absorbance). The difference in absorbance permitted the calculation of the concentration of PEP per weighed sample.

Statistics

All measurements were performed in duplicates. Average values for each animal were then used to calculate group averages at each time point. Differences among groups were evaluated using analysis of variance (anova) followed by least significant differences tests, and were considered significant if $P < 0.05$.

Results

Glucose

Intubation with vehicle or 750 mg/kg BW TRP resulted in a transient decline in circulating glucose ($F = 8.99$, δf 6, 42; $P < 0.0001$). TRP promoted a greater reduction in serum glucose 1 hr and 6 hr after treatment when compared with time matched controls. Furthermore, TRP treatment resulted in more rapid and more severe hypoglycemia than did agar control intubations (*Figure 1*). Glucose values of control rats killed at 1 hr were not significantly different from those of rats killed at 0 hr. Glucose values of control rats killed at 2 hr were different from those killed at 0 hr. By comparison, TRP treated rats were significantly hypoglycemic after 1 hr, and maintained hypoglycemia throughout the next hour, both at confidence levels of $P < 0.001$. The differences between TRP and controls did not attain statistical signifi-

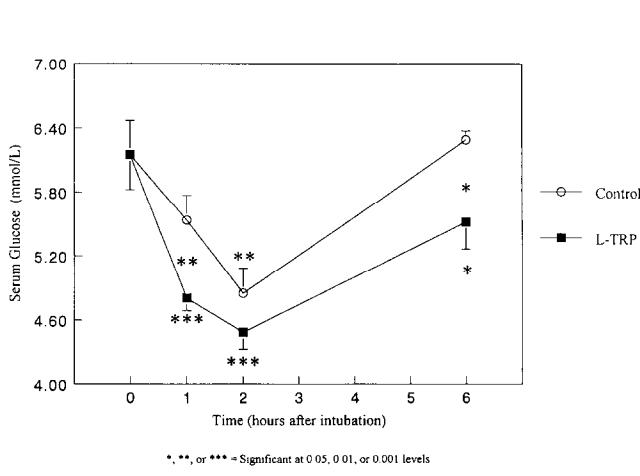

Figure 1 Serum glucose concentrations over the 6-hr monitoring period after intragastric intubation of 750 mg/kg BW L-TRP. All values are means ± SEM. Differences between means at any one time point are denoted by an asterisk placed between mean values. Differences between any value and serum glucose concentration observed at time 0 are denoted by asterisks placed above or below each point on the graph. Note that transient hypoglycemia was observed in both experimental and control groups, but that the hypoglycemia of the L-TRP treated animals was more severe.

cance at the 2-hr time point. TRP treated rats had significantly lower glucose concentrations at 6 hr than did controls; however, by 6 hr, only the control group was restored to euglycemia, as the average glucose of the 6-hr TRP group remained significantly lower than that of the 0-hr control ($P < 0.05$).

PYR

Intubation with either vehicle or TRP resulted in significantly lower circulating PYR concentrations throughout the 6-hr monitoring period ($F = 5.02$, δf 6, 41; $P < 0.001$). No effect of TRP was noted (*Figure 2*).

PEPCK

Intubation resulted in a transient increase in PEPCK activity in control rats. PEPCK activity of liver tissue taken from control treated rats 2 hr after intubation was significantly higher than that taken from rats killed at time 0 hr ($P < 0.05$) (*Figure 3*). By contrast, TRP treated rats initially experienced a decline in PEPCK activity, as revealed by a statistically significant suppression in activity noted at the 1 hr time point. This suppression was reversed thereafter. Hepatic PEPCK activity was higher in TRP treated rats 2 hr ($P < 0.05$) and 6 h ($P < 0.001$) post intubation when compared with time 0 controls ($F = 15.76$, δf 6, 43; $P < 0.0001$). Differences between TRP and control groups achieved significance at both the 1 hr and 6 hr time points. By 6 hr, TRP increased hepatic PEPCK activity by 74%.

PEP

Only small and inconsistent effects of TRP were found on PEP production as revealed by analysis of variance (ANOVA) ($F = 1.25$, δf 6, 41; P = n.s.). No differences in PEP production were found in control treated rats. By contrast, TRP administration resulted in a significant increase in PEP at the 1-hr and 2-hr time points when compared with levels found at time 0 ($P < 0.05$) (*Figure 4*). At no time were differences between TRP and time-matched control groups statistically significant.

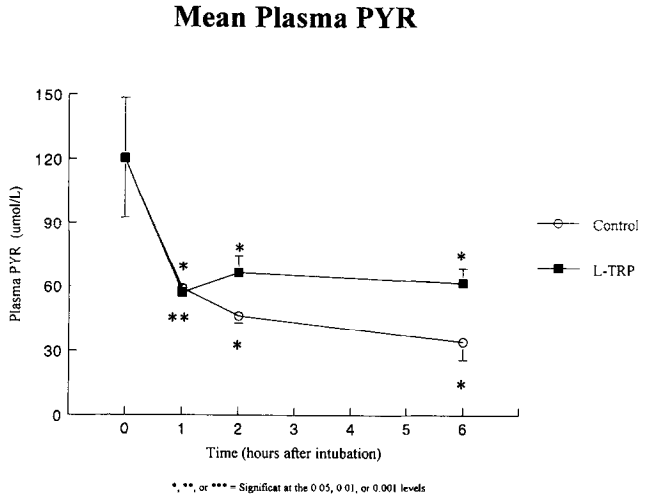

Figure 2 Plasma PYR concentrations over the 6 hr monitoring period after intragastric intubation with L-TRP. All values are means ± SEM. Differences between means at any one time point are denoted by an asterisk placed between mean values. Differences between any value and plasma PYR concentration observed at time 0 are denoted by asterisks placed above or below each point on the graph. During the first hour, plasma PYR concentrations of both groups fell. By the second hour, though PYR levels continued to decrease in control group, the TRP treated group did not. By the end of the experiment, TRP treated rats had significantly higher PYR levels than did controls.

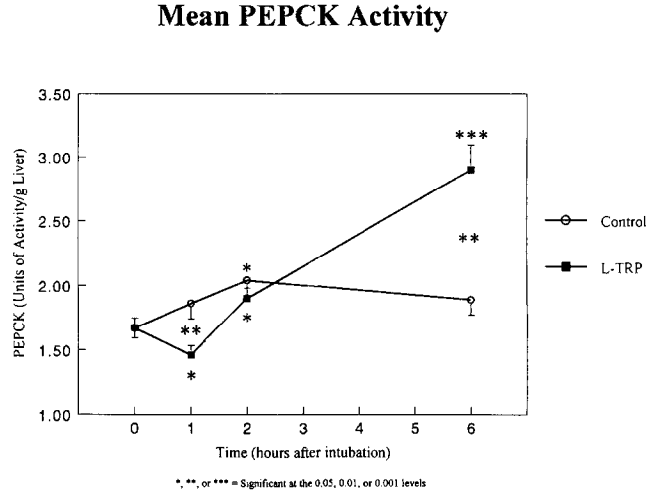

Figure 3 Hepatic PEPCK activity over 6 hr after treatment with either vehicle or TRP. All values are means ± SEM. Differences between means at any one time point are denoted by an asterisk placed between mean values. Differences between any value and PEPCK activity observed at time 0 are denoted by asterisks placed above or below each point on the graph. Note that 1 hr after intubation, PEPCK activity was significantly lower in TRP-treated group, but that by the end of the 6-hr monitoring period, TRP caused a profound increase in hepatic PEPCK activity.

Discussion

We had hypothesized that intragastrically administered TRP would interfere with gluconeogenesis. We set out to mea-

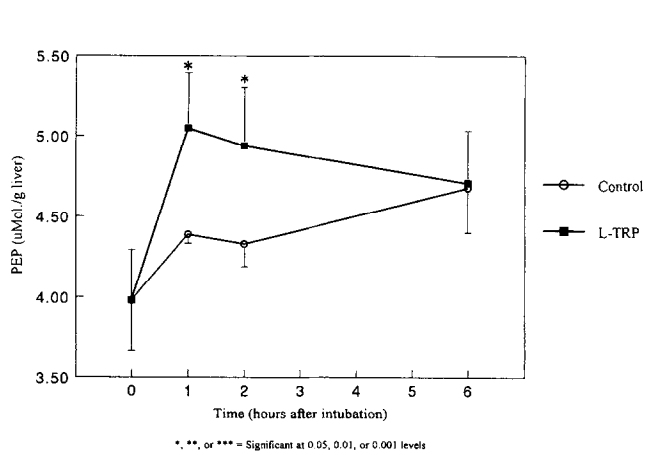

Figure 4 Hepatic PEP concentrations were significantly increased 1 hr after L-TRP intubation, and only slowly decreased over the next 5 hr. All values are means ± SEM. Differences between any value and hepatic PEP concentration observed at time 0 are denoted by asterisks placed above or below each point on the graph.

sure the time course for TRP induced hypoglycemia and to examine the role of gluconeogenesis in glycemic counter regulation. Our principal findings were that while TRP promoted significant hypoglycemia early after intubation (within 1 hr of treatment), hepatic PEPCK activity at that time was significantly lower than that found in control-treated animals. Paradoxically, hepatic PEP levels were significantly higher than levels measured for the 0-hr controls, but not significantly higher than time-matched controls. Euglycemia was approached in TRP treated rats, yet PYR concentrations were decreased after intubation and were not restored during the 6-hr experimental period. PEPCK activity was increased by 74% after TRP, yet PEP production was not simultaneously increased, suggesting that TRP inactivated PEPCK or in some way interfered with PEP production.

The present results replicate the observation that TRP administration can interfere with gluconeogenesis, but does so via an undetermined way, possibly in addition to altering PEPCK activity. Based on our time course observations, at least two effects exist. The first includes a decrease in PEPCK activity with its paradoxical concomitant increase in PEP that was observed early after TRP intubation. It may be that TRP interferes with gluconeogenesis subsequent to PEP production, but prior to final glucose production. This TRP-induced interference dissipated within the first few hours after treatment. The second effect resulted in a dramatic increase in PEPCK activity without any significant increase in PEP production that was observed at the end of the experimental period. This "inactivated" PEPCK has been previously described by Foster et al.[6]

Foster et al.[7] observed a strikingly elevated level of PEPCK in 30 min and a doubling of enzyme activity in intact fasted rats 1 hr after administration of L-TRP and a slower rate of increase thereafter. It was evident from the time course of the elevation of PEPCK after TRP administration that a major part of TRP-induced increase in activity does not represent an increase in enzyme concentration. We observed a suppression at the 1-hr time point for PEPCK activity, but an increase in activity at 2 hr after intubation of TRP followed by a dramatic increase at the end of the monitoring period.

The transient decrease in PEPCK during the first hour is possibly due to its conversion from an active form of the enzyme to an inactive form, as suggested by Ray et al.[5] However, the significance of our observation is suspect, as very high levels of PEP were observed at the 1-hr and 2-hr time points when compared with levels found at time 0 hr. These observations contrast with those of Foster et al.[7] who had reported that PEPCK activity increased within 30 min of TRP treatment.

Significantly lower circulating PYR concentrations throughout the 6-hr period in the TRP treated groups were observed when compared with levels measured at time 0. However, when compared with time-matched controls, the TRP treated rats had higher PYR concentrations at the 2-hr time point ($P < 0.05$). This observation suggests that TRP intubation spared some of the decline in PYR observed in controls. A decline in PEPCK activity would ordinarily result in higher PYR levels. Conversely, increased PEPCK would predict higher PEP production. Curiously, the biphasic PEPCK activity noted in TRP-treated rats resulted in neither predicted outcome. Early after TRP administration, PEPCK activity dropped, but pyruvate levels decreased and PEP levels increased. Six hr after TRP, PEPCK activity was increased, yet PYR levels remained low, and PEP levels fell back to baseline levels. Contrary to our findings, Ray et al.[5] observed a severe decrease of PEP and other intermediates toward glycogen and, conversely, a great increase in concentrations of lactate, PYR, citrate, aspartate, malate, and oxaloacetate within 30 min of administration of TRP. PEP levels were unaffected at 30 min, but dropped to 20% of normal at 1 hr.

These results demonstrate that TRP can have an effect on PEPCK activity. Our findings are not consistent with the prediction that increased PEPCK activity promotes increased PEP production. Factors that are responsible for this observation include TRP interaction with the enzyme, and possibly other enzymes, as well as potential unknown physiological factors that inhibit PEP production in vivo. Future investigations should include the measurement of not only activity, but amount of enzyme available for PEP production.

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