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GLUCOCORTICOID INDUCTION OF TRYPTOPHAN OXYGENASE

ATTENUATION BY INTRAGASTRICALLY ADMINISTERED CARBOHYDRATES AND METABOLITES

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Abstract—*In vivo* tryptophan 2,3-dioxygenase (TPO) activity in male rats was estimated from the rate of production of $^{14}CO_2$ after intragastric administration of [^{14}C-2]tryptophan. The synthetic glucocorticoids hydrocortisone-21-sodium succinate or Triamcinolone acetonide were injected to elevate hepatic TPO activity on an acute (1–6 hr) or chronic (24 hr) basis. Glucose, fructose, or glycerol was intragastrically intubated in doses ranging from 4 to 16 mmoles to assess their abilities to attenuate acute or chronic increases of TPO activity by these glucocorticoids. Hydrocortisone-21-sodium succinate at doses of 0, 25, and 50 mg/kg produced dose-dependent elevations of TPO. A 50 mg/kg dose produced a 3-fold elevation of enzyme activity when measured *in vitro* as product produced by liver homogenates and a 2-fold elevation when assessed from expired radioactive carbon dioxide from radiolabeled tryptophan *in vivo*. Enzyme activity measured by $^{14}CO_2$ production reached peak values in 2–3 hr and returned to baseline in 5 hr. Glucose, fructose or glycerol completely prevented the rise in conversion of [^{14}C-2]tryptophan produced by hydrocortisone hemisuccinate when administered at doses of 12 or 16 mmoles 0.5 hr before the steroid. Lower doses had less effect. The potencies of the compounds in inhibiting acute increases in TPO activity produced by hydrocortisone hemisuccinate were in the order glycerol > fructose > glucose. Chronic Triamcinolone treatment elevated *in vivo* TPO activity by 2.5-fold and *in vitro* TPO activity by 5-fold. The chronic elevation of *in vivo* TPO by Triamcinolone could be arrested within 1 hr by an intragastric fructose load. The present finding, that acute or chronic glucocorticoid-induced increases in *in vivo* TPO activity were rapidly blocked by intragastric carbohydrate loads, is consistent with the view that dietary carbohydrates modulate hepatic TPO activity via feedback repression and not by a cessation of TPO enzyme synthesis.

The hepatic enzyme tryptophan 2,3-dioxygenase [TPO; L-tryptophan oxidoreductase (decyclizing), EC 1.13.11.11] regulates the major pathway of tryptophan catabolism in the rat [1, 2] by cleaving the indole ring of L-tryptophan to produce L-*N*-formylkynurenine [3, 4]. As measured by *in vitro* [5] and *in vivo* [6] techniques, the synthesis of TPO enzyme protein is enhanced following glucocorticoid administration [5, 7–9], while the rate of TPO degradation can be decreased by protection of the enzyme with its substrate, tryptophan [7, 8, 10]. Interestingly, tryptophan oxygenase activity is also subject to inhibitory feedback control without necessarily inhibiting TPO synthesis [4, 11]. The activity of pre-existing TPO enzyme appears to be decreased by the reduced nucleotide end-products of the kynurenine pathway, NADH and NADPH [4, 11, 12]. Since hepatic NADH and NADPH concentrations are also increased by glucose metabolism [4, 13], dietary glucose may repress TPO by enhancing the Kreb's cycle generation of NADH and NADPH. Indeed, it has been demonstrated that chronic consumption of glucose [4, 14–16] or nicotinamide [4] in drinking water can decrease basal activity of liver TPO assessed by *in vitro* enzymatic assays as well as decrease the elevation in enzyme activity which follows administration of glucocorticoids. Administration of glucose, nicotinamide, sucrose, fructose [2] or ethanol [4] has been associated also with increases in the ratio of reduced to oxidized NADPH and/or NADH in liver. Together, these results suggest that dietary glycolytic intermediates should also block the effects of glucocorticoids in raising TPO activity when activity is measured by *in vivo* procedures. While several studies have reported carbohydrate repression of steroid-induced elevations of tryptophan oxygenase activity [4, 6, 17, 18], *ad lib.* consumption of carbohydrates in the drinking water in these studies prevented precise control over dose and time conditions of carbohydrate administration. Delivery of carbohydrates by gavage [18] or injection

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[12], while allowing such control, may introduce extraneous stressors into the procedure. Finally, many of these compounds, when added to the drinking water in high concentrations, are not readily consumed by rats. In the present study, we have administered carbohydrates via a chronically implanted intragastric cannula to minimize the stress of gavage, to control accurately the amount and duration of carbohydrate administration, and to determine (1) whether the acute or chronic elevation of TPO activity by glucocorticoids can be demonstrated *in vivo*; (2) whether carbohydrates which regenerate hepatic NADH and NADPH can repress such glucocorticoid induction *in vivo*; (3) the dose and time relationships between carbohydrate administration and the repression of glucocorticoid induction; and (4) whether intragastric carbohydrate administration can interrupt chronic glucocorticoid induction of TPO activity.

MATERIALS AND METHODS

Animals. Adult, male, Sprague–Dawley rats (200–250 g) derived from Charles River stock were housed singly in an animal colony maintained at 22 ± 3° and 50% relative humidity during a 14:10 hr light–dark (LD) cycle. Water was available *ad lib.* as were Purina Laboratory Chow pellets (Ralston Purina Co., St. Louis, MO) except when animals were food-deprived the night before *in vivo* or *in vitro* TPO determinations. Neither food nor water was available during *in vivo* TPO determinations.

Surgical techniques. Following an overnight fast, animals were injected intraperitoneally (i.p.) with sodium pentobarbital (Nembutal; 50 mg/kg) and atropine sulfate (0.5 mg/kg), and each animal was fitted with a chronic subcutaneous gastric cannula according to the procedure of Altar [19]. The intragastric cannula makes possible the infusion of compounds that might otherwise be rejected for oral consumption and permits precise control of the timing and quantity of substances delivered to the stomach.

Estimates of in vivo *or* in vitro *TPO activity.* The *in vivo* TPO activity of each rat was estimated by a modification of the procedure of Moran and Sourkes [20] based upon the rate of evolution of expired $^{14}CO_2$ following the intragastric administration of [2-^{14}C]L-tryptophan.

Animals were individually contained in Plexiglas cylinders (20 cm long by 9 cm diameter) (Fig. 1). One end of the cylinder was sealed with a No. 15 rubber stopper with two 1 cm diameter glass air inlet tubes. The other end of the cylinder was sealed with a flat Plexiglas plate through which a glass tube protruded. The glass tube was connected by a 30 cm piece of Tygon tubing to a glass tube with a fritted glass end which was immersed in 50 ml of a 3:1 monoethanolamine–2-ethoxyethanol (Mallinckrodt, St. Louis, MO) CO_2 trapping mixture contained within a separatory funnel. An air flow meter (Matheson Gas Products Co., Houston, TX) was connected to the separatory funnel via a calcium chloride drying tube. The flow meter was connected to the inlet valve of a one-third horsepower vacuum pump (Gast Co., Benton Harbor, MI) which maintained a flow of 1.4 to 1.5 l air/min through the entire system.

A 1-ml aliquot of the trapping mixture was removed every 15 min after animals were placed in the plastic cylinder and was replaced with 1 ml of fresh mixture. The aliquot containing radioactive carbon dioxide was added to 10 ml of Insta-gel (Packard Instrument Co., Downers Grove, IL) and 4.5 ml of distilled water in a glass scintillation vial. TPO activity was calculated from the rate of $^{14}CO_2$ evolution during the period between 0.5 and 1 hr after animals were placed in the metabolic chamber.

In some instances, hepatic TPO activity of intact animals receiving similar dietary or hormonal treat-

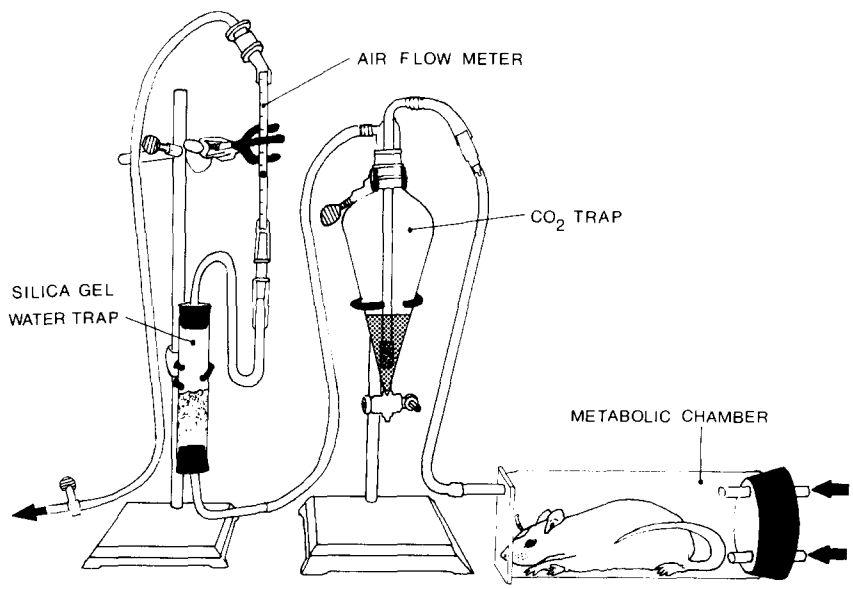

Fig. 1. Apparatus for determination of *in vivo* TPO activity. Arrows on the right indicate room air intake; arrow on the left leads to vacuum pump.

ment was measured by the *in vitro* procedure of Knox [21] in the presence of excess heme.

Drugs administered. Animals were given 0.5 μCi of L-tryptophan (2-ring-^{14}C, 36.4 mCi/mmole; ICN, Irvine, CA) in 0.5 ml of water followed by 0.5 ml of distilled water (to clear the subcutaneous cannula of any remaining radioactivity) immediately before being placed in the metabolic chamber.

Hydrocortisone-21-sodium succinate (hydrocortisone hemisuccinate; Sigma Chemical Co., St. Louis, MO) was injected i.p. in doses of 0 (1 ml of 0.9% saline/kg body wt), 25, or 50 mg/kg at either 0, 1, 2, 3, 4, 5, 5.5, or 6 hr before [^{14}C]tryptophan. Triamcinolone acetonide suspension (E. R. Squibb & Sons Inc., Princeton, NJ) was injected i.p. in a dose of 40 mg/kg at 9:00 a.m. and 4:00 p.m. the day before [^{14}C]tryptophan intubations.

D-Glucose (Baker, Phillipsburg, NJ) or D-fructose (Sigma Chemical Co.) was intragastrically intubated as a 70% solution. Glycerol (Allied Chemical Co., Morristown, NJ) was diluted by 50% with distilled water. D-Glucose, D-fructose, or glycerol was intubated 0.5 hr before [^{14}C]tryptophan unless otherwise indicated, in doses of 4, 8, 12, or 16 mmoles dissolved in 1, 2, 3, or 4 ml of distilled water respectively. Control animals received 2 ml of distilled water instead of carbohydrates at the same time before [^{14}C]tryptophan intubations. All carbohydrates were administered to freely moving animals via the gastric cannula using a 10 ml syringe connected to the cannula inlet by a 30-cm section of PE 60 tubing.

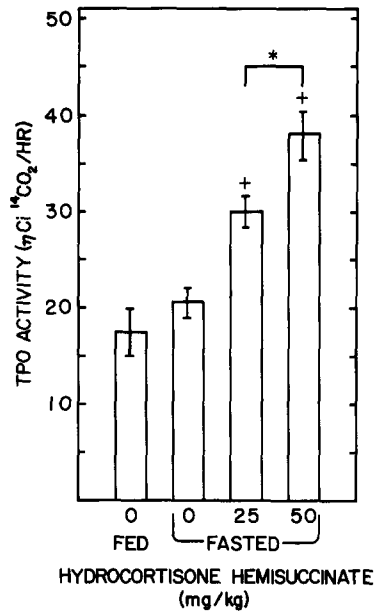

Fig. 2. TPO activity in fed or fasted rats measured over a 90-min period beginning 3.5 hr after injection of 0, 25, or 50 mg/kg of hydrocortisone hemisuccinate. Bars represent means ± S.E.M. Key: (*) $P \leq 0.05$, (†) $P \leq 0.01$ compared with fasted control. The number of control fed animals was 5; of control fasted, 18; of fasted 25 mg/kg, 14; and of fasted 50 mg/kg, 25.

RESULTS

In agreement with previous *in vitro* observations [16, 17], 24 hr of fasting failed to augment basal TPO *in vivo* above levels seen in animals fed *ad lib.* during the same period (Fig. 2). Hydrocortisone-21-sodium succinate elevated TPO activity by 44 or 84% above the fasting basal level at the 25 or 50 mg/kg doses respectively. A 50 mg/kg dose of the steroid produced a detectable increase in TPO activity by 1 hr, peak activity was reached in 2 hr, and activity returned to that of vehicle-injected controls by 5 hr (Fig. 3). Enzyme activities in comparable animals, when assessed by an *in vitro* procedure [21], were: controls, 3.5 ± 0.5 (6); 2 hr, 7.2 ± 0.8 (6); 4 hr, 10.6 ± 1.0 (5); 6 hr, 6.3 ± 0.5 (4); and 10 hr, 4.9 ± 0.5 (4); in μmoles kynurenine formed per hr per g liver (mean ± S.E.M.).

When given 0.5 hr before [^{14}C]tryptophan, 12 or 16 mmoles of intragastrically administered glucose, fructose, or glycerol prevented the steroid-induced increase in TPO activity (Table 1). Lesser doses of either carbohydrate had lesser effects and, in general, the extent of TPO inhibition was directly proportional to the infused dose. Of the compounds tested, glycerol was the most potent in preventing the increase in TPO activity due to hydrocortisone hemisuccinate and was effective at the 4 mmole dose. Fructose and glucose were less potent: doses of at least 8 and 12 mmoles of each, respectively, were required in order to prevent steroid-induced increases in TPO activity. Table 2 presents data on the relationship between the time of glucose administration relative to the time of testing and the effectiveness of steroid in elevating TPO activity. Coadministration of glucose and hydrocortisone did not prevent the hormonally induced increase in TPO activity measured 4 hr later whereas glucose attenuated the TPO elevation if glucose was infused closer to the time of TPO measurement. The implication of these results, that glucose does not act on transcription or translation but on the activity of existing

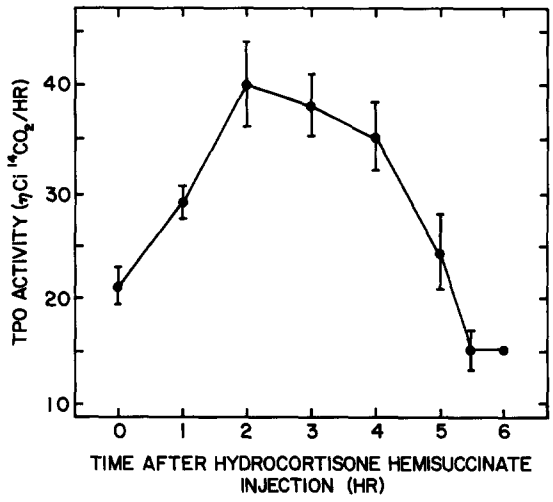

Fig. 3. TPO activity at various times after injection of 50 mg/kg hydrocortisone hemisuccinate. Animals were fasted overnight (16 hr) before steroid injection. The number of animals at zero time was 17; at 1 hr, 9; at 2 hr, 6; at 3 hr, 5; at 4 hr, 24; at 5 hr, 5; at 5.5 hr, 5; and at 6 hr, 1.

Table 1. Suppression of *in vivo* tryptophan oxygenase (TPO) activity by intragastrically administered compounds*

Carbohydrate load (mmoles)	TPO activity (nCi $^{14}CO_2$/hr)		
	Glucose	Fructose	Glycerol
0	35.6 ± 3.2 (39)	35.6 ± 3.2 (39)	35.6 ± 3.2 (39)
4	50.6 ± 8.4 (6)	29.2 ± 6.7 (5)	17.0 ± 5.4‡ (4)
8	26.2 ± 6.1 (3)	14.6 ± 2.8‡ (5)	23.0 ± 3.6§ (8)
12	8.3 ± 3.4‡ (6)	12.5 ± 4.4‡ (3)	
16	7.4 ± 2.1† (5)	4.8 ± 1.2‡ (5)	11.8 ± 5.7‡ (7)

* Glucose, fructose, or glycerol (0, 4, 8, 12, or 16 mmoles) was administered by gastric cannula 0.5 hr before [^{14}C-2]tryptophan and 4 hr after 50 mg/kg hydrocortisone-21-sodium succinate to animals that had been fasted for 16 hr before the experiment. TPO activity was estimated from expired radioactivity accumulated 30, 45, and 60 min after carbohydrate intubation. All values are means ± S.E.M. for TPO activity. Numbers in parentheses are the numbers of animals.

† $P < 0.02$, compared with vehicle (0 mmole) intubation.

‡ $P < 0.01$, compared with vehicle (0 mmole) intubation.

§ $P < 0.05$, compared with vehicle (0 mmole) intubation.

enzyme, is also supported by the results in Fig. 4. TPO activity was also determined on some comparably treated animals (using method in [21]); control values were 3.2 ± 0.5 (3) μmoles kynurenine formed per hr per g liver, compared with 17.2 ± 0.9 (6) for those treated with Triamcinolone. TPO activity was elevated at 12 and 24 hr following subcutaneous Triamcinolone. This chronic elevation was blocked within 45 min following a 16 mmole intragastric fructose load whereas the blockade diminished at 2.75 or 4.75 hr compared to that obtained by 1.25 hr, at which time the blockade was maximal.

DISCUSSION

Hydrocortisone-induced changes in *in vivo* TPO activity, estimated by the expiration of labeled carbon dioxide, followed a time course similar to that reported using *in vitro* measures of TPO activity [16]. The two estimates differ quantitatively, however. Triamcinolone raised TPO activity 2.5-fold when measured by the *in vivo* procedure (from 5.5 to 13.8 μmoles of radioactive $^{14}CO_2$/hr) but by 5.4-fold when measured by the *in vitro* procedure using comparably treated animals (from 3.2 to 17.2 nmoles kynurenine formed per hr per g liver). Similarly, hydrocortisone-21-sodium succinate (50 mg/kg) increased *in vivo* TPO activity by 1.7- to 2-fold (Figs. 2 and 3), whereas *in vitro* activity was increased 3-fold. Similar quantitative differences between *in vitro* TPO activity measurements and *in vivo* measurements of tryptophan-derived $^{14}CO_2$ have been reported previously [6, 22]. Most likely, these reflect the differences in enzymic environment in these two preparations. Additionally, however, the specific activity of liver tryptophan following *in vivo* tryptophan administration might be expected to fall steadily, both because of catabolism and because of dilution with incoming endogenous tryptophan. As a result, the *in vivo* procedure most likely underestimated true TPO activity. Nevertheless, both the general similarity in the time course for corticoid induction of TPO and the demonstration of glucose repression of such induction support the view [6, 20] that *in vivo* production of carbon dioxide from intragastrically administered [2-^{14}C]tryptophan reflects TPO activity.

The present results also indicate that *in vivo* glucose repression of steroid-induced elevations in tryptophan-derived $^{14}CO_2$ was produced by fructose and glycerol. The rate of the decrease in TPO activity following administration of glucose, fructose or glycerol (Table 1 and Fig. 4) is in accord with a feedback-repression mechanism rather than inhibition of polynucleotide or protein synthesis which requires several hours to decrease TPO activity [7]. This conclusion is further supported by the observations that concurrent administration of glucocorticoids and glucose did not prevent a subsequent rise in TPO activity (Table 2) (as it does for tyrosine transaminase [18]), that fructose transiently but rapidly inhibited the chronic increase in TPO activity

Table 2. Effect of time of glucose administration on degree of TPO inhibition*

Time (hr)	Inhibition (%)
No glucose	—
0	5
2.0	9
3.0	51
3.5	73

* Glucose (7.8 mmoles; 1.4 g/2 ml H_2O) was intubated at the time indicated after injection of hydrocortisone-21-sodium succinate (25 mg/kg). [2-^{14}C]Tryptophan (1 μCi; 38.4 Ci/mole) was intubated 4 hr later, and the expiration of radioactive carbon dioxide was measured over the next 2 hr. Liberation was linear over the period between 0.5 and 1.5 hr and the slope of the line was taken as measure of enzyme activity. The rate for animals given hydrocortisone-21-sodium succinate but not glucose (—) was 18 nmoles/hr, while that for animals given glucose and hydrocortisone hemisuccinate together (0 time) was 17 nmoles/hr. Percent inhibition was calculated as 100 times (1 minus the ratio of the rate for glucose-treated animals to that for the animals receiving steroid alone). Animals were fasted overnight before beginning the experiment.

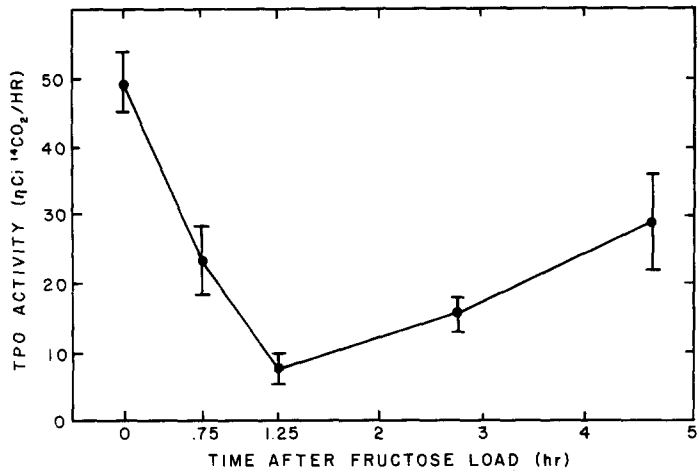

Fig. 4. Time course of fructose disruption of Triamcinolone-induced increases in TPO activity. Triamcinolone (40 mg/kg) was administered 24 and 16 hr before animals were given 16 mmoles of fructose by gavage. The number of animals at zero time was 5; at 0.75 hr, 3; at 1.25 hr, 3; at 3 hr, 3; and at 4.75 hr, 4.

produced by the synthetic glucocorticoid, Triamcinolone (Fig. 4), and that enzyme activity returned toward its initial elevated level within 5 hr after carbohydrate administration (Fig. 4). It is unlikely that this is the time required for renewed hepatic TPO synthesis to occur following a carbohydrate block in synthesis, since glucocorticoids can clearly elevate TPO activity in 1 hr (Fig. 3). Rather, it is more likely that this delay represents the period required for termination of the metabolic actions of the carbohydrate.

Feedback-repression generally involves an end-product of a metabolic pathway and, in the present experiments, the state of this product must be influenced by glucose, fructose and glycerol. The most likely candidates for feedback repressors in this instance are the nucleotides of the respiratory chain, NADH and NADPH [4, 11, 12]. Since the repressing potency of the compounds used followed the order glycerol > fructose > glucose and this order is opposite to their position in the glycolytic pathway, it would be interesting to determine whether the ability of these compounds to regenerate NADH or NADPH after their intragastric administration follows the same order as their repressive potency.

The present findings have demonstrated that intragastric carbohydrate infusions attenuate the increase of TPO activity that follows acute or chronic glucocorticoid treatment. Such modulation of *in vivo* TPO activity by the intake of dietary carbohydrates may be one example of how specific preventative and reparative actions ensue following glucocorticoid administration [23] or general adrenocortical activation by stress [24]. For example, serum tryptophan concentration is a determining factor for the aggregation of hepatic polyribosomes [25] and brain serotonin biosynthesis [26, 27]. One of the many possible actions of the carbohydrate repression of TPO appears to be a diversion of tryptophan from degradation by the kynurenine pathway [5, 6] and, therefore, possibly towards greater utilization in protein anabolism or brain serotonin biosynthesis. Relationships between hepatic, plasma, and brain tryptophan levels and TPO activity following glucocorticoid or carbohydrate administration are presently under investigation.

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