The Role of Dietary Protein in Generating Daily Rhythms in Rat Liver Tryptophan Pyrrolase and Tyrosine Transaminase

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Groups of rats exposed to light for 12 hr daily were given access to an 18% casein diet or to a protein-free diet at different times of day or night, and killed 24 hr later. Tryptophan pyrrolase activity rose significantly during the dark period (by 34%–67%) in all animals. Tyrosine transaminase activity, however, rose during the dark period (by 163%) *only* among those eating protein. In rats given access to a protein-containing chow diet in the afternoon following an overnight fast and killed 2 or 4 hr later, tryptophan pyrrolase activity did not increase after food consumption, but tyrosine transaminase activity rose by 120%–140%. If animals consumed chow for 12 hr/day, starting either at the onset of darkness or 6 hr later, the daily increases in tryptophan pyrrolase began several hours before animals were given access to food rather than after food ingestion, while the daily increases in tyrosine transaminase were always noted immediately after eating. These observations confirm findings which indicate that the cyclic ingestion of dietary protein is of major importance in generating the tyrosine transaminase rhythm, but is of little significance in producing the tryptophan pyrrolase rhythm. Variations in plasma tryptophan concentration did not correlate well with tryptophan pyrrolase activity in any group fed a diet containing protein; plasma tryptophan concentrations did not rise prior to the increases in tryptophan pyrrolase activity, nor did they fall after these increases. Hence, the normal daily rhythm in tryptophan pyrrolase activity apparently neither results from nor elicits the plasma tryptophan rhythm.

A CTIVITIES of the hepatic enzymes, tyrosine transaminase (L-tyrosine: 2-oxoglutarate amino transferase, E C 2.6.1.5)[1-4] and tryptophan pyrrolase (L-tryptophan: oxygen oxidoreductase, E C 1.13.1.12),[5-7] show characteristic daily rhythm in rats. The rhythm in tyrosine transaminase activity is primarily due to the cyclic ingestion of dietary protein,[8,9] whereas the mechanisms responsible for the diurnal variation in tryptophan pyrrolase activity are not fully understood. Because the injection of corticosterone[10] or tryptophan[11] increases tryptophan pyrrolase activity as measured in vitro, cyclic daily variations in the amounts of these compounds normally delivered to the liver via the systemic or portal circulations may influence tryptophan pyrrolase periodicity.

This report compares the roles of dietary protein in generating the rhythms in tyrosine transaminase and tryptophan pyrrolase activity. It also examines the correlations between physiologic variations in tryptophan pyrrolase activity and plasma tryptophan concentration.

MATERIALS AND METHODS

Treatment of Animals

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass.) weighing 150–250 g or 250–350 g were housed six per cage, exposed to light (Vita-Lite, Duro-Test Corporation, North Bergen, N.J.; 40 to 60 μW/sq cm) for 12 hr daily, and except where noted, given free access to water and Big Red Laboratory Animal Chow (Agway Company, Syracuse, N.Y.; 24% protein). Animals used in rhythm studies were maintained under the specified conditions for at least 3 wk before being killed.

Compositions of Diets

The casein diet was composed of 180 g casein, 207 g dextrose, 167 g sucrose, 207 g dextrin, 150 g Mazola oil, 10 g vitamin mix,[8] 40 g Rogers–Harper salt mix,[12] 4 ml choline (50% wt/vol), 35 g agar, and 1000 g water. (Casein was 18% of the diet weight before water was added; 15% of the dry weight was fat.)

The protein-free diet contained 220 g sucrose, 207 g dextrin, 207 g dextrose, 150 g Mazola oil, 10 g vitamin mix, 40 g Rogers-Harper salt mix, 4 ml choline, 40 g agar, and 1000 g water (17% of dry weight was fat).

Analytical Procedures

Animals were decapitated, and their blood collected from the cervical wound in heparinized tubes and then centrifuged. Plasma was frozen and subsequently assayed for tryptophan according to Denckla and Dewey.[13] The livers were quickly removed, weighed, frozen on dry ice, and then assayed for tryptophan pyrrolase activity by the method of Knox and Mehler,[14] as modified by Feigelson and Greengard,[15] and for tyrosine transaminase activity according to Diamondstone.[16] We obtained the tryptophan pyrrolase activities by assaying the 20,000 g supernatant of a liver homogenate in the absence of saturating concentrations of hematin, so that only the holoenzyme present at the time of death would be measured. Duplicate assays were performed in the presence of saturating amounts of cofactor; tryptophan pyrrolase activity responded similarly to all experimental manipulations regardless of the assay procedure. Food consumption was recorded in all experiments; since liver weight varies diurnally,[17] partly due to changes in glycogen content,[17,18] we corrected hepatic enzyme activities for changes in organ weight. We also corrected for variations in body weight since liver weight represents a constant proportion of body weight in adult rats, and since the animal weights in some of our experiments varied widely. Enzyme activities are thus expressed as μmoles/min/liver/100 g body weight. (The conclusions drawn from the data are the same whether activities are expressed in this manner or per gram of tissue.)

RESULTS

Persistence of Tryptophan Pyrrolase Rhythm in Rats Consuming a Diet Lacking Protein

Studies have shown that hepatic tyrosine transaminase activity is depressed and lacks a 24-hr periodicity among rats kept on a protein-free diet,[8] and that tryptophan pyrrolase activity is decreased in rats on a low-protein diet (6%).[19] This latter observation suggested that the cyclic ingestion of dietary protein might participate in eliciting the tryptophan pyrrolase rhythm, as well as the transaminase rhythm.

To examine this possibility, animals were presented with a casein diet ad lib. for 2 days, after which controls continued on this diet. At different times of day and night, experimental animals were switched to the protein-free diet for a 24-hr period, then killed. Food consumption was rhythmic (animals consumed more food at night) and the amount of food consumed over corresponding intervals was similar for both casein-fed animals and animals given the protein-
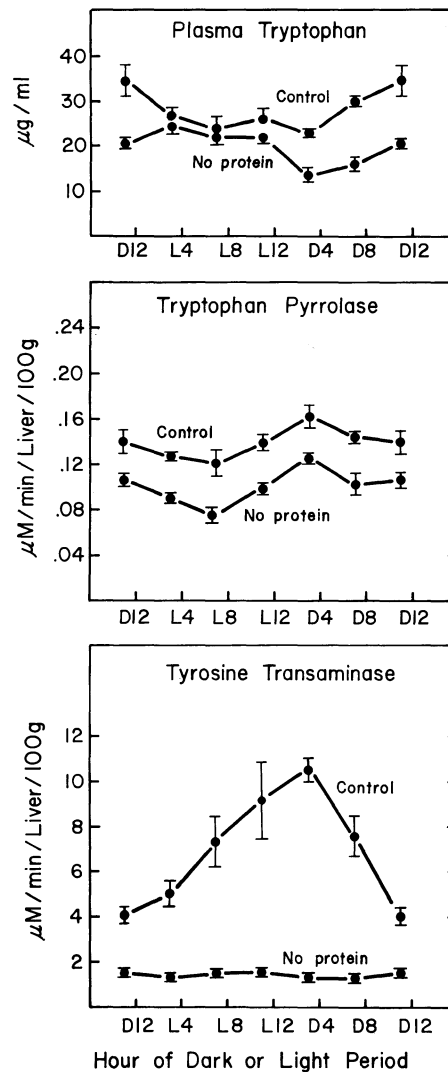
Fig. 1. Effects of protein deprivation of the daily rhythms in tryptophan pyrrolase and tyrosine transaminase activity and plasma tryptophan concentration. Rats had access to diets containing either 18% (control) or 0% (no protein) casein for 24 hr before death. L and D indicate light and dark periods, respectively, followed by the number of hours since the beginning of each period. Vertical bars represent standard errors of the mean.

free diet. The phasing and amplitude of the tryptophan pyrrolase rhythm did not differ between rats fed the two diets (Fig. 1). The increase in tryptophan pyrrolase activity over nadir values in casein-fed rats was 34% ($p < 0.01$) compared with a 67% ($p < 0.001$) increase in those on the protein-free diet. Tyrosine transaminase activity (Fig. 1) exhibited a diurnal variation of 163% ($p < 0.001$) in the casein-fed animals but did not vary significantly in the rats on the protein-free diet. Animals on the protein-free diet showed a 27% ($p < 0.001$) decrease in tryptophan pyrrolase activity averaged over the 24-hr period, and a much greater decrease in mean tyrosine transaminase activity (80%, $p < 0.001$). These observations suggest that although consumed dietary protein influences the *average* daily activity of both enzymes, it is a major factor in generating only the tyrosine transaminase rhythm; the daily pyrrolase rhythm persists without dietary protein.

Variations in plasma tryptophan concentration among control animals

(Fig. 1) did not correlate remarkably with tryptophan pyrrolase activity. Plasma tryptophan increased during the dark period, although pyrrolase was most active during the first hours of darkness. Thus, increases in pyrrolase activity did not precede falls in plasma tryptophan; the daily period of low pyrrolase activity was not associated with peak plasma tryptophan levels; and increases in plasma tryptophan levels did not precede the peak in pyrrolase activity.

Responses of Tryptophan Pyrrolase and Tyrosine Transaminase Activities in Fasted Rats to the Ingestion of Dietary Protein

Casein hydrolysates given by stomach tube increase rat hepatic tyrosine transaminase activity without altering tryptophan pyrrolase.[20] We examined the effects on the two enzymes of the consumption of diets either containing or lacking protein.

Animals were placed in clean cages and deprived of food at the onset of darkness (9 p.m.). Between noon and 2 p.m. the next day, groups of rats were continued on the fast, or given free access to chow or the protein-free diet, and killed 2 or 4 hr later. After 4 hr, animals given the protein-free diet had consumed an identical amount of food (expressed as dry weight) as animals fed the chow diet. Tryptophan pyrrolase activities in rats on the protein-containing chow diet did not increase 2 or 4 hr after food presentation compared with activities of fasted animals or animals on the protein-free diet (Fig. 2). In marked contrast, tyrosine transaminase activity rose sharply within 2 hr in rats given the chow diet to values 140% above those of fasted animals ($P < 0.001$), and 113% above those of animals on the protein-free diet ($P < 0.001$), and remained elevated at 4 hr (123% above fasted animals and 290% above animals on the protein-free diet [$p < 0.001$]). Tyrosine transaminase activities in animals given the protein-free diet did not significantly exceed those of fasted animals at either 2 or 4 hr after the presentation of food. Thus, dietary protein can rapidly increase the activity of tyrosine transaminase, but not of tryptophan pyrrolase.

Effects of Dissociating the Daily Lighting and Feeding Cycles

Rats allowed to eat only during the first 4 hr of the daily light period develop a 12-hr phase shift in the daily activity rhythms of both tyrosine transaminase and tryptophan pyrrolase compared with animals eating ad lib.[7,21] Moreover, animals kept on a reversed lighting schedule and given food ad lib. also show a 12-hr shift in the transaminase rhythm.[22] Under the latter experimental conditions, the daily rhythm in food consumption also exhibits a 12-hr phase shift. Hence, the entrainment of both the transaminase and pyrrolase rhythms by the light/dark cycle could reflect the known behavioral relationship of eating to the daily dark period, rather than changes in a cyclic humoral or neural input to the liver.

To determine whether the light/dark cycle or the food intake rhythm is the primary regulator of both hepatic enzyme rhythms, these two rhythms were experimentally dissociated. Controls were exposed to light from 9 a.m. to 9 p.m. and given free access to chow only during darkness. Experimental animals were exposed to light from 3 a.m. to 3 p.m. (i.e., 6 hr out of phase with
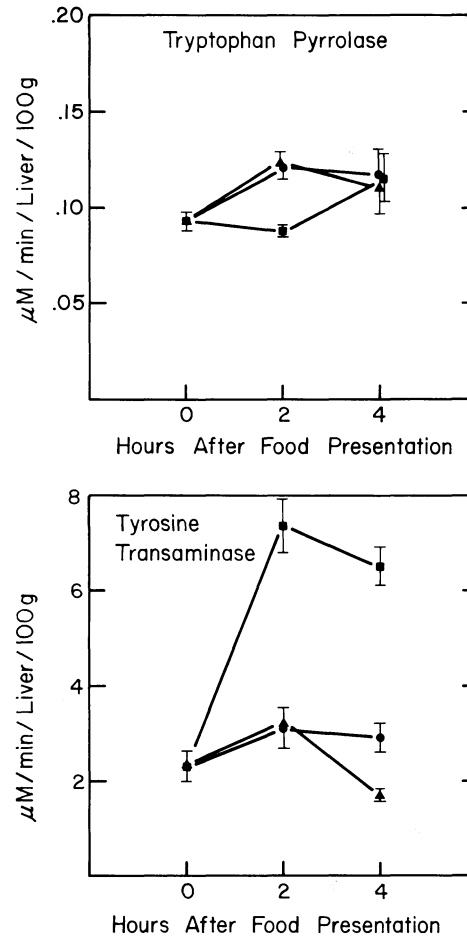
Fig. 2. Acute changes in the activities of tryptophan pyrrolase and tyrosine transaminase in fasted rats given access to a diet either containing or lacking protein. Rats fasted overnight were presented with either normal laboratory animal chow (24% protein) (squares) or a protein-free diet (triangles) the next afternoon; other animals continued to fast (circles). Vertical bars are standard error of the mean.

control animals) and presented with food from 9 p.m. to 9 a.m. In both groups the daily rhythm in tyrosine transaminase activity was phase locked with the time of food consumption (Fig. 3), that is, transaminase activity did not increase until after the ingestion of food. However, tryptophan pyrrolase activity in both control and experimental animals began to increase *before* food presentation (Fig. 3). Further, when food was *not* available during the first 6 hr of darkness, the elevation in pyrrolase activity, which had begun during the last hours of light, continued into the dark period such that most of the total daily increase occurred prior to food consumption. These results support the notion that the daily increase in tryptophan pyrrolase is not generated by food consumption. Thus, the daily oscillation in pyrrolase activity is not necessarily phase locked with food consumption, even though its daily peaks normally coincide with the time of maximal food consumption.

Plasma tryptophan concentration was phase locked with the ingestion of dietary protein (Fig. 3). Tryptophan levels were lowest just before food presentation, and increased to peak values soon after food became available. Variations in plasma tryptophan concentration were therefore poorly correlated with
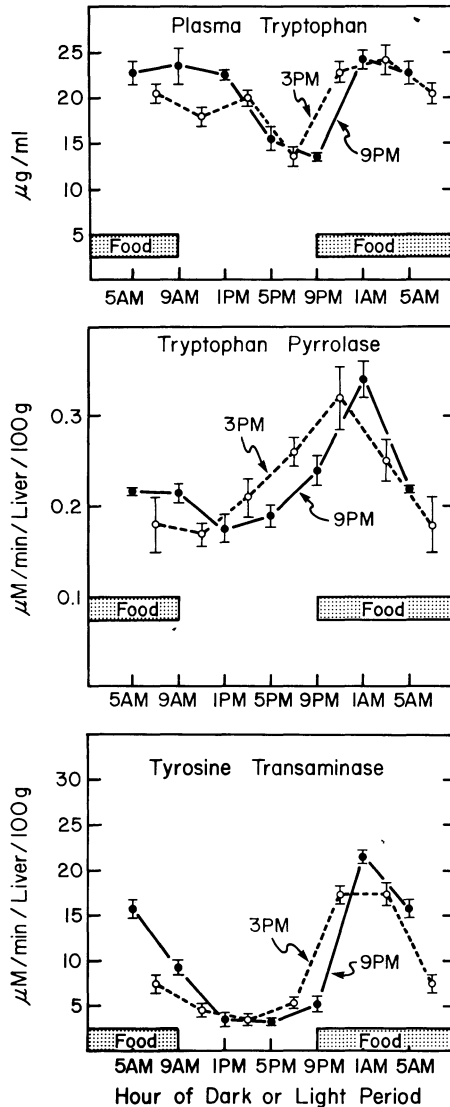
Fig. 3. Effects of dissociating daily lighting and feeding cycles on the phase relationships of the tryptophan pyrrolase and tyrosine transaminase rhythms. All rats had access to rat chow for 12 hr, beginning at 9 p.m. The daily dark period for control animals was between 9 p.m. and 9 a.m. (solid circles) (food and light schedule in phase), and between 3 p.m. and 3 a.m. for the experimental animals (open circles) (food and light schedule for 6 hr out of phase). Vertical bars represent standard error of the mean.

tryptophan pyrrolase activity: The rise in plasma tryptophan did not precede the rise in tryptophan pyrrolase activity, nor did the rise in pyrrolase activity precipitate a fall in plasma tryptophan concentration. These results indicate that variations in the tryptophan level in the systemic circulation probably do not produce the diurnal variations in tryptophan pyrrolase activity, and, further, that physiologic variations in tryptophan pyrrolase activity do not seem to affect plasma tryptophan concentrations appreciably.

DISCUSSION

These findings confirm previous observations that the daily rhythm in hepatic tyrosine transaminase activity results primarily from the cyclic ingestion of dietary protein,[8] and not from endocrine or neural signals to the liver.[9] They

further demonstrate that the tryptophan pyrrolase rhythm is generated by a different mechanism and is not primarily a response to cyclic food consumption, and that the daily rhythm in tryptophan pyrrolase activity neither results from nor causes the normal rhythm in plasma tryptophan concentrations.

The daily rhythm in tyrosine transaminase activity disappears in starved animals or those consuming only protein-free diets,[8] but persists in adrenalectomized,[1] hypophysectomized,[1] or reserpinized rats so long as the daily rhythm in protein consumption is maintained.[23] Among animals trained to eat equal amounts of food each hour of the day and night, the transaminase rhythm either disappears or persists at a considerably reduced amplitude.[24] Adrenalectomy abolishes the small residual rhythm.[24] However, any contribution of the daily rhythm in plasma glucocorticoids to the transaminase rhythm is usually masked by the larger inductive effect of dietary amino acids, peak concentrations of which are normally transported to the liver at about the time of day that plasma glucocorticoid levels are highest. These findings confirm previous observations that rats given access to food only during specified daily intervals exhibit corresponding shifts in tyrosine transaminase periodicity,[21] i.e., enzyme activity always rises maximally just after the animals eat protein (Figs. 2 and 3). Thus, the daily rhythm in tyrosine transaminase activity seems principally dependent on cyclic food consumption. Other environmental inputs such as the light/dark cycle influence the transaminase rhythm, but only indirectly, by influencing the pattern of food consumption.

The administration of casein hydrolysates by stomach tube elevates tyrosine transaminase activity by accelerating the synthesis of the enzyme protein.[20] The daily enzyme periodicity in untreated animals also appears to reflect changes in the rate of enzyme protein synthesis.[25] The transaminase rhythm may be destroyed by the elimination of a single amino acid, tryptophan, from the diet, and this rhythm may be restored by feeding rats diets lacking protein and containing only this amino acid.[8] Tryptophan is the least abundant amino acid in most proteins[26] and limits polysome aggregation in the liver.[27] By increasing the flow of tryptophan and other potentially limiting amino acids to the liver, the cyclic ingestion of dietary protein probably accelerates the synthesis of tyrosine transaminase in normal animals, thus producing the enzyme rhythm.[9]

The mechanism responsible for the daily rhythm in tryptophan pyrrolase activity is different from the mechanism that causes the transaminase rhythm. The pyrrolase rhythm persists among animals consuming protein-free diets (Fig. 1), and the daily increase in enzyme activity can be dissociated from the time of initial food consumption (Fig. 3). Furthermore, although mean daily pyrrolase activity is somewhat depressed in animals on protein-free diets (Fig. 1), the ingestion of dietary protein in the afternoon following an overnight fast fails to elevate tryptophan pyrrolase activity (Fig. 2). These observations all suggest a minor role for dietary protein in the generation of tryptophan pyrrolase periodicity. It seems paradoxical that increased tryptophan availability following the ingestion of a protein-containing meal should stimulate the activity of tyrosine transaminase, an enzyme that metabolizes a different amino acid, but have little physiologic effect on the activity of tryptophan pyrrolase, for which it is the natural substrate.

The primary signal to the liver cells that causes the daily rhythm in trypto-

phan pyrrolase activity remains to be elucidated; the most likely candidate at the present time would appear to be the rhythm in plasma glucocorticoid concentration. Pharmacologic doses of glucocorticoid do cause rapid and major increases in the activity of this enzyme,[10] probably by accelerating its rate of synthesis.[28] However, there is no conclusive evidence that changes in plasma glucocorticoid concentrations of the magnitude likely to occur normally have any effect on tryptophan pyrrolase. The daily peak in plasma corticosterone does precede the peak in pyrrolase activity observed in untreated rodents,[5] and removal of the adrenals does depress, but not extinguish, the pyrrolase rhythm.[5]

On the basis of the observation that rats fed a single daily meal during the first 4 hr of the light period exhibited a 12-hr shift in tryptophan pyrrolase activity without a clear shift in the corticosteroid rhythm, it was concluded that the hormone rhythm does not generate the enzyme rhythm.[7] However, in that study, tryptophan pyrrolase activity at most of the times tested was found to be higher in animals fed only at the beginning of the light period than in controls fed ad lib. This suggests that the experimental manipulation might have stressed the animals and thereby influenced enzyme activity. Indeed, plasma corticosteroid levels in these animals peaked during the end of the dark period rather than at the end of the light period as in normal rats, and thus might have produced the subsequent rise in enzyme activity.

In our experiments, plasma tryptophan concentrations did not correlate well with changes in tryptophan pyrrolase activity. In no group given access to a normal protein-containing diet was a rise in pyrrolase activity preceded by peak plasma tryptophan concentrations or followed by a fall in plasma tryptophan. When the daily feeding and lighting cycles were separated, the daily increases in tyrosine transaminase activity and plasma tryptophan concentration occurred immediately after protein consumption, while the increase in tryptophan pyrrolase activity began prior to the ingestion of dietary protein.

The functional significance of the daily rhythms in tryptophan pyrrolase and tyrosine transaminase activity remain to be elucidated. It is not even certain that increases in tyrosine transaminase or tryptophan pyrrolase activity measured in vitro are associated with increases in the rates at which the liver degrades tyrosine or tryptophan in vivo. If the activities of these enzymes are elevated by treating animals with pharmacologic doses of glucocorticoids, the in vivo conversion of DL-tryptophan-2-^{14}C or L-tyrosine-1-[14]CO_2 is not appreciably accelerated, nor is the ability to clear tryptophan proportionately enhanced in isolated, perfused livers taken from such animals.[29] In contrast, induction of tryptophan pyrrolase by another pharmacologic agent, alpha-methyl tryptophan, is associated with major reductions in circulating tryptophan.[30] The daily rhythm in plasma tyrosine persists in subjects deprived of dietary protein,[31] while the enzyme rhythm in rats is largely or totally extinguished:[8] The present studies indicate that plasma tryptophan is similarly independent of tryptophan pyrrolase activity in the normal animal (Figs. 1 and 3). Thus, these hepatic enzymes are probably not of major significance in generating the normal variations in their circulating substrates.

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