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DAILY VARIATIONS IN THE CONCENTRATIONS OF INDIVIDUAL AMINO ACIDS IN RAT PLASMA

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Summary

Daily rhythms have been described in the plasma concentrations of tyrosine and phenylalanine in the rat (1), and of tryptophan and total amino acids in the mouse (2,3). However, no data appear to be available on daily variations in the concentrations of other plasma amino acids except in the chicken and the human (4,5,6,7). The determination of whether diurnal rhythmicity is common to the plasma levels of most amino acids, and most mammals, or whether such temporal changes are associated in rats only with tyrosine and phenylalanine is essential for the characterization of the physiological mechanisms that control plasma amino acid levels. It would also be useful in assessing the significance of changes in plasma amino acid levels observed in rats several hours after particular experimental manipulations. This study describes the daily rhythmic changes that occur in the concentrations of 12 amino acids in rat plasma.

Materials and Methods

Seventy-two Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass.) weighing 150-200 g were caged in groups of four and exposed to light (Vita-Lite, Duro-Test Corp., North Bergen, N. J.) between 9AM and 9PM daily for two weeks. Big Red Lab Chow (Country Best, Agway, Syracuse, N. Y.) and water were provided ad libitum. On the day of the experiment, groups of 12 rats were decapitated at 4-hour intervals. Blood was collected from the cervical wound in heparinized tubes, placed in an ice bath, and centrifuged at the end of each collection period. Plasma was then frozen until assay.

The concentrations of all of the amino acids studied except tryptophan were measured using a Beckman Model 120C Amino Acid Analyzer. Tryptophan was determined by the fluorimetric method of Denckla and Dewey (8).

Results

Most of the plasma amino acids studied attained their lowest values around 6PM, 3 hours before the onset of darkness, and their peaks 12 hours later, around 6AM (Table 1). The concentrations of glutamic acid and glycine reached their nadirs at 10PM (i.e., at the end of the first hour of darkness: D_1). The daily rhythm in plasma tryptophan was somewhat out of phase with the rhythms of the other amino acids: tryptophan levels were highest at 2AM (D_5) and lowest 8 hours later at 10AM (L_1). Except for leucine, the concentrations of individual amino acids in plasma obtained at 10AM (L_1) and 2PM (L_5) were all within the ranges observed by other investigators who sampled blood at a single time of day (9,10,11,12). Most papers describing the concentrations of leucine in rat plasma have reported levels of 12 to 15 μmoles/100 mg (10,11). The factors responsible for the higher levels observed in our animals are not apparent.

The ratios of the 6AM to the 6PM plasma concentrations and of the highest to lowest plasma concentration were calculated for each amino acid studied (Table 1). These ratios were greatest for threonine, valine, leucine, and phenylalanine, and least for glycine, alanine, and tyrosine.

TABLE 1

Daily Variations in the Concentrations of 12 Amino Acids in Rat Plasma*

Amino Acid	Time of Day						Ratios	
	10AM	2PM	6PM	10PM	2AM	6AM	High/Low	6AM/6PM
Threonine	.467±.04	.303±.02	.269±.02	.335±.01	.470±.02	.466±.02	1.75†	1.73
Serine	.302±.02	.241±.01	.247±.01	.283±.01	.323±.01	.331±.01	1.37†	1.34
Glutamate	.090±.01	.104±.01	.108±.01	.085±.003	.102±.02	.135±.01	1.59†	1.25
Glycine	.349±.01	.321±.02	.350±.02	.285±.004	.314±.02	.338±.015	1.23†	0.97
Alanine	.572±.04	.518±.02	.495±.03	.609±.03	.617±.04	.640±.02	1.29§	1.29
Valine	.258±.02	.235±.01	.206±.01	.224±.01	.306±.03	.360±.01	1.75†	1.75
Methionine	.071±.003	.060±.002	.055±.001	.067±.004	.076±.005	.080±.004	1.46†	1.46
Isoleucine	.124±.01	.110±.01	.100±.002	.119±.01	.136±.01	.165±.004	1.65†	1.65
Leucine	.214±.01	.185±.01	.170±.003	.210±.01	.226±.01	.275±.005	1.62†	1.62
Tyrosine	.127±.01	.105±.003	.089±.005	.096±.01	.106±.01	.109±.007	1.43§	1.23
Phenylalanine	.078±.002	.066±.002	.063±.001	.079±.003	.086±.01	.092±.003	1.46†	1.46
Tryptophan	.098±.004	.105±.003	.105±.002	.125±.005	.132±.006	.122±.004	1.35†	1.16

*Data are expressed as μmoles of amino acid/ml of plasma ± S.E.M.
†$P<0.001$; highest concentration compared with lowest concentration.
§$P<0.01$; highest concentration compared with lowest concentration (Student's t-test).

Discussion

These data suggest that in rats, as in humans (4,5,6), the concentrations of most or all of the amino acids in plasma exhibit characteristic diurnal rhythms. Also as in humans, most of these rhythms are in phase with each other. The amino acids measured fall into several groups, defined by the amplitudes of their daily rhythms: for example, those amino acids having relatively great daily fluctuations (greater than 60%; threonine and the branched-chain amino acids, valine, isoleucine, and leucine) and those showing small variations (less than 30%; glycine and alanine). In general, amino acids which are essential or which are present in the body only in relatively small amounts show the greatest tendency to temporal variability (6). A similar correlation between total body pool size and amplitude of the plasma amino acid rhythm has been noted in humans (6). Plasma amino acid concentrations in humans tend to reach their nadirs around 2 to 4 AM or 6 to 12 hours after the corresponding nadirs observed in the rat (5). This temporal difference probably reflects the tendency of the rat to consume most of its food during the dark portion of each 24-hour period (13).

That the plasma levels of all of the amino acids studied here varied diurnally does not seem surprising, inasmuch as most of the processes known to cause free amino acids to enter or leave the plasma also undergo daily fluctuations in activity (6). For example, food consumption occurs cyclically; thus the overflow of dietary amino acids from the portal to the systemic circulation might be expected to be greater in rats soon after the onset of darkness than at times of day when the animals eat less. This phenomenon would then cause significant nocturnal increases in the plasma levels of most amino acids; and these elevations do, in fact, occur (Table 1).

Several hormones that influence plasma amino acid levels by facilitating or suppressing amino acid uptake into tissues are known to be secreted preferentially at certain times of day or night. For example, the insulin secreted following food consumption increases the net tissue uptake, and thereby depresses the plasma levels of most amino acids (14). Insulin exerts the opposite effect on plasma tryptophan in the rat; it causes tryptophan concentrations to rise significantly (15). This difference may explain the failure of the plasma tryptophan rhythm to be in phase with the other amino acid cycles. Finally, the activities of at least three hepatic enzymes that metabolize amino acids [tyrosine transaminase (16); tryptophan pyrrolase (2); and phenylalanine hydroxylase (17)] have major temporal fluctuations. The tyrosine transaminase rhythm appears to be a response to the consumption of protein (18).

The physiological significance of most of these rhythms in plasma amino acid concentration awaits discovery. However, one physiological consequence of the tryptophan rhythm already seems apparent. Very small changes in plasma tryptophan concentration which are similar in magnitude to those occurring normally in our animals due to the daily rhythm have recently been shown to "drive" corresponding increases in brain tryptophan and brain serotonin contents (19). This may reflect the disparity between the levels of tryptophan needed to saturate tryptophan hydroxylase (20) and those normally present in the brain (19,21).

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