Effects of Sleep Deprivation on the Periodicity of Tyrosine Metabolism in Mice

We have been using the hepatic enzyme tyrosine aminotransferase (EC 2.6.1.5, L-tyrosine: 2-oxoglutarate aminotransferase) to assess the magnitude of metabolic response to environmental stresses[1]. This enzyme is instrumental in the metabolism of its substrate, tyrosine, initiating its conversion in the liver to carbohydrate intermediates. Although many investigators have reported on the hormonal induction[2–4], diurnal rhythmicity[5–7] and chemical alteration of enzymatic activity[8,9] few studies have examined the potentially important relationship between large increases in enzyme activity and substrate availability[10,11] Accordingly, in this investigation we have determined the effects of prolonged sleep deprivation on the periodicity and activity of tyrosine aminotransferase as well as substrate concentration in both liver and plasma.

Materials and methods. Experimental animals. Adult, male, Swiss Webster mice (Type CD-1, Charles River Breeding Laboratories, Wilmington, Massachusetts) were allowed free access to food (Purina Lab Chow) and water for 1 week prior to the start of the experiment. The animals were held in a room at 70 °F ± 2 with alternating light (06.00–18.00 h) and dark (18.00–06.00 h) so that naturally occurring periodic oscillations might be established and stabilized. Animals were sacrificed at 08.00 h and 20.00 h, times of minima and maxima, respectively, of enzymatic activity.

Sleep deprivation. Mice were deprived of sleep by a slight modification of the technique reported by MARK et al.[12]. Corks (2 cm diameter) are fastened to a metal plate which is placed in a large Plexiglas tank. The water level in the tank is brought to within 1 cm of the top of the corks on which the animals are placed. Hence, the mice must maintain a degree of body tone to avoid toppling into the water. Food and water bottles are suspended on a wire mesh approximately 12 cm above the dry surface of the corks, and the animals have no difficulty in feeding. The mice weighed 30–35 g at sacrifice. Although quantification of food intake was not attempted, animals seemed to take food reasonably well particularly through the first 72 h of sleep deprivation with no significant weight losses during this time. They were exsanguinated by cardiac puncture, and the blood was collected in heparinized tubes. The animals were then sacrificed by cervical dislocation, and the livers were removed. Both plasma and liver samples were frozen until assay.

Assays. Tyrosine aminotransferase was assayed according to the method of DIAMONDSTONE[13] with diethyldithiocarbamic acid incorporated into the assay to prevent the spontaneous oxidation of *p*-hydroxyphenylpyruvic acid. Specific activity is expressed in terms of μmoles of product formed per hour per g liver wet weight.

Tyrosine in liver and plasma was assayed fluorometrically with a Farrand ratio fluorometer using glass filters. Concentrations were determined by the method of WAALKES and UDENFRIEND[14] with filters CS 4–76 and CS 3–110 providing excitation and emission wavelengths of 460 nm and 570 nm.

Results. Figure 1 illustrates that although levels of hepatic tyrosine aminotransferase (TAT) are significantly increased at each of the sampling periods ($p < 0.025$), the periodicity is maintained through 60 h of sleep deprivation with a greater than normal amplitude. However, it should be noted that between 72–84 h periodicity is abolished as a result of marked increases in enzyme induction culminating at 96 h with a specific activity of nearly 2100 units. Thus, the intensity of the stress response increases with time, and maximal enzyme activity occurs between 72–96 h of sleep deprivation.

Figure 2 shows that during the first 60 h of sleep deprivation, hepatic levels of tyrosine are significantly elevated, and at 72 h are nearly 100% above control values ($p < 0.001$). However, between 72 and 96 h values drop precipitously possibly as a result of the marked increases in enzyme activity noted at 84 and 96 h (Figure 1).

Figure 3 demonstrates that no significant alterations occur in plasma tyrosine content through 72 h of sleep deprivation; however, thereafter its concentration sharply decreases while hepatic levels of TAT are increasing 5 to 7 times above control values.

Discussion. The results indicate that sleep deprivation affects the regulation and periodicity of the metabolism of

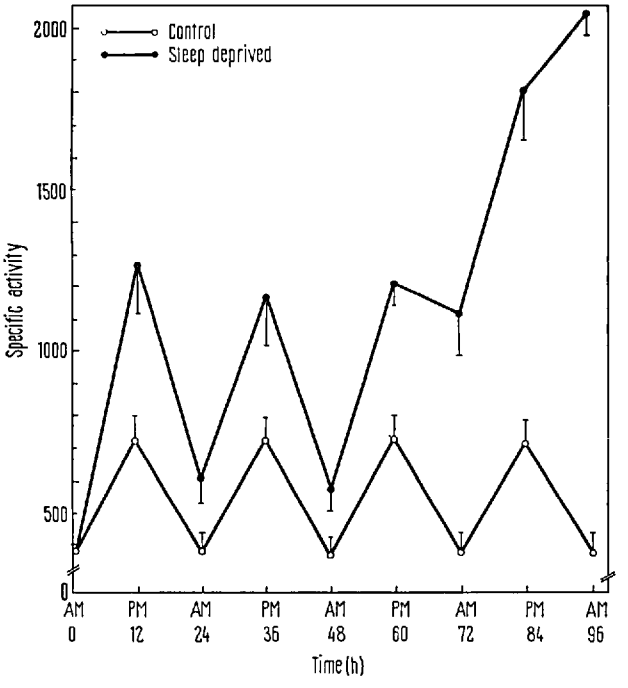

Fig. 1. Effects of sleep deprivation on the daily rhythmicity of hepatic tyrosine aminotransferase. Specific activity units are μmoles *p*-hydroxy-phenylpyruvic acid formed/h/g liver wet weight. Each point represents the mean of a minimum number of 5 animals ± 1 S.E.M.

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tyrosine. Moreover, the magnitude of the effect increases with prolonged sleeplessness particularly in the time period between 72 and 96 h. In a separate experiment, we have noted extremely high levels for plasma corticosterone at these time intervals which correlate with and can explain the high levels of enzyme activity noted. Tyrosine aminotransferase seems to be particularly sensitive to sleep deprivation resulting in extremely high levels of enzyme activity. Similarly, we had previously noted that when mice are exposed to acute cold stress (0–4 °C), there is also a marked effect upon the activity and periodicity of TAT.

Interestingly, hepatic tyrosine concentration is elevated through 84 h of sleep deprivation culminating in a sharp decline at 96 h. In view of the increased activity of tyrosine aminotransferase, it, at first, appears plausible that hepatic and plasma tyrosine levels may decline. However, tyrosine concentrations can be maintained by ingestion, catabolism of protein or conversion from phenylalanine[10], and our work with other enzyme-substrate systems indicates the latter mechanism is probably most responsible for maintaining hepatic and plasma tyrosine.

The method of sleep deprivation used in this study disturbs the periodicity of tyrosine via the induction of its catabolic enzyme during the first 60–72 h of sleeplessness. However, it appears plausible that plasma levels of this amino acid are being maintained most importantly by hepatic repletion (phenylalanine → tyrosine). Further, it is apparent that with prolonged sleep deprivation the stress response intensifies with concommitant increases in plasma corticosterone and enzyme activity. Thus, with limited amino acid availability, enzyme induction may reduce tyrosine concentrations in the liver and plasma, and hence its availability for thyroxin, protein, and catecholamine synthesis. In fact, CURZON and GREEN[15] relate decreases in brain serotonin to an increase in liver tryptophan oxygenase activity in immobilized rats. In a very recent publication, FERNSTROM and WURTMAN[16] confirm these results suggesting that plasma tryptophan concentrations may affect brain serotonin levels. Hence, it appears likely that the precipitous decrease in hepatic and plasma tyrosine concentrations during the final 72–96 h of sleep deprivation may be closely related to the sharp increases in enzyme activity observed during this interval.

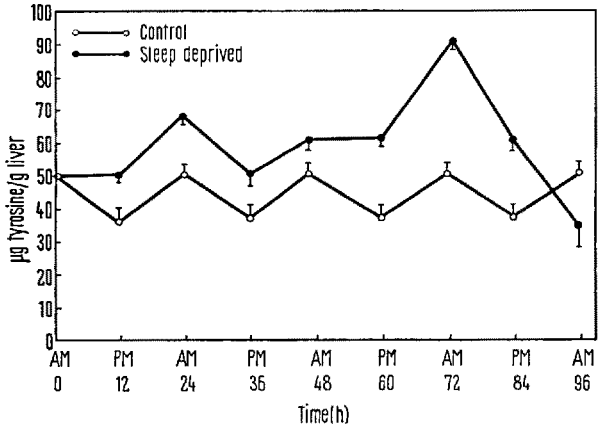

Fig. 2. Effects of sleep deprivation on hepatic levels of tyrosone (µg tyrosine/g liver wet weight). Each point represents the mean of a minimum number of 5 animals, and vertical lines denote ± 1 S.E.M.

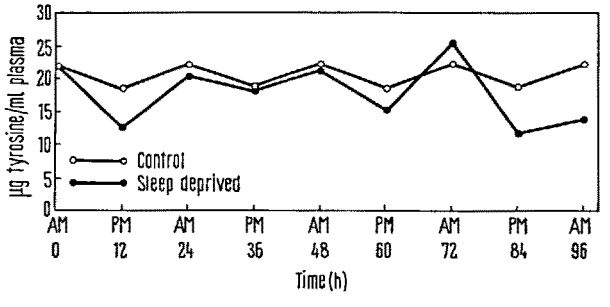

Fig. 3. Effects of sleep deprivation on plasma levels of tyrosine in terms of µg tyrosine/ml plasma. Each point represents the value of pooled plasma from at least 5 animals performed in duplicate.

Résumé. La privation de sommeil pendant 96 h a crée des ruptures dans la périodicité d'enzyme-tyrosine-aminotransférase du foie, à cause de l'induction. Des concentrations hépatiques de tyrosine ont augmenté alors que sa présence dans le plasma fut sans effet. La privation de sommeil affecte non seulement les rhythmes quotidiens naturels, mais, si elle est prolongée, elle intensifie la réponse de «stress» généralisé peut-être au point d'affecter notablement l'effet de l'acide amidé.

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Cumulation of Liver Lipids after Administration of Iron

When peroxidative degradation of polyenic fatty acids in the liver tissue in vivo is activated by divalent iron and ascorbic acid administration, a simultaneous accumulation of neutral liver lipids was found[1]. This effect of iron has not been described sofar. That is the reason why we studied the influence of iron on the cumulation of different liver lipid fractions.

Male white mice (strain H – Konárovice) weighing 20–25 g kept on a standard laboratory diet were used in the experiments.

Iron was administered in solution with ascorbic acid (Fe-AA complex); containing 0.1*M* $FeCl_2$ and 0.2*M* ascorbic acid neutralized by sodium hydroxide solution to pH 7.4. The dose was 0.5 mmol Fe^{2+}/kg. The animals