Pharmaceutical Biology
2004, Vol. 42, No. 1, pp. 1–7

Taylor & Francis
Taylor & Francis Group

Circadian Variations of Biochemical Variables in Aspartame Treated Rats

P. Rajasekar, P. Subramanian and T. Manivasagam

Department of Biochemistry, Faculty of Science, Annamalai University, Annamalainagar, Tamil Nadu, India

Abstract

In a study of the impact of aspartame on biochemical oscillations, we examined the rhythms of blood glucose, plasma cholesterol, and protein and serum aspartate transaminase (AST) in experimental rats. Our results showed acrophase delays in glucose, total protein and advances in AST rhythms and increased mesor (in AST), amplitude (in cholesterol) and decreased amplitude values (in glucose, AST) in aspartame treated animals. Oral administration of aspartame might lead to increased levels of aspartate in brain which could alter the characteristics of biochemical variables possibly by modulating the transmission in several areas/nuclei in brain including retinohypothalamic tract (RHT) and suprachiasmatic nuclei (SCN).

Keywords: Aspartame, biochemical variables, circadian rhythms.

Introduction

The biological clock in mammals is located in the suprachiasmatic nuclei (SCN). (Hannibal, 2002). Circadian clocks govern the timing of development, behaviour, physiology, endocrinology and biochemistry, as well as photoperiodic events (Forster et al., 2001). These biological rhythms are adjusted daily (entrained) to the environmental light/dark cycles via retinohypothalamic tract (RHT) (Hannibal, 2002). Many putative neurotransmitters have been identified in the SCN (Vandenpol, 1980). Aspartate has been reported to be a putative excitatory neurotransmitter in retina (Yagub & Eldred, 1991) in the RHT (Honma et al., 1996) and SCN (Liou et al., 1986; Csaki et al., 2000). This excitatory amino acid is involved in the transmission of light information from retina to SCN via RHT (Takeuchi & Takahashi, 1994). Further, derivatives of aspartate (like *N*-acetyl aspartyl glutamate) also act as a neurotransmitter in RHT (Hannibal, 2002). Ingestion of L-aspartate into the SCN results in minor phase advances during a subjective day (Devries & Meijer, 1991).

Aspartame (ASP) is a dipeptide artificial sweetener; on oral administration, it is hydrolysed in the gastrointestinal tract to its constituent amino acids, L-phenylalanine and L-asartic acid (Ranney & Oppermann, 1979). Previous studies showed that dietary aspartame could alter the diurnal feeding patterns, meal size, distribution of diurnal pattern of spontaneous motor activity (Torie et al., 1985). ASP reduced aggressive attack via a serotonergic meachanism (Goerss et al., 2000). The influences of chronic ASP ingestion on brain neuropeptide Y (NPY) concentration, plasma hormone, food intake and body fat in rats have been reported (Beck et al., 2002).

The circadian nature of cholesterol synthesis (Jones & Schoeller, 1990) and total protein rhythms in humans and mice (Berezkin et al., 1992) and circadian variation of AST (Coll et al., 1993) were documented. However, the influences of ASP on biochemical circadian rhythms has not been investigated intensively. In the present study, ASP administration to Wistar rats and its influence on circadian rhythms of blood variables (glucose, cholesterol, total protein and AST) were monitored.

Accepted: May 4, 2004

Address correspondence to: P. Subramanian, Department of Biochemistry, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India. E-mail: psub@rediffmail.com

DOI: 10.1080/13880200390500885 © 2004 Taylor & Francis Ltd.

Materials and methods

Animals

Male Wistar rats (180–190 g) were obtained from the Central Animal House, Annamalai University. They were housed in polypropylene cages and provided food pellets (Agro-corporation Private Limited, Bangalore, India) as a basal diet during the experiment. Animals were maintained at room tempertature (25 ± 2 °C) and under LD (12 : 12) conditions (Rajakrishnan et al., 1999; Subramanian & Balamurugan, 1999; Subramanian et al., 1998, 2000, 2001). Food and water were available *ad libitum* throughout the experimental period.

Aspartame was obtained from SRL and all other biochemicals used in the study were of analytical grade.

Treatment schedule

The animals were randomized and grouped into experimental and control rats (n = 6 in each group). Group I rats act as control, which received standard food pellets. Group 2 animals were treated orally with aspartame (500 mg/kg body weight) every day (Sharma & Coulombe, 1987; Goerss et al., 2000) throughout the experimental period (11 weeks).

Biochemical oscillations

At the end of the experimental period, a minimal amount of blood (0.75 ml) was collected from the orbital sinus using heparinized tubes from normal and aspartame treated groups every 4 hour (00:00, 04:00, 08:00, 12:00, 16:00, 20:00 and 24:00 hour) throughout the 24 hour period (Subrmanian & Balamurugan, 1998; Subramanian et al., 2000). Blood glucose (Fings et al., 1970), plasma cholesterol (Zlatkis et al., 1953), protein (Lowry et al., 1951) and serum AST (Reitman & Frankel, 1957) were estimated after blood collection. At the end of the study both control and ASP treated rats were killed by decapitation and aspartate levels in brain tissues (Pfleiderer, 1969) were measured.

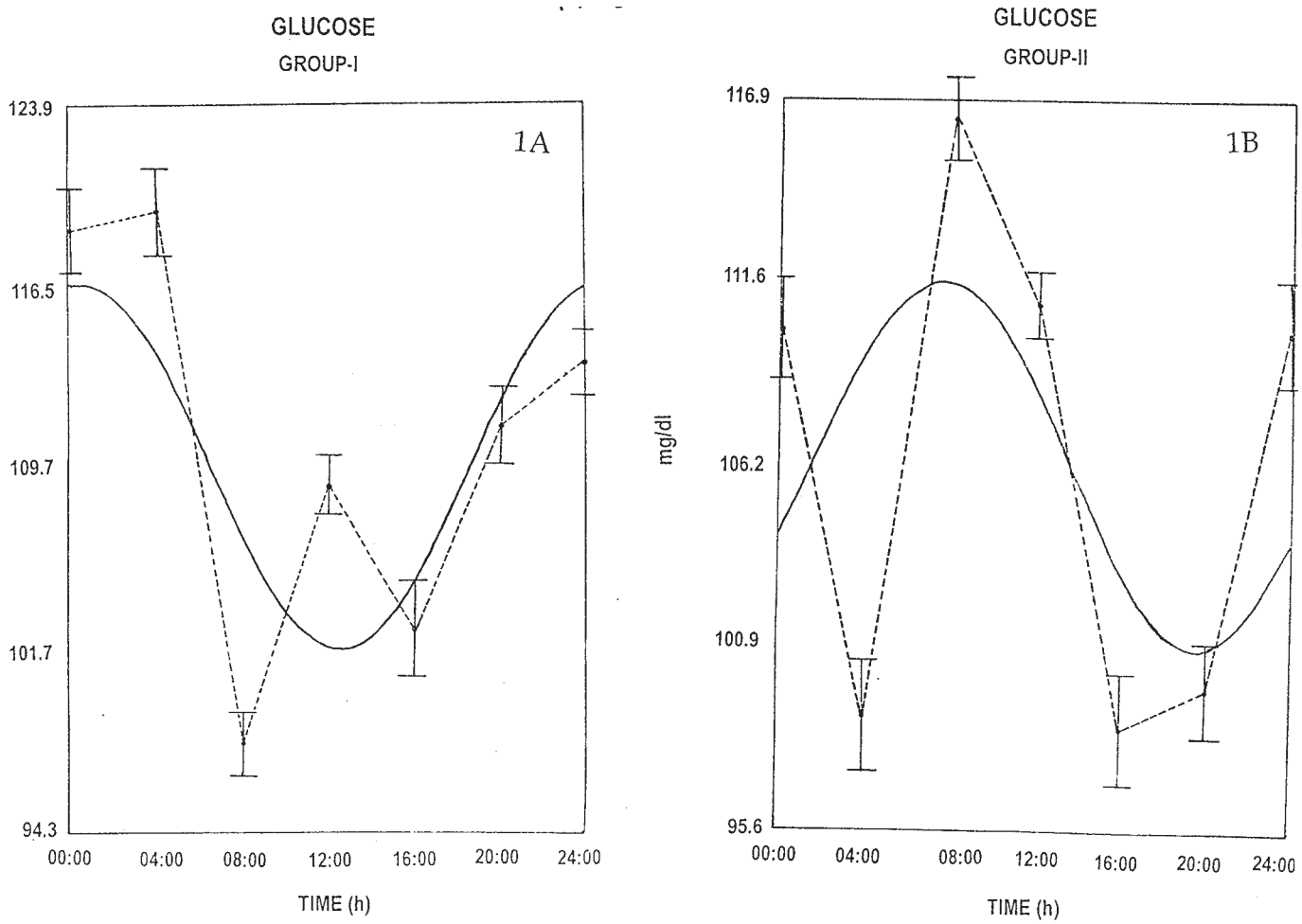

Figure 1. Temporal oscillations of glucose at 4 hour intervals for a period of one day in Wistar rats [control (1A) and aspartame treated (1B)]. Dotted lines represent the raw data and solid lines represent the best fitting cosinor curves (obtained using "cosinorwin" computer software program). Note 6 hour delay of the acrophase in aspartame treated animals (Figure 1B).

Time series analysis

Time series analysis of the oscillation (measurements of acrophase, amplitude and mesor) were done by using "cosinorwin" computer software program. Acrophase is the measure of peak time of the total rhythmic variability in a 24 hour period. Amplitude corresponds to half of the total rhythmic variability in a cycle. Mesor (M) is the rhythm adjusted mean. It is equal to the arithmetic mean for equidistant data covering the 24 hour period. The acrophase is expressed in h; mesor and amplitude values are expressed with the same units as the documented variables.

Table 1. Changes in the rhythm characteristics of glucose and cholesterol in control and aspartame treated rats.

Biochemical variable	Groups	Acrophase (h)	Amplitude (mg/dl)	Mesor (mg/dl)	r-value	p-value
Glucose	I	00:35	7.3	109.1	0.71^{dr}	($p < 0.05$)
	II	7.32	5.3	106.2	-0.34^{ns}	($p < 0.50$)
Cholesterol	I	18:41	8.2	51.9	-0.67^{dr}	($p < 0.05$)
	II	18:49	8.7	51.3	-0.67^{dr}	($p < 0.05$)

dr – detectable rhythmicity.
ns – no significant rhythmicity.

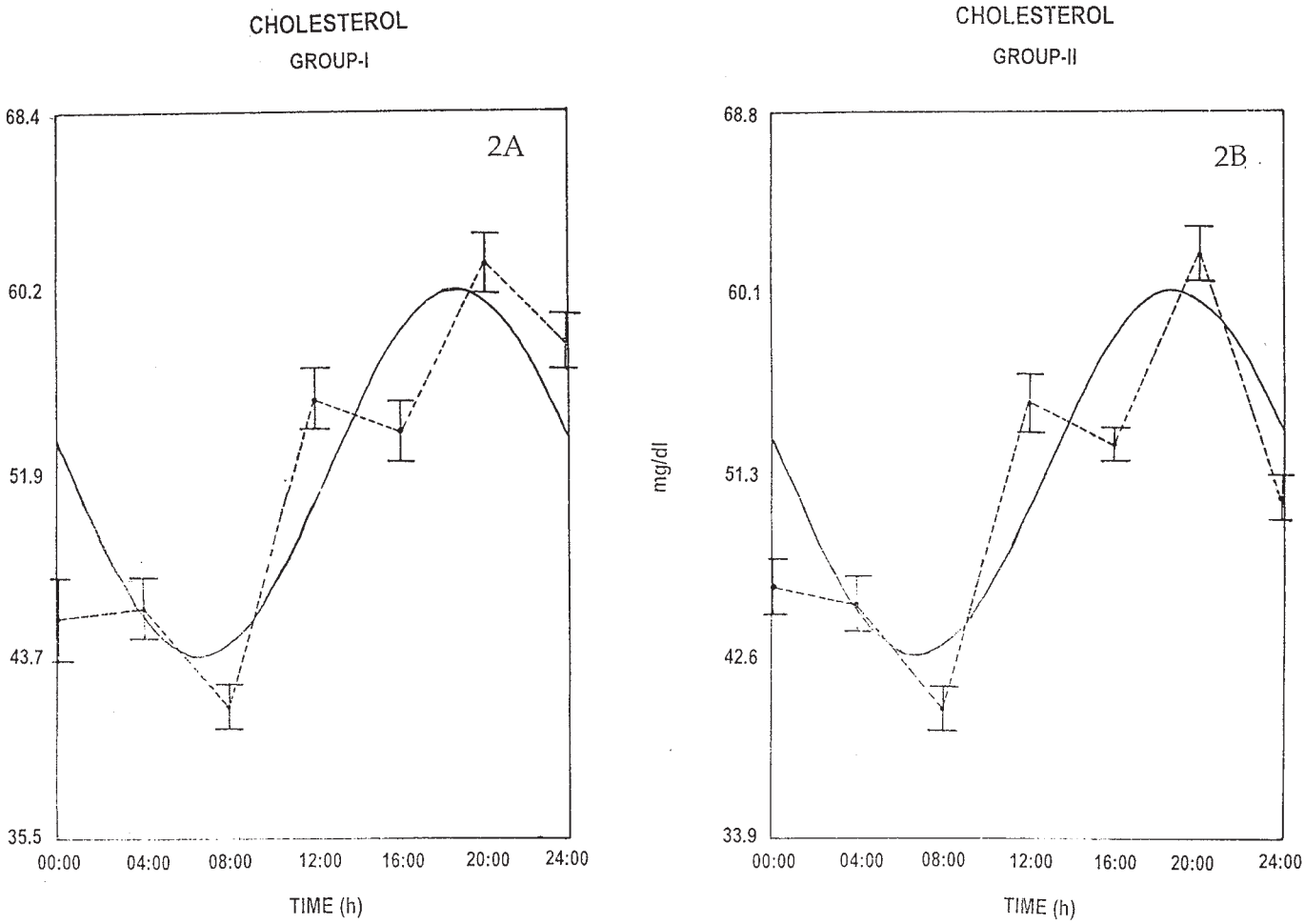

Figure 2. Diurnal rhythms of cholesterol at 4 hour intervals for a period of one day in Wistar rats [control (2A) and aspartame treated (2B)]. Note modulatory increase in amplitude of the rhythm. No significant changes in acrophase was observed in aspartame treated animals (Figure 2B). Other details as in Figures 1A and 1B.

Results

Aspartate levels in brain tissues (mean ± SD) were increased (4.212 ± 0.41) significantly ($p < 0.001$) in aspartame treated animals when compared to controls (1.40 ± 0.15). The endogenous circadian rhythms of glucose showed acrophase at 00:35 hour in normal rats; in the case of aspartame treated animals (group II), maximum levels were found at 7:32 hour (Figure 1A, B). The amplitude, mesor and r-values were decreased significantly in group II when compared with controls (group I). Consinor analysis revealed detectable rhythmicity in normal group and this rhythmicity is affected in aspartame treated group (Table 1). Cholesterol levels were maximum at 18:41 hour in normal and at 18:49 hour in aspartame treated animals (Figure 2A, 2B). Increased amplitude and decreased mesor values were found in aspartame treated rats than that of normal ones. Detectable cholesterol rhythms were observed in two groups (Table 1).

Table 2. Changes in the rhythm characteristics of total protein and AST in control and aspartame treated rats.

Biochemical variable	Groups	Acrophase (h)	Amplitude (mg/dl)	Mesor (mg/dl)	r-value	p-value
Total protein	I	08:48	1.0	5.0	0.02^{ns}	($p < 0.5$)
	II	12:00	0.9	5.2	0.97^{dr}	($p < 0.001$)
AST	I	23:49	10.1	50.9	0.88^{dr}	($p < 0.002$)
	II	12:33	4.0	61.9	0.91^{dr}	($p < 0.001$)

dr – detectable rhythmicity.
ns – no significant rhythmicity.

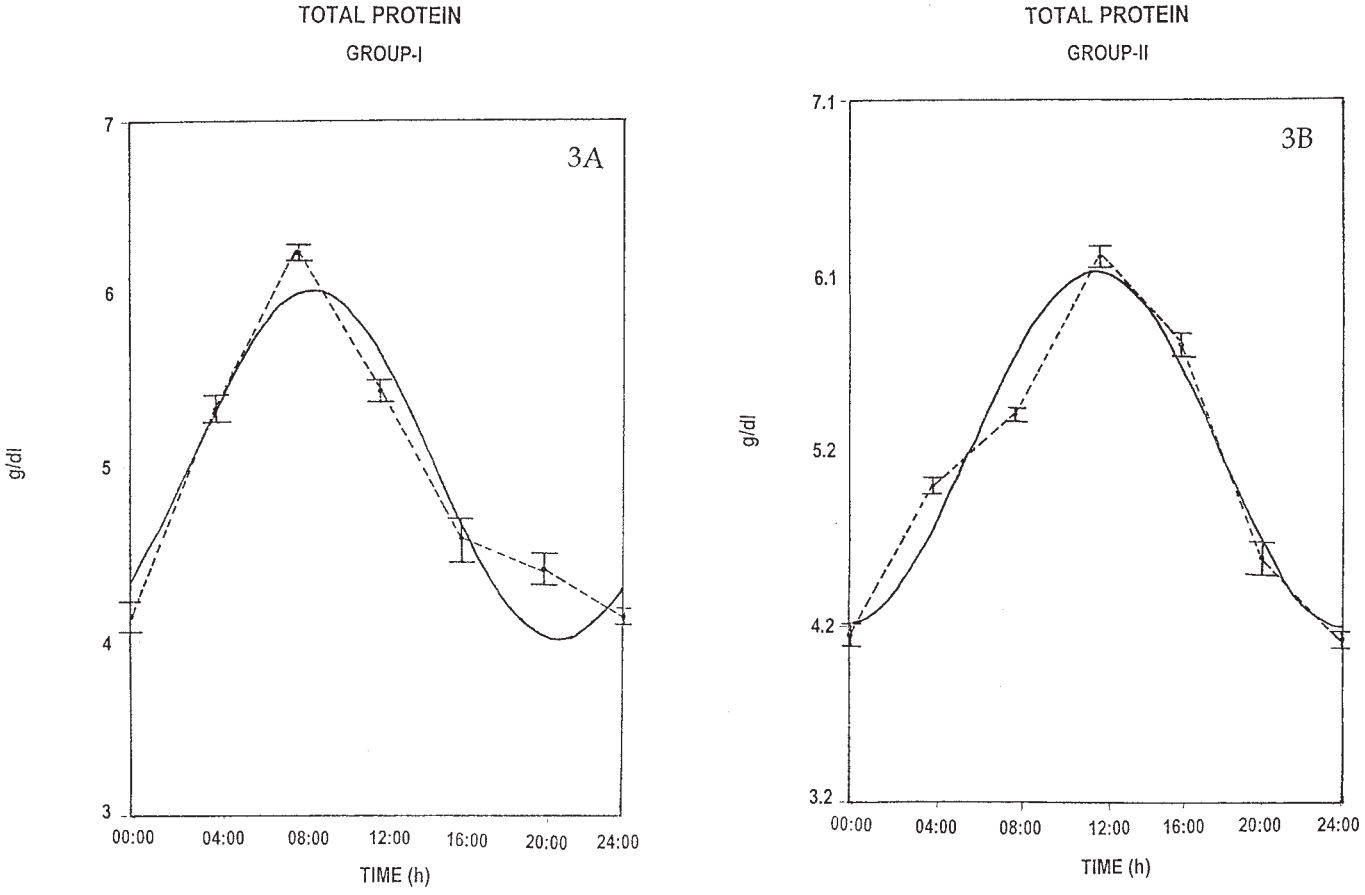

Figure 3. Temporal oscillations of total protein at 4 hour intervals for a period of one day in Wistar rats [control (3A) and aspartame treated (3B)]. Note 4 hour delay of the acrophase in aspartame treated animals (Figure 3B). Other details as in Figures 1A and 1B.

Total protein levels showed the peak value at 8:48 hour and maximum value of aspartame treated animals was observed at 12:00 hour (Figure 3A, 3B). The amplitude and mesor values did not alter significantly in both cases. The r-value is increased in treated animals than that of normal (Table 2). Analysis of serum AST activity over the 24 hour period revealed that the maximum activity at 23:49 hour in normal and at 12:33 hour in aspartame treated animals (Figure 4A, B). Low amplitude values and increased mesor and r-values were observed in aspartame treated animals, when compared with normal animals (Table 2).

Discussion

Neurotransmitters involving in regulation of circadian rhythms were normally used to probe the nature of circadian rhythms. Presence of aspartate in RHT (Liou et al., 1986), horizontal, amacrine and ganglion cells, in some photoreceptors and in some unidentified cells in the peripheral retina (Yagub & Eldred, 1991) was reported. The biochemical parameters chosen for this study showed marked fluctuations over a 24 hour period. From this study it can be concluded that light dark cycles are the most effective synchronizers for biochemical circadian rhythms studied in Wistar rats.

In the present study, peak time of glucose was at 3:00 hour in normal and at 7:32 hour in treated animals; this could be attributed to the food intake, digestion, and accumulation of glucose in blood. Rats administered aspartame with showed about 3 hour delays in glucose rhythms. However, no mesor value change was observed in group II when compared with normal rats. Aspartame could not alter glycemia (Ngugen et al., 1998).

Cholesterol levels in rats in the present study were increased at night as reported previously (Ueberberg et al., 1984). The whole body, free and total cholesterol syntheses oscillate periodically and predictably (Jones & Scholler, 1990). In rats, the rate limiting enzyme (HMG CoA reductase) in the cholesterol synthesis pathway peak its activity at midnight (Mietenen, 1982; Pappu & Illingworth, 1994). The peak level of transcription of cholesterol-7α-hydroxylse (7αH) gene was reported to occur in the evening. All these factors may contribute to the nocturnal increase of cholesterol.

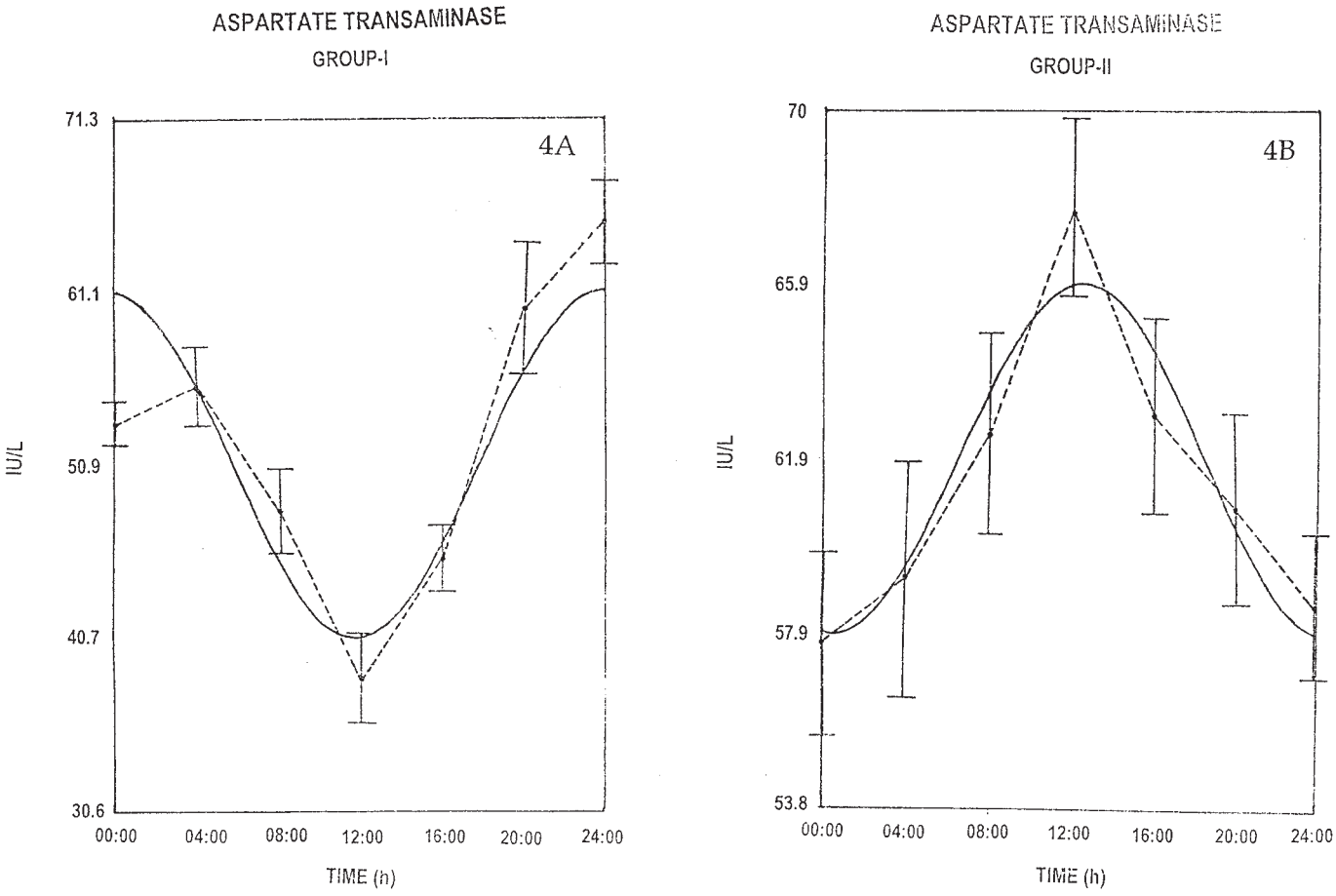

Figure 4. Temporal variation of AST activity at 4 hour intervals for a period of one day in Wistar rats [control (4A) and aspartame treated (4B)]. Note 11 hour advance of the acrophase in aspartame treated animals (Figure 4B). Other details as in Figures 1A and 1B.

Circadian rhythms in total protein were reported in humans and mice (Touitou et al., 1986; Berezkin et al., 1992). The positive and negative balance between synthesis and degradation of protein might be responsible for the rhythmic phenomenon. In our experiment, AST levels are maximum at 23 hour in control and 11 hour advance in aspartame treated animals. The significant increase in mesor and amplitude of AST rhythm indicated that this may be due to the aspartame metabolites; aspartate might enter the TCA cycle via transamination of aspartate to oxaloacetate (Ranney & Oppermann, 1979). In the present study, administration of aspsartame increased the brain aspartate levels corroborating the previous results (Moller, 1991; Burns et al., 1991). This increased brain aspartate might favour the transmission of light information to the SCN (Takeuchi & Takahashi, 1994)

Further, aspartame is known to act on NMDA receptors and has some neurological effects seen with glutamate (Disk, 2000; Abdollahi et al., 2001). Activation of NMDA receptors tends to transmit photic information to the SCN (Mintz et al., 1999). This activation leads to increased production of nitric oxide (NO) (Meller & Gebhart, 1993). Involvement of NO in the transmission of light information to SCN is also suggested (Caillol et al., 2000). Previous results showed that administration of aspartate into the SCN induced phase shifts in the free running rhythms of hamsters (Smith, 2000). Hence, we speculate that increased aspartate levels in brain could alter the characteristics of biochemical rhythms studied, possibly by modulating the transmission in several areas/nuclei in brain including RHT and SCN.

References

Abdollahi, Nikfar, Abdoli (2001): Potentiation by nitric oxide synthase inhibitor and calcium channel blocker of aspartame induced antinociception in the neouse formalin test. *Blackwell Sci Funda Clin Pharmacol 15*: 117–123.

Beck B, Urlet A, Pierre Man J, Krongrad A (2002): Effects of long-term ingestion of aspartame on hypothalamic neuropeptide Y, pasma leptin and body weight gain and composition. *J Physiol Behav 75*: 41–47.

Berezkin MV, Grastisinskii EN, Kudinova VF, Batygov AN, Ponomareve LE, Prikazchikova O, Zhunkova GN (1992): Seasonal and circadian fluctuactions in blood biochemical indicators in mice in natural conditions and exposed to constant light. *Bull EKSP Biol Med 114*: 75–78.

Burns TS, Stargel WW, Tsehanz C, Kotdonis FN, Hurwitz A (1991): Aspartame and sucrose produce a similar increase in the plasma phenylalanine to large neutal amino acid ratio in healthy subjects. *Pharmacology 43*: 210–219.

Caillol M, Devinoy E, Lacroise MC, Schirar A (2000): Endothelial and neuronal nitric oxide synthases are present in the suprachiasmatic nuclei of syrian hamsters and rats. *Eur J Neurosci 12*: 649–61.

Coll AR, Arderiux F, Noguera AD (1993): Circadian rhythms of serum concentrations of 12 enzymes of clinical interest. *Chronobiol Int 10*: 190–202.

Csaki A, Kocsis K, Halasz B, Kiss J (2000): Localization of glutamatergic/ aspartatergic neurons projecting to the hypothalamic paraventricular nucleus studied by retero grade transport. *J Neurosci 101*: 637–655.

Devries MJ, Meijer JH (1991): Aspartate injections into the suprachaismatic region of Syrian hamsters do not mimic the effects of light on the circadian activity rhythys. *Neurosci Lett 127*: 215–218.

Disk (2000): Micromedex international health series. Aspartame neurologic clinical effects. *Toxicol Inf Poison*: 105.

Fings CS, Toltiff CR, Duonin RT (1970): Glucose determination by *o*-toluidine method using glacial acetic acid. In: G Toro PG Ackermann eds., *Practical Clinical Chemistry.* Boston. Little Brown and Bronon CO 115–118.

Forster CH, Winter C, Hofbauer A, Hall JC, Stanewsky R (2001): The circadian clock of fruit flies is blind after elimination of all known photoreceptor. *J Neuron 30*: 249–261.

Goerss AL, Wagner GC, Wendy L (2000): Acute effects of aspartame on aggression and neurochemistry of rats. *Life Sci 67*: 1325–1329.

Hannibal J (2002): Neurtransmitters of the retinohypothalamic tract. *J Cell Tissue Res 309*: 73–88.

Honma S, Katsuno Y, hinohara K, Abe H, Honma K [1996]: Circadian rhythm and response to light of extracellular glutamate and aspartate in rat suprachiasmatic nucleus. *Am J Physiol* 271: *[Regulatory Integrative Comp Physiol 40]* R579–R585.

Jones PJH, Schoeller DA (1990): Evidence of diurnal periodicity in human cholesterol synthesis. *J Lipid Res 37*: 667–693.

Liou SY, Shibata S, Iwasaki K, Ueki (1986): Optic nerve stimulation-induced increase of release of ^{3}H-glutamate and ^{3}H-aspartate but not ^{3}H-GABA from the suprachiasmatic nucleus in slices of rat hypothalamus. *Brain Res Bull 16*: 527–531.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951): Protein measurement with the Folin-phenol reagent. *J Biol Chem 193*: 265–275.

Meller ST, Gebhart GF (1993): Nitric oxide (NO) and nociceptive processing in the spinal cord. *Pain 2*: 127–136.

Mietencen TA (1982): Diurnal variation of cholesterol precursors squalene and methyl sterols in human plasma lipoproteins. *J Lipid Res 23*: 466–473.

Mintz EM, Marvel CL, Gillespie CF, Price KM, Albers HE (1999): Activation of NMDA receptors in the suprachiasmatic nucleus produces light-like phase shifts of the circadian clock in vivo. *J Neurosci 19*: 5124–5130.

Moller SE (1991): Effect of aspartame and protein administered in phenylalanine equivalence doses, on plasma netural amino acids, aspartate, insulin, and glucose in man. *Pharmacol Toxicol 68*: 408–412.

Nguyen UN, Dumoulin G, Henriet MT, Reganrd J (1998): Aspartame ingestion increases urinary calcium, but not oxalate excretion, in healthy subjects. *Clin Endocrinol Metabol 83*: 165–168.

Pappu AS, Illingworth DR (1994): Diurnal variations in the plasma concentrations of mevalonicacid in patients with abeta lipoproteinaemia. *Eur J Clin Invest 24*: 698–702.

Pfleiderer G (1969): In: *Methods of Enzymatic Analysis.* Bergmeyer HU Ed, New York and London, Academic Press, pp. 381–383.

Rajakrishnan V, Subramanian P, Viswanathan P, Menon VP (1999): Effect of chronic ethanol ingestion on biochemical circadian rhythms in Wistar rats. *Alcohol 18*: 147–152.

Ranney RE, Oppermann JA (1979): A review of the metabolism of the aspartyl moiety of aspartame in experimental animals and man. *J Environ Pathol Toxicol 2*: 979–985.

Reitman S, Frankel AS (1957): A colorimetric method for the determination of serum glutamic, oxaloacetic and glutamic pyruvic transaminases. *Am J Clin Path 28*: 53–56.

Sharma KP, Coulombe RA (1987): Effects of intense sweeteners on hunger, food intake and body weight. *Food Chem Toxicol 25*: 565–568.

Smith RQ (2000): Transport of glutamate and other aminoacids at the blood brain barrier. *J Nutr 30*: 1016S–1022S.

Subramanian P, Balamurugan E (1999): Temporal oscillations of serum electrolytes in *N*-phathaloyl GABA-treated rats. *Pharmacol Biochem Behav 62*: 551–514.

Subramanian P, Menon VP, Arokiam FV, Rajakrishnan V (1998): Lithium modulates biochemical circadian rhythms in Wistar rats. *Chronobiol Int 15*: 29–38.

Subramanian P, Sivabalan S, Menon VP, Vasudevan K (2000) Influence of chronic zinc supplementation on biochemical variables and circadian rhythms in Wistar rats. *J Nutr Res 20*: 413–425.

Subramanian P, Sunderasen S, Balamurugan E (2001): Temporal oscillations of phosphatases in *N*-phthaloyl gamma-aminobutyric acid treated rats. *Indian J Exptl Biol 36*: 1141–1143.

Takeuchi Y, Takahashi K (1994): Circadian variations of amino acid content of suprachiasmatic nucleus in rats. *Neuroscience Lett 178*: 275–278.

Torie K, Mimura T, Takasaki Y, Ichimura M (1985): Dietary aspartame with protein on plasma and brain amino acids, brain monoamines and behaviour in rats. *J Physiol Behav 36*: 765–771.

Touitou Y, Rein berg A, Bogdan A, Auzeby A, Beck H, Touitou C (1986): Differences between young and elderly subjects in variations of total plasma proteins and blood volume as reflected by hemoglobin, hematocrit and erythrocyte counts. *Clin Chem 32*: 801–804.

Ueberberg H, Laque K, Trieb G (1984): Comparative studies on the circadian rhythm of conticosterone lipid and cholesterol levels in adrenals and blood of rats. *Chronobiol Int 1*: 41–49.

Vandenpol AN (1980): The hypothalamic suprachiasmatic nucleus of the rat intrinsic anatomy. *J Comp Neurol 191*: 661–702.

Yagub A, Eldred WD (1991): Localization of aspartate like immuno reactivity in the retina of the turtle *(Pseudemys scripta)*. *J Comp Neurol 312*: 584–598.

Yokogoshi H, Roberts CH, Caballero B, Wurtman RJ (1984): Effects of aspartame and glucose administration on brain and plasma levels of large neutral amino acids and brain 5-hydroxy indoles. *Am J Clin Nutr 40*: 1–7.

Zlatkis A, Zak B, Boyle AJ (1953): A new method for the direct determination of serum cholesterol. *J Lab Clin Med 45*: 486.

Copyright of Pharmaceutical Biology is the property of Swets & Zeitlinger, BV and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.