Polish Journal of Pharmacology
Pol. J. Pharmacol., 2004, 56, 79–84
ISSN 1230-6002

MONOSODIUM GLUTAMATE AFFECTS THE TEMPORAL CHARACTERISTICS OF BIOCHEMICAL VARIABLES IN WISTAR RATS

Thamilarasan Manivasagam, Perumal Subramanian[#]

Department of Biochemistry, Faculty of Science, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India

Monosodium glutamate affects the temporal characteristics of biochemical variables in Wistar rats. T. MANIVASAGAM, P. SUBRAMANIAN. Pol. J. Pharmacol., 2004, 56, 79–84.

Monosodium glutamate (MSG) was administrated chronically for 60 days to Wistar rats and 24 h rhythms of glucose, cholesterol, total protein and alkaline phosphatase were studied. MSG treatment was found to cause acrophase delays in the glucose and alkaline phosphatase rhythms and advances in acrophases of cholesterol and total protein levels. Amplitude and mesor values of these rhythms were found to be altered during MSG treatment. Glutamate levels in the brain were found to be significantly increased, which could alter these biochemical rhythms by modulating the transmission in retinohypothalamic tract and in the hypothalamic nuclei, probably including suprachiasmatic nuclei.

Key words: *circadian rhythm, monosodium glutamate, glucose, cholesterol, total protein, alkaline phosphatase.*

[#] *correspondence;* e-mail: psub@rediffmail.com

INTRODUCTION

The endogenous circadian rhythms govern most aspects of physiological and biochemical processes in mammals including body temperature levels, endocrine functions and enzyme levels [6, 9]. The suprachiasmatic nucleus (SCN) constitutes the circadian pacemaker in mammals [22] including humans [15]. Synchronization of the bodily processes is the ablity of the body's internal clock (SCN) to reset itself to external cues. The primary external signal that entrains the biological clock is light-dark cycle [21]. The photic information is transmitted to the SCN from the retinohypothalamic tract (RHT) through glutamate [6, 23]. The glutamate phase shifting effects on activity rhythms of hamsters [17], and on feeding and food anticipatory rhythms [20] in rats were documented.

Monosodium glutamate (MSG), the sodium salt of glutamate, is commonly used as a flavor enhancer especially in Chinese, Thainese and Japanese foods. Schaumburg et al. [30] reported that MSG could produce symptoms such as numbness, weakness, flushing, sweating, dizziness and headaches that commenced between 10 min and 2 h after start of the MSG containing meal and lasted 4 h or less [8].

Olney [25] reported that the subcutaneous injection of MSG could cause brain lesions leading to acute neuronal necrosis in several regions of the developing brain of the neonatal mice and acute lesions in the brain of adult mice. Such brain lesions were reported to cause the alterations in the levels of growth hormone [16], sex hormones [18] and thyroid hormones [19]. Alterations in the levels of thiobarbituric acid reactive substances (TBARS) and antioxidants like reduced glutathione, catalase and superoxide dismutase were reported in adult mice during MSG treatment [2, 5]. Furthermore, disruption in the levels of biochemical parameters such as carbohydrates, lipids and proteins in MSG-treated rats were also well documented [1]. However, the temporal patterns of these biochemical variables during MSG treatment were not investigated so far.

It was demonstrated that in a rat brain slice preparation containing the SCN, stimulation of the optic nerve induced the release of [^{3}H]glutamate [13]. SCN neurons were found to be responsive to glutamate [31, 37]. These findings suggest that glutamate could be involved in the photic transmission to the SCN.

The main objective of the present study is to investigate the influence of glutamate on the characteristics (acrophase, amplitude and mesor) of circadian rhythms of glucose, cholesterol, total protein and alkaline phosphatase (ALP) and to investigate whether glutamate could affect these rhythms differently.

MATERIALS and METHODS

Adult male Wistar rats were obtained from Central Animal House, Faculty of Medicine, Annamalai University. The rats were housed in polypropylene cages at room temperature (30 ± 2°C) under seminatural [28, 32–35] conditions. In Annamalainagar, the LD cycle is almost 12:12 h throughout the year. Animals were maintained under seminatural light-dark conditions in an experimental room [28, 32–35].

The experimental animals were divided into two groups (n = 6 in each group): control (group I) and glutamate-treated (group II). MSG, 600 mg/kg [4], was injected subcutaneously to group II rats every day (at irregular intervals) for 60 days.

Blood samples were collected from animals (groups I and II) at 4h intervals (00:00–24:00) throughout the 24 h period continuously. Minimal amount of the blood was collected from the orbital sinus with great care using heparinized tubes. The levels of glutamate in the brain were also estimated [3]. Glucose [7] was estimated in blood, cholesterol [38] and total protein [14] were measured in plasma and ALP in serum [11] was estimated at the above-mentioned time intervals. The values of the variables (means ± SD) were plotted versus the time of blood collection. Measurements of acrophase (φ-measure of peak time of the variable studied), amplitude (A–corresponds to half the total rhythmic variability in a cycle), mesor (M-rhythm adjusted mean) and r value (correlation coefficient) were done by cosinor analysis using "cosinorwin" computer software program.

RESULTS

Glutamate level was found to be increased in brain tissues of group II animals (18.44 ± 2.89 μmol/g tissue) compared to normal (group I) animals (10.45 ± 1.33 μmol/g tissue). Control animals showed the maximum levels of glucose at 04:39 h; in MSG-

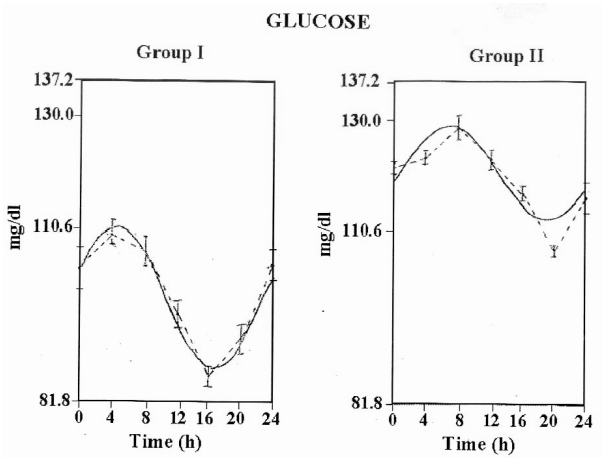

Fig. 1. Temporal oscillation of glucose level measured at 4 h intervals in control (1a) and MSG-treated (1b) Wistar rats. Dotted line represents the raw data and solid line represents the best fitting cosine curve (obtained using "cosinorwin" computer software program). Note ~2.30 h delay in the acrophase in MSG-treated animals

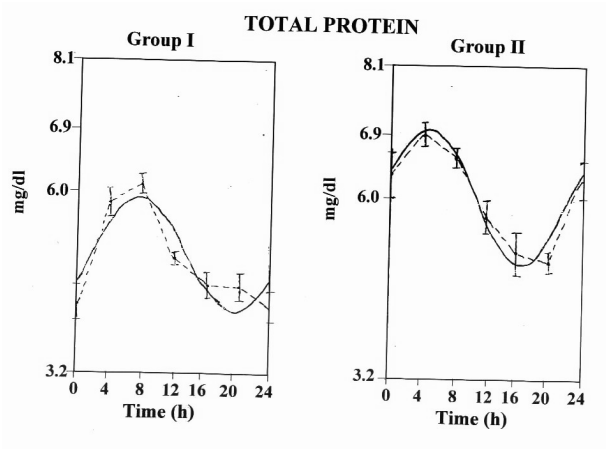

Fig. 3. Diurnal rhythm of total protein level measured at 4 h intervals in control (3a) and MSG-treated (3b) rats. Acrophase of total protein in MSG-treated animals was advanced by ~2.30 h. Other details as in Figure 1 a, b

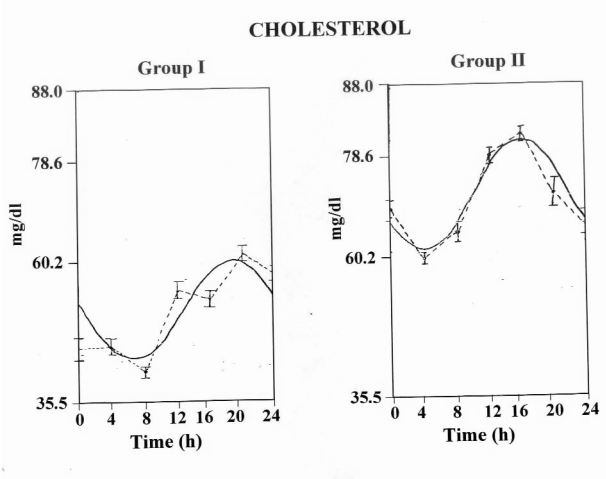

Fig. 2. Temporal oscillation of cholesterol measured at 4 h intervals control (2a) and in MSG-treated (2b) Wistar rats. Note ~1.30 h advance of the rhythm in MSG-treated animals. Other details as in Figure 1a, b

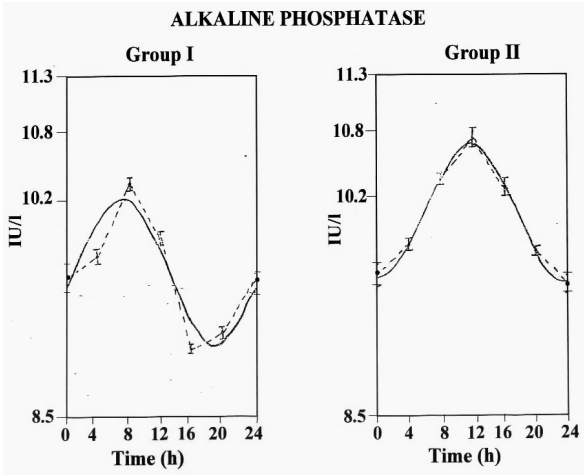

Fig. 4. Diurnal variation of alkaline phosphatase (ALP) activity measured at 4 h intervals in control (4a) and in MSG-treated (4b) Wistar rats. The ~4 h delay in acrophase of ALP was observed in MSG-treated animals. Other details as in Figure 1a, b

treated animals, the peak time was found at 07:00 h (~2.30 h delay) (Fig. 1a, b). Mesor value was increased and the amplitude was decreased in group II animals (Tab. 1). Cholesterol levels showed peak at 18:41 h (normal animals) and they were maximal at 17:10 h in MSG-treated rats (~1.30 h advance) (Fig. 2a, b). Further, the mesor and amplitude values were increased (Tab. 1). Peak time of total protein lied at 07:35 h in normal animals. Their levels were maximal at 04:57 h in MSG-treated rats (~2.30 h advance) (Fig 3a, b); mesor and amplitude were increased in group II animals (Tab. 1). ALP levels showed peak at 07:90 h in normal animals and it was maximum at 11:48 h in MSG-treated rats (~4 h delay) (Fig. 4a, b); mesor and amplitude values were higher in group II animals (Tab. 1).

DISCUSSION

Russell et al. [29] showed that some circadian rhythms were synchronized by meal timing. It has also been reported that the timing of food availabil-

Table 1. Characteristics of temporal patterns of biochemical variables in MSG treated rats

Biochemical variables	Characteristics of rhythm	Normal animals	Glutamate-treated animals
Glucose	Acrophase ϕ (h)	04:39	07:00
	Amplitude	9.6	7.2
	Mesor (mg/dl)	101	122
	r – value	0.69 ^{dr} ($p < 0.05$)	0.72 ^{dr} ($p < 0.025$)
Cholesterol	Acrophase ϕ (h)	18:41	17:10
	Amplitude	8.2	9.3
	Mesor (mg/dl)	51.9	69.2
	r – value	–0.67^{dr} ($p < 0.05$)	–0.38 ^{ns} ($p < 0.25$)
Total protein	Acrophase ϕ (h)	07:35	4.57
	Amplitude	0.9	1.0
	Mesor (g/dl)	5.0	5.9
	r – value	0.54 ^{ns} ($p < 0.20$)	0.8 ^{dr} ($p < 0.05$)
Alkaline phosphatase	Acrophase ϕ (h)	07:90	11:48
	Amplitude	0.5	0.5
	Mesor (IU/L)	9.7	10.2
	r – value	0.71^{dr} ($p < 0.02$)	0.99 ^{dr} ($p < 0.01$)

dr – detectable rhythmicity, ns – no significant rhythmicity

ity and intake exerted powerful effects on the temporal characteristics of biochemical rhythmic phenomena [29].

Adult Wistar rats normally commence eating soon after 18:00 h. Eating would be most pronounced during the hours of dark phase [29]. The peak of activity of enzymes involved in glycolysis were found to lie at the dark phase [24]. In the present study the acrophase of glucose (at 04:39 h) can be attributed to food intake, digestion and accumulation of glucose in blood [24]. Increase in the mesor value of glucose in group II might be due to the inhibitory effect of MSG on growth hormone, thereby decreasing glycogenesis in the liver and inactivating the gluconeogenesis from amino acids [1].

Earlier studies in our laboratory showed that the peak levels of cholesterol occurred at night [28, 34]. The whole body free and total cholesterol synthesis oscillated periodically [10] within a day. Circadian patterns of transcription of cholesterol 7-α hydroxylase gene in liver were found to reach peak levels in the evening, adding evidence that several factors could be involved in the temporal organization of cholesterol levels [12]. Endogenous cholesterol synthesis was also known to exhibit a diurnal rhythm (more than twice at night) but different from endogenous fat synthesis in several ways [12]. In rats the rate limiting enzyme (HMG CoA reductase) in the cholesterol synthesis pathway peaks its activity at midnight [26]. The cholesterol rhythm in MSG-treated rats was disturbed (r – 0.38; $p > 0.2$). This may be due to the destruction of the arcuate nucleus in the hypothalamus, which could function in a regulatory manner towards fat metabolism [1].

Circadian rhythms in total protein were reported in humans and mice [36], and in the present study, acrophase of the protein level occurred at 07:35 h. The positive and negative balance between synthesis and degradation of proteins might be responsible for this rhythmic phenomenon. In group II animals, peak levels of total protein were found at 04:57 h. Administration of MSG caused the increased levels of total protein in RBC [2] and liver [5] which might lead to elevated mesor and amplitude values in group II animals.

Diurnal rhythms of plasma ALP were well documented in Wistar rats [35]. It was already reported that ALP rhythm was not affected by the activity status of the animal but was synchronized by the environmental factors like light-dark cycles [34]. N-phthaloyl-γ-aminobutyric acid (involved in the transmission of dark information to the clock) was also found to affect the characteristics of ALP rhythm [35]. Exogenously administered MSG

could alter the intestinal function and release the intestinal ALP [27] and thereby might affect the temporal characteristics of ALP rhythmicity.

Glutamate was reported as a putative transmitter of RHT [6, 9]. Glutamate injection in SCN caused phase shifts that were dependent on the circadian time at which glutamate is applied [18]. Furthermore, phase shifting effects of glutamate [20] suggest that glutamate could be involved in photic entrainment of circadian pacemaker. Hence, we hypothesize that increased glutamate levels in brain could alter the characteristics of biochemical rhythms by modulating the transmission in several areas/nuclei in brain probably including RHT and SCN.

REFERENCES

1. Ahluwalia P, Malik VBT: Effects of monosodium glutamate (MSG) on serum lipids, blood glucose and cholesterol in adult male mice. Toxicol Lett, 1989, 45, 195–198.
2. Ahluwalia P, Tiwari K, Choudhary P: Studies on the effects on monosodium glutamate (MSG) on oxidative stress in erythrocytes of adult male mice. Toxicol Lett, 1996**,** 84, 161–165.
3. Bernet E, Bergmeyer HU: L-glutamate: determination with glutamic acid dehydrogenase. In: Methods of Enzymatic Analysis; Ed. Bergmeyer HU. Academic Press, NewYork, London, 1963, 384–388.
4. Bopanna KN, Balaraman R, Nadig RS: Antioxidant status of S-allyl cysteine sulphoxide on monosodium glutamate potentiated atherogenesis. Indian J Pharmacol, 1998, 30, 73–81.
5. Choudhary P, Malik VBT, Puri S, Ahluwalia P: Studies on the effects of monosodium glutamate on hepatic microsomal lipid peroxidation, calcium, ascorbic acid and glutathione and its dependent enzymes in adult male mice. Toxicol Lett, 1996**,** 89, 71–76.
6. Ebling FJP: The role of glutamate in the photic regulation of the suprachiasmatic nucleus. Neurobiology, 1996, 50, 109–132.
7. Fings CS, Toltiff CR, Duonin RT: Glucose determination by o-toluidine method using glacial acetic acid In: Practical clinical chemistry. Ed. Toro G, Ackermann PG, Little Brown and Bronnon Co, Boston, 1970, 115–118.
8. Geha RS, Beiser A, Ren C, Patterson R, Grammar LC, Ditto AM, Harris KE et al.: Review of allergic reaction to monosodium glutamate and outcome of a multicenter double blind placebo-controlled study. J Nutr, 2000, 130, 1032S–1038S.
9. Gillette MU, Tischkau SA: Suprachiasmatic nucleus: the brain's circadian clock. Recent Prog Horm Res, 1999, 54*,* 33–58.
10. Jones PJH, Schoeller DA: Evidence of diurnal periodicity in human cholesterol synthesis. J Lipid Res, 1990, 37, 667–673.
11. King EJ, King PR: Colorimetric estimation of alkaline phosphatases. J Clin Pathol, 1954, 7, 322.
12. Lavery DJ, Schibler U: Circadian transcription of the cholesterol 7alpha hydroxylase gene may involve the liver-enriched bZIP protein DBP. Genes Develop, 1993, 7, 1871–1884.
13. Liou SY, Shiabata S, Iwasaki K, Ueki S: Optic nerve stimulation-induced increase in 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, 1986, 16, 527– 531.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin-phenol reagent. J Biol Chem, 1951, 193, 265–275.
15. Lydick R, Schoene WC: Suprachiasmatic region of the human hypothalamus: homolog to the primate circadian pacemaker? Sleep, 1985, 2, 355–361.
16. Maiter D, Underwood LE, Martin JB, James I: Neonatal treatment with monosodium glutamate: effects of prolonged growth hormone (GH)-releasing hormone deficiency on pulsatile GH secretion and growth in female rats. Histol Histopathol, 1991, 128, 1100–1106.
17. Meijer JH, Vanderzee EA, Dietz M: Glutamate phase shifts circadian activity rhythms in hamsters. Neurosci Lett, 1988, 86, 177–183.
18. Miskowiak B, Kesa B, Limanowski A, Partyaka M, Filipiak B: Long-term effect of neonatal monosodium glutamate (MSG) treatment on reproductive system of the female rat. Folia Morphol, 1999, 58, 105–113.
19. Misckowiak B, Partyka M: Neonatal treatment with MSG (monosodium glutamate) on thyroid of the adult male rats. Histol Histopathol, 1999, 15, 415–419.
20. Mistlberger RE, Antle MC: Neonatal monosodium glutamate alters circadian organization of feeding, food anticipatory activity and photic masking in the rat. Brain Res, 1999, 842, 73–83.
21. Moore RY: Circadian rhythms: basic neurobiology and clinical applications. Annu Rev Med, 1997, 48, 253–266.
22. Moore RY, Eichler VB: Loss of circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rats. Brain Res, 1972**,** 42*,* 201–206.
23. Neison HS, Georg B, Hannibal J, Fahrenkrug J: Homer-mRNA in the rat suprachiasmatic nucleus is regulated differently by the retinohypothalamic tract transmitters, pituitary adenylate cyclase activating polypeptide and glutamate at time points where light phase-shifts the endogenous rhythms. Brain Res Mol Brain Res, 2002, 105, 79–85.
24. North C, Feurers SJ, Scheving LE, Pauly JE, Tsai TH, Casciano A: Circadian organization of thirteen liver and six brain enzymes of the mouse. Am J Anat, 1981, 162, 183–199.
25. Olney J: Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. Science, 1969, 164, 719–721.
26. Pappu AS, Illingworth DR: Diurnal variations in the plasma concentrations of mevalonic acid in patients

with abetalipoproteinaemia. Eur J Clin Invest, 1994, 24, 698–702.
27. Racek L, Lenharalt L, Mozes S: Effect of feeding and refeeding on duodenal alkaline phosphatase activity in monosodium glutamate obese rats. Physiol Res, 2001, 50, 365–372.
28. Rajakrishnan V, Subramanian P, Viswanathan P, Menon VP: Effect of chronic ethanol ingestion on biochemical circadian rhythms in Wistar rats. Alcohol, 1999, 18, 147–152.
29. Russell JE, Simmons DJ, Huber B, Roos BA: Meal timing as a *zeitgeber* for skeletal deoxyribonucleic acid and collagen synthesis rhythm. Endocrinology, 1983, 113, 2035–2042.
30. Schamburg HH, Byck R, Gersti R, Mashman JH: Monosodium L-glutamate; its pharmacology and role in the Chinese restaurant syndrome. Science, 1969, 163, 826–828.
31. Shibata S, Liou SY, Ueki S: Influence of excitatory amino acid receptor antagonist and of baclofen on synaptic transmission in optic nerve to the suprachiasmatic nucleus in slices of rat hypothalamus. Neuropharmacology, 1986, 25, 403–409.
32. Subramanian P, Balamurugan E: Temporal oscillations of serum electrolytes in N-phthaloyl GABA-treated rats. Pharmacol Biochem Behav, 1999, 62, 511–514.
33. Subramanian P, Menon VP, Arokiam FV, Rajakrishnan V: Lithium modulates biochemical circadian rhythms in Wistar rats. Chronobiol Int, 1998, 15, 29–38.
34. Subramanian P, Sivabalan S, Menon VP, Vasudevan K: Influence of chronic zinc supplementation on biochemical variables and circadian rhythms in Wistar rats. Nutr Res, 2000, 20, 413–425.
35. Subramanian P, Sunderasen S, Balamurugan E: Temporal oscillations of phosphatases in N-phthaloyl-gamma-aminobutyric acid-treated rats. Indian J Exp Biol, 2001, 36, 1141–1143.
36. Touitou Y, Rein Berg A, Bogdan A, Auzeby A, Beck H, Touitou C: 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, 1986, 32, 801–804.
37. Vandenpol AN: Cellular communication in the circadian clock, the suprachiasmatic nucleus. Neuroscience, 1993, 56, 793–811.
38. Zak B, Boyle AJ, Zaltkis A: A method for the determination of serum cholesterol. J Clin Med, 1953, 41, 486–492.

Received: May 8, 2003; in revised form: December 15, 2003.