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RESEARCH ARTICLE

Influence of N-Phthaloyl GABA on the Circadian Rhythms of Lipid Peroxidation and Antioxidants in Wistar Rats under Constant Light

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ABSTRACT

N-Phthaloyl GABA was administrated daily (50mg/Kg body weight-i.p) to Wistar rats for 21 days and circadian rhythms of thiobarbituric acid reactive substances (TBARS) and antioxidants such as reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were studied under constant light (LL) conditions. Delayed acrophase of TBARS and advanced acrophase of antioxidants (GSH, CAT and SOD) were found in experimental groups as compared with control rats. LL-exposed rats showed increased mesor of TBARS and decreased mesor of antioxidants. Administrations of GABA reversed all the changes to the near normal. Since GABA is being involved in conveying dark information to clock, exogenous administration of P-GABA might reduce the photic information received by the clock. The exact mechanism is still unclear and further research is needed to elucidate the mechanism(s) involved.

Keywords: *Circadian, P-GABA, Constant Light, Lipid peroxidation, Antioxidants*

In mammals, the suprachiasmatic nucleus (SCN) in the anterior hypothalamus is the master circadian clock that drives the daily (24-hour) regulation of physiology, biochemistry, endocrine rhythms, metabolism, cell division, visual activity, renal activity, gene regulation, blood pressure and heart beat in mammals [1-2]. The intrinsic pacemaker activity of the SCN is autonomous in nature and is entrained to the 24-hour period by environmental factors of which the light-dark cycles are most effective [3]. Light is the principal *zeitgeber* for the generation of rhythms; photic information being relayed to the SCN from the retina via the retina hypothalamic tract and indirectly from the intergeniculate leaflet of thalamus via the geniculohypothalamic tract [4].

Light exposure at night seems to be associated with a number of serious behavioral and health problems, including cancer [5]. Results from the human experiments have reported that night shift work (exposure to light at night) was associated with an increased risk of breast cancer and other cancers [6-8]. However, the bright light pulses could induce phase shifts in circadian rhythms, which are used to treat the disorders such as delayed sleep phase syndrome [9], seasonal affective disorder [10] and maladaptation to night work [11].

Gamma-aminobutyric acid (GABA) is found to be the principal neurotransmitter of the circadian system [12,13] and suggested to be involved in conveying dark information to the circadian clock via the afferent pathways [14]. Exogenously-administered GABA does not cross the blood-brain barrier [15]. To overcome this, Bhowmick et al. [16] and Sen et al. (1994) [17] synthesized derivatives of GABA-like N-Phthaloyl GABA (P-GABA) and N-Octanoyl GABA. Both chemicals mimic the action of GABA, are more lipophilic than GABA, and ultimately increase brain GABA level [16]. It has been demonstrated that P-GABA possesses anticonvulsant [16], antinociceptive [18] and antiulcer activities [19,20]. Many GABA analogues have been shown to prevent several manifestations of stress in experimental studies [21]. However, the influence of GABA on the lipid peroxidation products and antioxidants level under LL condition has not still been elucidated.

24-hour rhythms of TBARS [22], catalase and GSH [23], SOD [24], glucose [25], cholesterol synthesis [26], total protein [27], and AST [28] were well documented in many laboratories. The main objective of the present study is to investigate the influence of P-GABA exposure on the characteristics (acrophase, amplitude and mesor) of circadian rhythm of lipid peroxidation prod-

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Table 1. Temporal patterns of TBARS, GSH, Catalase and SOD in control and experimental rats

Biochemical variables	Characteristics of rhythm	Group I (Control)	Group II (Constant Light)	Group III (LL + P-GABA-treated))	Group IV (P-GABA-treated)
TBARS (Thiobarbituric acid reactive substances)	Acrophase ϕ (h)	19:16	21:56	23:59	22:00
	Amplitude (A)	0.5	0.3	0.4	0.3
	Mesor (nmole/m1)	2.2	3.4	2.7	2.0
	r – value	-0.59^{ns}	-0.42^{ns}	-0.44^{ns}	-0.62^{dr}
	p-value	<0.50	<0.50	<0.50	<0.50
GSH (Reduced glutathione)	Acrophase ϕ (h)	7:20	4:40	3:10	4:21
	Amplitude (A)	2.8	1.2	0.3	0.7
	Mesor (mg/d1)	27	22	24	29.1
	r – value	0.7^{dr}	0.63^{dr}	0.27^{dr}	0.83^{dr}
	p-value	<0.02	<0.05	<0.05	<0.05
Catalase	Acrophase ϕ (h)	7:25	4:27	3:53	5:10
	Amplitude (A)	0.8	0.3	0.5	0.4
	Mesor (mg/mg of Hb)	6.2	5.5	6.0	7.1
	r – value	-0.62^{dr}	0.56^{ns}	0.22^{ns}	0.66^{dr}
	p-value	<0.50	<0.50	<0.05	<0.50
SOD (Superoxide dismutase)	Acrophase ϕ (h)	12:24	10:48	11:41	1:33
	Amplitude (A)	0.2	0.1	0.2	0.3
	Mesor (mg/mg of Hb)	2.7	2.0	2.5	2.9
	r – value	0.9^{dr}	0.85^{dr}	0.34^{ns}	0.92^{dr}
	p-value	<0.01	<0.05	*p*<0.25	<0.001

ucts and antioxidants under continuous light exposure (LL).

MATERIALS AND METHODS

Animals

Adult albino male Wistar rats (body weight range from 160-180g) obtained from Central Animal House, Faculty of Medicine, Annamalai University. The rats were housed in polypropylene cages (45×24×15cm) at room temperature (30±2^{o}C) under semi-natural conditions (LD 12:12) as mentioned previously [29]. The animals were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai, India) and water was available *ad libitum.* Food and water were replenished daily. All animal experiments were approved by the Ethical Committee at Annamalai University and were in accordance with the guidelines of the National Institute of Nutrition (NIN), Indian Council of Medical Research (ICMR), Hyderabad, India.

Preparation of N-Phthaloyl GABA

GABA and phthalic anhydride were obtained from Sigma Chemical Co., USA. An intimate mixture of 6.18g GABA and 8.95g of finely ground phthalic anhydride was heated for 30 minutes with stirring in an oil bath at 145-150^{o}C. After cooling, the solid material was dissolved in hot methanol. The filtrate was diluted with 20 ml of water and the product was identified as reported previously [30, 31].

Experimental design

The animals were randomized and divided into four groups (Experimental period: 21 days; n=6 in each group)

Group– I control (no LL condition, no P-GABA administration)
Group– II constant light (LL) condition
Group–III constant light condition + P-GABA administration (50mg/kg i.p)
Group– IV P-GABA administration (50mg/kg i.p)

Temporal biochemical determinations

After the experimental period (21 days), the blood samples were collected from control and experimental rats for biochemical estimations at 4-hour intervals (00:00, 04:00, 08:00, 12:00, 16:00, 20:00 and 24:00) throughout 24-hour period continuously. Minimal amount of the blood (0.75 ml) was collected from the orbital sinus with great care using heparinized tubes [30,32]. Plasma TABRS [33] and GSH [34] were measured. Catalase [35] and superoxide dismutase [36] were assayed in hemolysate. The values of the variables (mean ± SD) were plotted verses the time of blood collection. Time series analyses of the oscillation (measurements of acrophase, amplitude and mesor) were done by using Cosinorwin Computer Software program [37]. Acrophase (ϕ - measure of peak time of the variable studied), amplitude (A – corresponds to half of the total rhythmic variability in a cycle), mesor (M – rhythm adjusted mean) and '*r*' values (correlation coefficient of the rhythm) were applied. Acrophase is expressed in

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hour(s) and amplitude values are expressed with the same units as the documented variables. From the *'r'* value, we calculate the p value as follows;

$$\left| \bar{t} \right| = \frac{r^2}{\sqrt{(1-r^2)}} \times \sqrt{n-2}$$

Where 'n' = number of samples taken; 'p' values were calculated from critical values of 't' distribution at level of 0.05 significance

RESULTS

The biochemical variables measured in this study showed marked fluctuations over a 24-hour period in all the groups. The characteristics of rhythm with r and p values indicating detectable rhythmicity or non-significant temporal variation over a 24-hour period were shown in Table 1. Acrophase of TBARS was delayed in group II, III and IV. Mesor Increased in group II and decreased in group IV, when compared to group I (control rats). Acrophases of GSH, catalase, SOD were advanced in group II, III and IV (Table 1). Decreased mesor in group III and IV (compared to controls) were also noted.

DISCUSSION

The lipid peroxidation products and antioxidants showed marked fluctuations over the 24-hour period. In our study, peak time of TBARS was found to be at 19:16 hours in control rats corroborating the previous results [37,38]. Previous studies have shown that healthy animals exposed to environmental factors such as oxygen or constant light display elevated malonaldialdehyde levels in liver, kidney and brain [38], suggesting a generalized increase in free radicals under LL prompted by formation of photooxidants. The higher plasma lipid peroxide concentrations (increased mesor values in group II rats) in our study might be due to leakage of the lipid peroxidation products from the organ or tissues into blood stream under constant light condition [39].

The decreased mesor of antioxidants (GSH, CAT and SOD) in group II rats may be due to over-utilization of antioxidants to scavenge LL-mediated lipid peroxidation. The antioxidants such as CAT and GSH show maximal activity around 08:00 h in normal animals and maximal levels occurred around 05:00 h in P-GABA-treated rats. Antioxidant enzymes, such as SOD, CAT and GPx are important for cellular protection due to their ability to detoxify free radicals, such as reactive oxygen species (ROS). SOD is involved in reducing superoxides to hydrogen peroxides, while both CAT and GPx are involved in detoxifying hydrogen peroxide to H_2O and O_2.

Due to LL exposure, stress-mediated free radical generation could occur, which could cause increased TBARS and depleted antioxidants due to anti-stress activity of GABA. Administration of GABA to LL-exposed rats normalizes the levels of lipid peroxidation products and antioxidants. The exact mechanism remains to be explored and further research is essential. Agonists and antagonists of GABA are widely used as tools to probe the nature of circadian rhythms [40, 41]. The presence of GABA in the retina [42], SCN [43] and lateral geniculate nuclei [44] strongly suggests the role of GABA in regulating the circadian rhythms [12]. Hence, we hypothesize that administration of P-GABA could alter the characteristics of biochemical rhythms, probably by modulating the transmission in several areas/nuclei in the brain.

Rhythmic alterations in the lipid peroxidation products and antioxidants during constant-Light-exposed rats (group II) may be due to the modulation of neuroendocrine levels under LL, which could lead to modulation of feeding rhythm, temporal locomotor pattern, production of nitric oxide and lipid peroxidation. Administration of GABA to LL-exposed rats reversed the above –mentioned changes by modulating carbohydrate and fat metabolism, feedings rhythms. It could also enhance the antioxidants levels and reduce lipid peroxidation levels. To elucidate the mechanism(s) involved, further research on neuroendocrine rhythms is needed.

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