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Temporal Oscillations of Serum Electrolytes in N-Phthaloyl GABA-Treated Rats

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SUBRAMANIAN, P. AND E. BALAMURUGAN. *Temporal oscillations of serum electrolytes in N-Phthaloyl GABA-treated rats.* PHARMACOL BIOCHEM BEHAV **62**(3) 511–514, 1999—N-Phthaloyl gamma-aminobutyric acid (P-GABA) has been known to cross the blood–brain barrier and ultimately to increase brain GABA level. In the present study, P-GABA was administered to Wistar rats for 21 days and circadian rhythms of sodium, potassium, and calcium levels in serum were studied under seminatural light–dark conditions. P-GABA administration caused desynchronization of the rhythms and advanced the peak times of serum electrolytes. Exogenously administered P-GABA could alter the photic information received by the clock. The results could be explained by slightly less or more than 1-h daily delays, which would bring the peak times to the points 21 days after the start of administration. The changes in amount of electrolytes after P-GABA administration are discussed. © 1999 Elsevier Science Inc.

Circadian N-Phthaloyl gamma-aminbutyric acid Sodium Potassium Calcium

A number of blood and serum variables are found to be circadian in nature (11,27,29). Circadian variations in urinary electrolytes (sodium, potassium, inorganic phosphorus, and magnesium) were demonstrated in young men (29).

Circadian rhythms of potassium have been demonstrated in plasma, erythrocytes, and in urine (19). Circadian variations of potassium metabolism were detected in fasting subjects, during total deprivation of potassium intake, and in subjects receiving equal quantities of food and potassium at equal intervals (19).

Twenty-four-hour rhythms in plasma calcium concentrations (13,20), in calcium metabolism (24), excretion by the kidney (15), and in the absorption through intestine (33) were documented. Circadian rhythms in calcium transfer into the bone, calcium release from bone, and bone reabsorbing activity in serum of rats were observed (13,22,23). All of these studies support the concept that circadian rhythmicity is an essential feature of calcium metabolism. Circadian rhythms of calcium oxalate and calcium phosphate levels were also documented in urinary specimens (15). A statistically significant circadian rhythm of serum magnesium was also detected in young males, elderly males, and females (28).

Gamma-aminobutyric acid (GABA) is reported to be the principal neurotransmitter of the circadian system (12,31). Agents that are known to affect GABA transmission have been shown to affect circadian rhythms (18,25,26,30). However, the influences of exogenously administered P-GABA on the circadian rhythms of serum electrolytes have not been studied so far.

Studies carried out on the noncircadian effects of P-GABA on serum electrolytes are also very limited. GABA has been known to stimulate the absorption and transport of electrolytes in guinea pigs (10), and to increase serum chloride level (32). It has been reported that $GABA_B$ receptors are indirectly coupled to K^+ channels via G proteins (2).

Exogenously administered GABA does not cross the blood–brain barrier (14). To overcome this, Bhowmick et al. (1) and Sen et al. (21) synthesized derivatives of GABA like N-Phthaloyl GABA and N-Octanoyl GABA. Both mimic the action of GABA, more lipophilic than GABA, and ultimately increase brain GABA level (1). In the present study, GABA was administered as N-Phthaloyl GABA (P-GABA) to Wistar rats, and its influence on circadian rhythms of sodium, potassium, and calcium levels in serum were monitored.

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METHOD

Animals and Housing

Adult male albino rats (Wistar strain) obtained from central animal house, Faculty of Medicine, Annamalai University, were used for the study. The rats were housed in polypropylene cages (45 × 24 × 15 cm) at room temperature (30 ± 2°C) under seminatural conditions (LD 12:12) (27). The animals were randomized and separated into normal, control, and experimental groups ($n = 5$ in each group; body weight ranged between 220–250 g). The animals received a diet of standard pellets (Hindustan Lever Ltd., Mumbai, India). Food and water were available ad lib and replenished daily.

Preparation of N-Phthaloyl GABA

GABA and phthalic anhydride were obtained from Sigma Chemical Co., St. Louis, MO. An intimate mixture of 6.18 g GABA and 8.95g of finely ground phthalic anhydride was heated for 30 min with stirring in an oil bath at 145–150°C. After cooling, the solid material was dissolved in hot methanol. The filterate was diluted with 20 ml of water and the product was allowed to crystallize. The product was identified by (a) measuring the melting point, (b) UV absorption, and IR spectroscopy. Thin-layer chromatography of the product was also performed using *n*-propanol: water (7:3), and R_f value was determined (1).

Drug and Dosage

P-GABA (25 mg P-GABA in 2 ml of 20% ethanol) and ethanol (2 ml; 20%) were administered to experimental and control animals, respectively, through intraperitoneal injections every day between 0800 and 1100 h. Hence, P-GABA was administered in an optimum dose of 5 mg/animal. LD_{50} of P-GABA is 327 ± 8.2 mg/kg IP (5). P-GABA treatment was continued until the end of the experiments. After 21 days of treatment, electrolyte determinations were done.

Serum Electrolyte Determinations

Blood samples were collected from each group of animals (normal, control and P-GABA treated) every 3 h (0300, 0600, 0900, 1200, 1500, 1800, 2100, and 2400) throughout the 24-h period continuously for 3 days (three cycles). A minimum amount of blood (0.5–0.75 ml) was collected from orbital sinus with great care, using heparinized tubes. All the chemicals used in the study were obtained from S. D. Fine Chemicals, India, and were of analytical grade. Sodium and potassium levels were determined by flame photometry (4). Serum calcium was estimated by the *0*-Cresolphthalein method (8).

At the end of the study, normal, control, and P-GABA–treated animals were killed by decapitation. Brain tissues were taken in ice-cooled trichloroacetic acid. The levels of GABA in brain tissues were measured by spectorfluorimetry (9).

TABLE 1

GABA LEVELS IN RAT BRAIN

Group ($n = 5$)	GABA Level (μmol/g Tissue) (Mean ± SD)
Normal	1.300 ± 0.21
Control (20% ethanol treated)	1.400 ± 0.15
P-GABA treated	3.212 ± 0.53*

*p-Value: < 0.001 vs normal and control.

Peak time, Range and 24-h Mean

Peak time (the time at which the level of the variable was highest over a 24-h period), range (half of the difference between maximum and minimum values of the variable over a 24-h period), and the 24-h mean (mean value of the variable for equidistant data covering a 24-h period) were calculated (27).

The range and 24-h mean were expressed with the same unit as the documented variable. The peak time was expressed in hours. The range of the rhythm was given in SD, while the 24-h mean was given in ±SEM (27). A Student's *t*-test was performed to detect the significant changes between control and P-GABA–treated groups.

RESULTS

White needle-shaped crystals of P-GABA with a high degree of purity were prepared by the method of Bhowmick et al. (1). The purity of the sample was checked by measuring the melting point (116–117°C), UV absorption peak at 215 nm, and the presence of functional groups were confirmed by IR spectra (1). The R_f value of the sample was found to be 0.21 (1). Brain GABA concentration was increased in P-GABA–treated animals (Table 1).

There were no significant body weight changes in P-GABA–treated animals when compared to normal and control groups. The time difference between maximum values was found to be 24 h in all cases. In P-GABA–treated animals there was a loss of synchrony to the LD cycle and between

TABLE 2

CHANGES IN THE CHARACTERISTICS OF SERUM ELECTROLYTE CIRCADIAN RHYTHMS DURING P-GABA TREATMENT

	Peak Time (h)				Range			24 h Mean		
Rhythm Studied	Normal	Control (20% Ethanol Treated)	P-GABA	Magnitude of Change	Normal (± SD)	Control (±SEM)	P-GABA Treated (±SEM)	Normal (±SD)	Control (±SEM)	P-GABA Treated (±SEM)
Sodium	1800	1200	1200	6 h advance	9.28 ± 0.58	6.93 ± 0.21	8.63 ± 1.38	144.45 ± 1.19	145.25 ± 1.19	149.52 ± 1.20*
Potasium	1200	1200	0300	9 h advance	1.04 ± 0.37	0.726 ± 0.36	0.832 ± 0.18	5.25 ± 0.11	4.95 ± 0.1	4.42 ± 0.12*
Calcium	1500	1500	0600	9 h advance	3.78 ± 0.35	4.28 ± 0.25	1.73 ± .63*	11.06 ± 0.54	11.11 ± 0.61	8.42 ± 0.22*

Peak time—the time at which the level of the variable is highest over a 24-h period; range—half of the difference between maximum and minimum values of the variable over a 24 h period; 24-h mean—mean value of the variable for equidistant data coveirng a 24-h period (27).

p-Values: * < 0.001 vs normal and control.

K^+, Ca^{2+}, and Na^+ rhythms, but possibly not between K^+ and Ca^{2+} rhythms. The changes in peak time, range, and 24-h mean values of the rhythms are shown in Table 2.

Sodium rhythms showed peak time at 1800 (ZT 12) in normal and control animals. The levels are lowest at 0600 h (ZT 0). Sodium levels are maximum at 1200 h (ZT 6; 6 h advance) in P-GABA–treated animals (Fig. 1). The levels are minimum at 0600 h (ZT 0). The 24-h mean value was significantly increased in P-GABA–treated animals when compared with normal and control groups, whereas range did not show a significant change (Table 2).

Potassium rhythms showed a peak time at 1200 h (ZT 6) in normal and control groups (Fig. 2). Maximum levels were found at 2400 h (ZT 18) in normal rats. In controls, the levels were minimum at 2400 h (first cycle) and at 0300 h (second and third cycles). In P-GABA–treated animals maximum levels were found at 0300 h (ZT 21; 9-h advance). The levels are minimum at 1800 h (ZT 12; first and third cycles) and at 1200 h (ZT 6, second cycle). The range did not show any significant changes, whereas 24-h mean value was significantly decreased in P-GABA–treated rats (Table 2).

The peak time of calcium rhythms lie at 1500 (ZT 9) in normal and control animals (Fig. 3). The levels were lowest at 0900 h (ZT 3; first cycle) and at 0600 h (ZT 0; second and third cycles) of the control animals. Calcium levels were maximum at 0600 h (ZT 0) in P-GABA–treated animals (9-h advance). The levels were low at 1200 h (ZT 6; Fig. 3). The range and 24-h mean values decreased significantly in P-GABA–treated animals when compared with normal and control groups (Table 2).

DISCUSSION

The serum electrolytes chosen for the study exhibit marked fluctuations over a 24-h period. From the study, it might be concluded that environmental light–dark cycles could act as synchronizers for the serum electrolyte rhythms studied in Wistar rats.

Agonists and antagonists of GABA are widely used as tools to probe the nature of circadian rhythms (18,25,26,30). The presence of GABA in the retina (34), SCN (3,6,31), and lateral geniculate nuclei (6) strongly suggests the role of GABA in regulating the circadian rhythms (12,31). In our study, administration of P-GABA increased the brain GABA levels corroborating previous results (1). P-GABA acts like a nonspecific agonist of GABA (16) and more lipophilic than GABA (1).

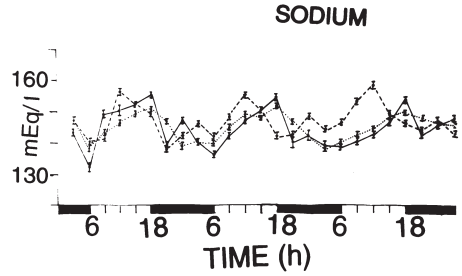

FIG. 1. Temporal oscillations of sodium at 3-h intervals for a period of 3 days in Wistar rats. The shaded portions indicate the nighttime of the LD cycle. Solid lines represent the normal animals, dotted lines represent the control animals, and broken lines represent the P-GABA–treated animals. Note the advances of peak times in P-GABA–treated animals.

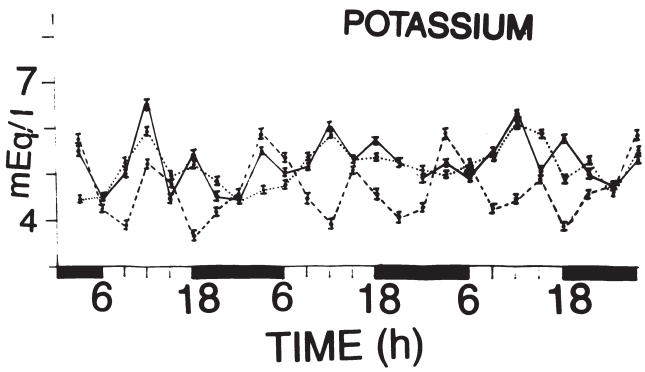

FIG. 2. Temporal oscillation of potassium at 3-h intervals for a period of 3 days in Wistar rats. Note the advances of peak times in P-GABA–treated animals. See Fig. 1 legend for particulars.

In the present study, P-GABA was administered in 20% ethanol. The volume of ethanol was reduced (0.2 ml) because ethanol might influence circadian oscillations (35). However, in the present study, no significant differences were detected in peak time, range, and 24-h mean values between normal and control (ethanol-treated) animals, suggesting that ethanol has no additive/interactive/synergistic action with P-GABA in our study.

In the present study, after long-term systemic administration of P-GABA, desynchronization of circadian rhythms of the electrolytes was observed. The peak time advances of electrolytes could be explained by a slightly less or more than 1-h daily delays, which would bring the peak times to the points after the start of administration.

In asiatic chipmunks, calcium levels are reported to be maximum at 1300 h (24). In our study, calcium levels are found to be maximum at 1500 h (ZT 9) in all the three cycles studied. Furthermore, calcium rhythms are not abolished by parathyroidectomy or thyroidectomy (33), suggesting that these rhythms might be controlled by the master oscillator, the SCN (17,24).

Agonists of GABA are known to evoke phase shifts similar to dark pulses (7,25,26). Hence, GABA might be involved in conveying dark information to the clock (SCN) via the afferent pathways (18). Furthermore, GABA has been reported to reduce the photic information transmitted to the clock (18). Hence, in the present study of exogenous administration of P-GABA, it might be speculated that the P-GABA's effects would be on the circadian system-on input pathway, on the clock or on output mechanisms.

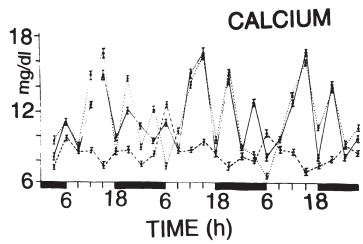

FIG. 3. Temporal oscillations of calcium at 3-h intervals for a period of 3 days in Wistar rats. Note the advances of peak times and reduction of ranges (peak-to-trough variations) in P-GABA–treated animals. See Fig. 1 legend for particulars.

Further, it might be speculated that the exogenous administered of P-GABA could reduce the photic information received by the clock, or it could mimic dark conditions in our study, which, would probably cause the loss of synchronization of the rhythms when compared to controls. However, it is desirable to carry out the experiments in constant dark conditions and examine the effect of single injection of P-GABA to firmly conclude that the changes of these parameters observed in the present study reflect circadian changes only.

Studies carried out on the noncircadian effects of P-GABA/GABA on serum electrolytes are very limited. GABA was known to stimulate the absorption and transport of electrolytes in guinea pigs (10). It has been reported that $GABA_B$ receptors are indirectly coupled to K^+ channels via G proteins (2).

Sodium is involved in the transport of GABA; potassium is involved in the $GABA_B$ receptor-mediated function (2), and GABA release into presynaptic terminals appears to be calcium dependent (2,32). Those interactions could alter the serum levels of these electrolytes. The significant decrease of range of calcium rhythms and significant decreases of 24-h mean values of sodium, potassium, and calcium rhythms might be due to the increased level of abovementioned interactions in the P-GABA–treated animals.

REFERENCES

1. Bhowmick, S.; Pal, M.; Pal, S. P.: Synthesis and anticonvulsant activity of N-phthaloyl GABA—a new GABA derivative. Indian J. Exp. Biol. 27:805–808; 1989.
2. Bittiger, H.; Froestl, W.; Mickkel, S. J.; Olpe, H. R.: $GABA_B$ receptor antagonists: From synthesis to therapeutic application. Trends Pharmacol. Sci. 14:391–394; 1993.
3. Card, J. P.; Moore, R. Y.: The suprachiasmatic nucleus of the golden hamster: Immunocytochemical analysis of cell and fiber distribution. Neuroscience 13:415–431; 1984.
4. Gownlock, A.: Electrolytes and acid base disturbances. In: Varley's clinical practical biochemistry. Boca Raton, FL: CRC Press, 1988: 550–600.
5. Habibuddin, M.; Pal, M.; Pal, S. P.: Neuropharmacology of amide derivatives of P-GABA. Indian J. Exp. Biol. 30:578–582; 1982.
6. Hendrickson, A. E.; Ogren, M. P.; Vaughan, J. E.; Barber, R. P.; Wu, J. Y.: Light and electron microscope immunocytochemical localization of glutamic acid decarboxylase in monkey geniculate complex: Evidence for GABAergic neurons and synapses. J. Neurosci. 3:1245–1262; 1983.
7. Houpot, T. A.; Boulos, Z., Moore-Ede, M. C.: Circadian phase response curve to diazepam for hamsters in contrast light. Soc. Neurosci. Abst. 12:1071; 1986.
8. Lorentz, K.: Improved determination of serum calcium with *o*-cresolphthalein complexone. Clin. Chim. Acta 126:327–334; 1982.
9. Lowe, I. P.; Robins, E.; Eyerman, G. S.: Determination of brain GABA level by spectrofluorimetry. J. Neurochem. 3:8; 1958.
10. MacNaughton, W. K.; Pineau, B. C.; Krantis, A.: Gamma-aminobutyric acid stimulates electrolyte transport in the guinea pig ileum *in vitro*. Gastroenterology 110:498–507; 1996.
11. Markowitz, M.; Rotkin, L. I.; Rosen, F.: Circadian rhythms of blood minerals in humans. Science 213:672–674; 1988.
12. Moore, R. Y., Speh, J. C.: GABA is the principal neurotransmitter of the circadian system. Neurosci. Lett. 150:112–116; 1993.
13. Muhlbauer, R. C.; Fleish, H.: A method for continual monitoring of bone resorption in rats: Evidence for a diurnal rhythm. Am. J. Physiol. 259:R679–R689; 1990.
14. Myers, R. D.: Blood brain barrier technique for the intracerebral administration of drugs. In: Iversen, L. L.; Iversen, S. D.; Sryder, S. H., eds. Handbook of psychopharmacology, vol. 2. New York: Plenum Press; 1975: 1–28.
15. Ogawa, Y.: Circadian rhythm of urinary saturation levels of calcium oxalate and calcium phosphate in normal male individuals. Hinyokikakiyo 39:785–789; 1993.
16. Pal, S. P.; Habibuddin, M.; Sen, S.: Anti-stress activity of N-phthaloyl gamma-aminobutyric acid in rats. Indian J. Exp. Biol. 33:585–588; 1995.
17. Perault-Staub, A. M.; Staub, J. F.; Milhaud, G.: Constancy and rhythmicity, a dual concept for homeostasis exemplified by 24 hour plasma calcium regulation in the rat. In: Talmage, R. V.; Owen, M.; Parson, J. A., eds. Calcium regulating hormones. New York: Elsevier; 1975:229–238.
18. Ralph, M. R.; Menaker, M.: GABA regulation of circadian responses to light. I. Involvement of $GABA_A$-benzodiazepine and $GABA_B$ receptors, J. Neurosci. 9:2858–2865; 1989.
19. Reinberg, A.: Biological rhythms of potassium metabolism: Proceedings of the 8th colloquium of the International Potash Institute. Uppsala, Sweden: International Potash Institute; 1971:160–180.
20. Roelfsema, F. D.; Van der Heide, D.; Paulis, J.; Smeenk, D.: Diurnal urinary calcium and phosphate excretion patterns in parathyroidectomized rats. J. Interdiscipl. Cycle Res. 14:305–314; 1983.
21. Sen, S.; Habibuddin, M.; Pal, S. P.: Antinociaptive action and N-octanoyl GABA-a new GABA mimetic agent. India J. Exp. Biol. 32:718–723; 1994.
22. Shinoda, H.; Okada, M.: Diurnal rhythms in the formation of lamellar bone in young growing animals. Proc. Jpn. Acad. 64:307–310; 1988.
23. Shinoda, H.; Stern, P. H.: Diurnal rhythms in calcium transfer into bone, calcium release from bone and bone reabsorbing activity in serum of rats. Am. J. Physiol. 262:R235–R240; 1992.
24. Staub, J. F.; Perault-Staub, A. M.; Milhaud, G.: Endogenous nature of circadian rhythms in calcium metabolism. Am. J. Physiol. 237:R311–R317; 1979.
25. Subramanian, P.; Subbaraj, R.: Diazepam phase shifts the circadian clock of the field mouse *Mus booduga*. J. Biosci. 18:103–110; 1993.
26. Subramanian, P.; Subbaraj, R.: Diazepam modulates the period of locomotor rhythm in mice (*Mus booduga*) and attenuates light-induced phase advances. Pharmacol. Biochem. Behav. 54:393–398; 1996.
27. Subramanian, P.; Menon, V. P.; Arockiam, F. V.; Rajakrishnan, V.; Balamurugan, E.: Lithium modulates biochemical circadian rhythms in wistar rats. Choronobiol. Int. 15:29–38; 1998.
28. Touitou, Y.; Touitou, C.; Bogdan, A. A.; Beck, H.; Reinberg, A.: Serum magnesium circadian rhythm in human adults with respect to age, sex and mental status. Clin. Chim. Acta 87:35–41; 1978.
29. Touitou, Y.; Touitou, C.; Bogdan, A.; Reinberg, A.; Motohashi, Y.; Auzeby, A.; Beck, H.: Circadian and seasonal variations of electrolytes in aging humans. Clin. Chim. Acta 180:245–254; 1989.
30. Turek, F. W.; Losee-Olson, S. H.: Dose response curve for the phase shifting effect of triazolam on the mammalian circadian clock. Life Sci. 40:1033–1038; 1987.
31. Wagner, S.; Castel, M.; Gainer, H.; Yarom, Y.: GABA in the mammalian suprachiasmatic nucleus and its role in diurnal rhythmicity. Nature 387:598–603; 1997.
32. Worrel, D. M.; Williams, D. C.: Sodium ion dependent transporters for neurotransmitters; A review of recent development. Biochem. J. 297:425–436; 1994.
33. Wrobel, J.: Diurnal variations of calcium transport in the small intestine of adrenalectomized, nephrectomized and vitamin D treated rats. Calcif. Tissue Res. 35:352–356; 1983.
34. Wu, J. Y.; Brandon, C.; Su, Y. Y. T.; Lam, D. M. K.: Immunocytochemical and autoradiographic localization of GABA system in the vertebrate retina. Mol. Cell. Biochem. 39:229–238; 1981.
35. Zucker, I.; Rusak, B.; King, R. G.: Neural bases for circadian rhythms in rodent behaviour. In: Riesen, A. H.; Thompson, R. F., eds. Advances in psychobiology, vol. III. New York: Wiley; 1976: 35–74.