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Gamma-butyrolactone Sleep: A 24-Hour Rhythm Paralleling Normal Sleep in the Rat and CNS Amine Changes[1]

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SPECIALE, S. G., JR. AND A. H. FRIEDMAN. *Gamma-butyrolactone sleep: a 24-hour rhythm paralleling normal sleep in the rat and CNS amine changes.* PHARMAC. BIOCHEM. BEHAV. 3(5) 761–764, 1975. – The duration of sleep induced by a fixed dose of gamma-butyrolactone (GBL) (350 mg/kg, IP) follows the normal circadian sleep pattern of rats. GBL sleep duration is maximal at 1800 hr and minimal at 0600 hr. CNS amine changes are not extensive, but when normal sleep is anticipated, GBL treatment increases dopamine and serotonin levels and decreases norepinephrine levels.

Gamma-butyrolactone Circadian Norepinephrine Dopamine Serotonin Drug-induced sleep

SHORT chain fatty acids (from C4 to C10) can produce narcosis [11, 20, 25]. Attention has been focused on the C4 compounds, gamma-hydroxybutyrate (GBH) and gamma-butyrolactone (GBL). Their demonstration in the mammalian brain [1,17] prompted speculation that one or both substances might be involved in the induction and maintenance of various sleep states. In vivo, GBL is rapidly hydrolyzed to GHB [18] by a lactonase present in the blood and liver. The onset and end of CNS depression correlate best with the GHB levels in the brain [7, 16], indicating that GHB is the active compound.

A circadian pattern in the duration of narcosis (loss of the righting reflex) produced by such hypnotics as pentobarbital [4, 6, 21] and hexobarbital [15] has been demonstrated. Therefore, it was of interest to examine the hypnotic effect, as well as CNS amine changes, induced by GBL, on a 24 hr basis.

METHOD

Animals

Male albino rats (Sprague-Dawley, Holtzman), ranging in weight from 300–400 g, were adapted to a programmed lighting schedule of 0800–2000 hr light, under conditions of stable ambient temperature, barometric pressure and relative humidity, for at least 3 weeks prior to use. Illumination from Vita-Lite fluorescent bulbs (supplied by Duro-Test Corp.) was utilized to provide a lighting spectrum approximating natural daylight. Animals, maintained in groups of 6 in stainless steel cages, over a bedding mixture of Pel-e-cel and Litter-green, were fed, ad lib at random times to prevent the induction of an exogenous rhythm. These procedures, as well as drug injections, were performed under dim red light during the dark phase of the illumination cycle.

Procedure

A dose of GBL (350 mg/kg, IP, in normal saline), shown to produce sleep of 60–90 min duration [19], was utilized. Several min after the injection of GBL, the rats exhibited behavioral depression and when placed on their backs, did not attempt to right themselves, presenting an appearance similar to the response to barbiturates. The duration of GBL sleep was determined at 0600, 1200, 1800 and 2400 hr, as measured as the time from the loss (onset) to the recovery of the righting reflex.

Groups of 3–5 animals received only GBL at 6 hr intervals; designated GBL (alone). Groups of 4–6 rats were pretreated one hour before GBL (for a related GBL-histidine experiment see [23]) and the results are designated GBL (pooled). Norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (5HT) concentrations were determined in the hypothalamus, midbrain, caudate nucleus and brain stem by a modification of the method of Shellenberger and Gordon [22]. For the amine studies, groups of 4–6 animals were sacrificed (by decapitation) at each of the four experimental times, midway through the previously-determined GBL-sleep periods. Control animals for the amine studies were sacrificed at the same times but received the saline vehicle only.

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RESULTS

The GBL-sleep pattern is circadian, with a peak duration at 1800 hr (110 ± 16 min) and a trough at 0600 hr (68 ± 9 min); Fig. 1 and Table 1, GBL (alone). Sleep is longest during the light phase of the illumination cycle, when these nocturnal animals normally sleep, and shortest during the dark phase, when they are normally active. The 24 hr pattern is similar to that obtained with pentobarbital [4,6] and hexobarbital [15]. The peak duration differs significantly from the trough at 0600 hr, as well as from the values at 1200 and 2400 hr (each $p<0.05$).

The GBL-sleep pattern of saline treated controls (Fig. 1 and Table 1), GBL (pooled) differed quantitatively but not qualitatively from GBL (alone). The peak duration of GBL-sleep occurred at 1200 hr and the trough at 2400 hr. The differences between the two patterns might be due to a perturbing effect on the rhythm of the pretreatment injection or the different days of injection of the two groups.

NE, DA and 5HT concentration in the midbrain, hypothalamus, caudate nucleus and brain stem were determined midway through the previously-determined sleep duration (GBL (pooled)) after injection at 0600, 1200, 1800 and 2400 hr, in GBL and control saline-treated animals. The significant changes (Table 2) are largely in the direction of increases in amine concentrations in the brains of GBL-treated animals, compared with controls, as follows: midbrain 5HT, 1200 hr (78 percent increase, $p<0.001$); caudate nucleus DA, 1200 hr (41 percent increase, $p<0.05$); brain stem 5HT, 1200 hr (50 percent increase, $p<0.05$); midbrain 5HT, 1800 hr (65 percent increase, $p<0.05$); hypothalamic DA, 1800 hr (128 percent increase, $p<0.02$); and hypothalamic NE, 2400 hr (20 percent decrease, $p<0.05$).

DISCUSSION

The duration of GBL-sleep is greater during the light than the dark phase of the illumination cycle. This pattern is similar to those found with such classic hypnotics as pentobarbital [4,6] and hexobarbital [15]. Rats adapted to a diurnal light-dark schedule have been shown to sleep significantly more during the light part of the cycle, than during the dark phase [14]. Thus, the 24 hr pattern of GBL-sleep, as well as that of other hypnotics, parallels the normal sleep pattern.

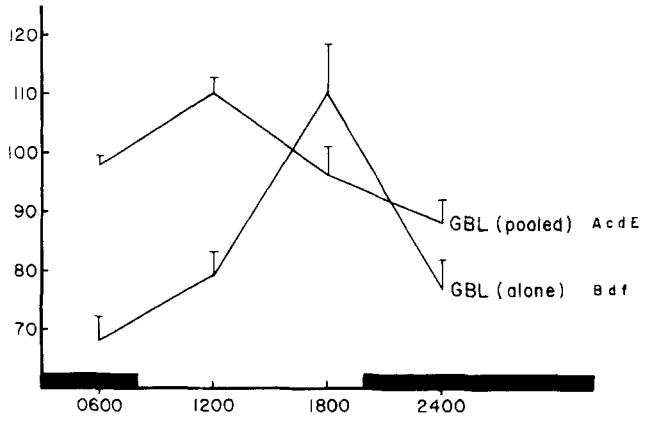

FIG. 1. The duration of GBL sleep in rats, determined over a 24 hr period. GBL (350 mg/kg, IP) was administered to groups of 3–5 (GBL (alone)) or 6–11 animals (GBL (pooled)) at 6 hr intervals. GBL (pooled) designates control animals used in a related GBL-histidine study, which received saline, one hour prior to GBL. The X-axis indicates time, in hours. The black bar represents the dark phase of the illumination, and the open area the light phase. The Y-axis represents the duration of GBL sleep, in minutes. The standard error of each value is indicated by the vertical brackets. Significant point to point statistical comparisons on the curve are indicated by lower and upper case letter designations, as follows: a A (0600 vs 1200); b B (0600 vs 1800); c C (0600 vs 2400); d D (1200 vs 1800); e E (1200 vs 2400) and f F (1800 vs 2400). Lower case letters indicate p values for statistical differences at the 0.05 level; upper case letters indicate the 0.01 level.

Several reports [26,27] have criticized the designation of the GBL (or GHB) effects as sleep. They found that GHB produced the following progression of EEG effects: hypersynchrony, spiking with polysynaptic bursts (7–15 Hz) and periods of electrical silence and hypersynchronous high frequency seizures. They designate this a "catatonic" state of "generalized non-convulsive epilepsy or seizure". Since the 24 hr pattern of GBL-sleep parallels the normal sleep pattern, the hypersynchronizing or sleep effect might be the predominating one, with the dose of GBL used in

TABLE 1

THE DURATION OF GBL-INDUCED SLEEP, IN MINUTES, FOR ANIMALS RECEIVING GBL ALONE OR WITH A SALINE PRETREATMENT (POOLED) ONE HR BEFORE GBL (350 mg/kg, IP)

	0600	1200	1800	2400	
GBL (alone)	68.2 ± 9.3* (5)†	79.3 ± 6.7 (3)	110.3 ± 16.3 (4)	77.0 ± 11.2 (5)	B d F‡
GBL (pooled)	98.0 ± 3.6 (6)	110.1 ± 4.5 (8)	96.4 ± 16.2 (11)	88.3 ± 10.0 (7)	A c d E

*Mean ± standard deviation †Number of animals ‡See Fig. 1 legend

TABLE 2

CONCENTRATIONS (μg/g, TISSUE WET WEIGHT) OF NOREPINEPHRINE (NE), DOPAMINE (DA) AND 5-HYDROXYTRYPTAMINE (5HT) IN THE MIDBRAIN (MB), HYPOTHALAMUS (HPTH), CAUDATE NUCLEUS (CN) AND BRAIN STEM (STEM)

		0600	(%)	1200	(%)	1800	(%)	2400	(%)
MB NE	C	0.80 ± 0.07* (4)†		0.90 ± 0.08 (4)		0.83 ± 0.04 (4)		0.87 ± 0.09 (4)	
	T	0.88 ± 0.05 (4)	(110)	0.91 ± 0.08 (5)	(101)	0.85 ± 0.09 (5)	(102)	0.88 ± 0.07 (5)	(101)
Hpth NE	C	2.39 ± 0.37 (4)		2.79 ± 0.49 (4)		2.74 ± 0.40 (4)		3.23 ± 0.16 (4)	
	T	2.33 ± 0.05 (3)	(98)	2.72 ± 0.51 (5)	(98)	2.43 ± 0.25 (5)	(89)	2.57 ± 0.47‡ (5)	(80)
CN NE	C	0.18 ± 0.02 (4)		0.20 ± 0.02 (4)		0.21 ± 0.02 (4)		0.17 ± 0.05 (4)	
	T	0.17 ± 0.007 (4)	(94)	0.22 ± 0.04 (4)	(110)	0.19 ± 0.05 (5)	(90)	0.23 ± 0.08 (4)	(135)
Stem NE	C	1.03 ± 0.13 (4)		1.05 ± 0.06 (4)		0.95 ± 0.08 (4)		1.21 ± 0.009 (3)	
	T	1.08 ± 0.13 (4)	(105)	1.21 ± 0.15 (5)	(115)	1.02 ± 0.09 (4)	(107)	1.14 ± 0.14 (5)	(94)
MB DA	C	0.18 ± 0.03 (3)		0.18 ± 0.05 (4)		0.20 ± 0.05 (4)		0.21 ± 0.04 (4)	
	T	0.25 ± 0.05 (4)	(139)	0.21 ± 0.05 (5)	(117)	0.26 ± 0.06 (5)	(130)	0.26 ± 0.06 (4)	(124)
Hpth DA	C	0.34 ± 0.05 (4)		0.61 ± 0.13 (3)		0.44 ± 0.12 (4)		0.44 ± 0.03 (3)	
	T	0.33 ± 0.13 (4)	(97)	0.46 ± 0.06 (4)	(75)	1.00 ± 0.32‡ (4)	(227)	0.44 ± 0.15 (4)	(100)
CN DA	C	6.46 ± 0.90 (4)		6.17 ± 0.66 (4)		7.75 ± 1.36 (4)		5.72 ± 1.61 (4)	
	T	7.72 ± 1.00 (4)	(120)	8.72 ± 1.66‡ (4)	(141)	9.40 ± 1.04 (5)	(121)	8.33 ± 2.22 (4)	(146)
Stem DA	C	0.20 ± 0.04 (4)		0.05 ± 0.01 (4)		0.11 ± 0.01 (4)		0.06 ± 0.03 (3)	
	T	0.16 ± 0.06 (4)	(80)	0.05 ± 0.007 (4)	(100)	0.14 ± 0.03 (4)	(127)	0.10 ± 0.03 (4)	(168)
MB 5HT	C	0.67 ± 0.10 (3)		0.35 ± 0.05 (3)		0.31 ± 0.14 (4)		1.09 ± 0.23 (4)	
	T	0.54 ± 0.30 (4)	(81)	0.62 ± 0.07‡ (5)	(177)	0.52 ± 0.07‡ (5)	(168)	1.42 ± 0.44 (4)	(130)
Hpth 5HT	C	0.78 ± 0.28 (3)		0.71 ± 0.14 (4)		0.74 ± 0.36 (3)		1.20 ± 0.22 (3)	
	T	0.86 ± 0.07 (4)	(110)	0.82 ± 0.14 (5)	(116)	0.81 ± 0.18 (5)	(110)	1.32 ± 0.45 (5)	(110)
CN 5HT	C	0.36 ± 0.06 (2)		0.37 ± 0.06 (3)		0.48 ± 0.13 (4)		0.34 ± 0.16 (3)	
	T	0.28 ± 0.08 (4)	(79)	0.40 ± 0.03 (3)	(108)	0.67 ± 0.31 (5)	(140)	0.38 ± 0.07 (3)	(112)
Stem 5HT	C	0.53 ± 0.13 (4)		0.54 ± 0.06 (3)		0.53 ± 0.13 (4)		0.60 ± 0.08 (4)	
	T	0.47 ± 0.04 (3)	(89)	0.81 ± 0.15‡ (4)	(150)	0.62 ± 0.03 (4)	(117)	0.73 ± 0.10 (4)	(122)

Control (C, saline) or treated (T, GBL (350 mg/kg, IP)) rats were injected at the six-hour intervals and were sacrificed midway through the previously-determined GBL-sleep duration (see Table 1, GBL (pooled)). The values in parentheses after each T value were obtained by dividing each amine concentration value by its respective 24-hr mean and taking the ratio of treated to controls.

*Mean ± standard deviation

†Number of animals

‡Significant change ($p<0.05$) from controls, calculated from the absolute amine concentrations

the present study. However, if GBL produces catatonia, such a catatonia follows a circadian pattern.

The duration of GBL-sleep obtained in this study is similar to that found by Roth and Suhr [19], but they used much higher IP doses of GBL (750 or 1000 mg/kg) and GHB (1500 mg/kg, 3 consecutive doses of 500 mg/kg) to examine amine changes. In the present study, DA levels increased only in the caudate nucleus at 1200 hr and in the hypothalamus at 1800 hr. In addition to the differences in dosages, their amine determinations were made on either whole or large portions of rat brain. They found that whole brain 5HT was significantly elevated after GHB. In the segment they designated midbrain-diencephalon-basal ganglion, DA increased, homovanillic acid decreased, while 5HT was unchanged, after GBL (750 mg/kg). In the present study amine changes were few: NE decreased, 1/16 instances; DA increased, 2/16 instances and 5HT increased, 3/16 instances. The reduction in NE levels and the increase in 5HT favor sleep [6]. Although the exact relationship between the neurochemical and behavioral effects of GBL remains unresolved, the GBL-induced increase in neostriatal DA in the rat has been shown to be due to an inhibition of impulse flow in the nigro-striatal pathway and an increase in DA synthesis [24]. DA is capable of forming in vitro, the metabolite dihydroxyphenylethanol (DOPET), which has soporific actions [2]. A conversion of DA to DOPET should be considered in an assessment of the hypnotic activity of GBL.

REFERENCES

1. Bessman, S. P. and W. N. Fishbein. Gamma-hydroxybutyrate, a normal brain metabolite. *Nature* **200:** 1207–1208, 1963.
2. Blum, K., J. H. Merritt, J. E. Wallace, R. Owen, J. W. Hahn and I. Geller. Effects of catecholamine synthesis inhibition on ethanol narcosis in mice. *Curr. ther. Res.* **14:** 324–329, 1972.
3. Courville, J., J. Walsh and J. P. Cordeau. Functional organization of the brain stem reticular formation and sensory input. *Science* **138:** 973–975, 1962.
4. Davis, W. M. Day-light periodicity in pentobarbital response of mice and the influence of socio-psychological conditions. *Experientia* **18:** 235–237, 1962.
5. DeFeudis, F. V. and B. Collier. Amino acids of brain and gamma-hydroxybutyrate-induced depression. *Archs int. Pharmacodyn.* **187:** 30–36, 1970.
6. Friedman, A. H. and C. A. Walker. Rat brain amines, blood histamine and glucose levels in relationship to circadiam changes in sleep induced by pentobarbital sodium. *J. Physiol., Lond.* **202:** 133–146, 1969.
7. Giarman, N. J. and R. H. Roth. Differential estimation of gamma-butyrolactone and gamma-hydroxybutyric acid in rat blood and brain. *Science* **145:** 583–584, 1964.
8. Giarman, N. J. and K. F. Schmidt. Some neurochemical aspects of the depressant action of gamma-butyrolactone on the central nervous system. *Br. J. Pharmac.* **20:** 563–568, 1963.
9. Godin, Y. and J. Mark. Modifications de la biosynthèse des acides aminés du cerveau du rat sous l'effect de l'acide 4-hydroxybutyrique. *C. r. Séanc. Soc. Biol.* **161:** 1392–1395, 1967.
10. Godin, Y., J. Mark and P. Mandel. The effects of 4-hydroxybutyric acid on the biosynthesis of amino acids in the central nervous system. *J. Neurochem.* **15:** 1085–1091, 1968.
11. Holmquist, B. and D. H. Ingvar. Effect of short chain fatty acid anions upon cortical blood flow and EEG in cats. *Experientia* **13:** 331–333, 1957.
12. Lamson, P. D., M. E. Grieg and C. J. Hobdy. Modification of barbiturate anesthesia by glucose, intermediary metabolites and certain other substances. *J. Pharmac. exp. Ther.* **103:** 460–470, 1951.
13. Leonard, B. E. and W. P. Wilkinson. Some effects of 4-hydroxybutyric acid on brain carbohydrate metabolism. *Life Sci.* **10:** 713–719, 1971.
14. Mouret, J. R. and P. Bobillier. Diurnal rhythms of sleep in the rat: augmentation of paradoxical sleep following alterations of the feeding schedule. *Int. J. Neurosci.* **2:** 265–270, 1971.
15. Nair, V. R. and R. Casper. The influence of light on daily rhythm in hepatic drug metabolizing enzymes in rat. *Life Sci.* **8:** 1291–1298, 1969.
16. Roth, R. H., J. M. R. Delgado and N. J. Giarman. γ-Butyrolactone and γ-hydroxybutyric acid. II. The pharmacologically active form. *Int. J. Neuropharmac.* **5:** 421–428, 1966.
17. Roth, R. H. and N. J. Giarman. Natural occurrence of gamma-hydroxybutyrate in mammalian brain. *Biochem. Pharmac.* **19:** 1087–1093, 1970.
18. Roth, R. H. and N. J. Giarman. Preliminary report on the netabolism of gamma-butyrolactone and gamma-hydroxybutyric acid. *Biochem. Pharmac.* **14:** 177–178, 1965.
19. Roth, R. H. and Y. Suhr. Mechanism of the γ-hydroxybutyrate-induced increase in brain dopamine and its relationship "sleep". *Biochem. Pharmac.* **19:** 3001–3012, 1970.
20. Samson, F. E., N. Dahl and D. N. Dahl. A study on the narcotic action of the short chain fatty acids. *J. Clin. Invest.* **35:** 1291–1298, 1956.
21. Scheving, L. E., D. F. Vedral and J. E. Pauly. A circadian susceptibility rhythm is rats to pentobarbital sodium. *Anat. Rec.* **160:** 741–750, 1968.
22. Schellenberger, M. K. and J. H. Gordon. A rapid, simplified procedure for simultaneous assay of norephinephrine, dopamine and 5-hydroxytryptamine from discrete brain areas. *Anal. Biochem.* **39:** 356–372, 1971.
23. Speciale, S. G., Jr. and A. H. Friedman. Does histamine play a role in sleep induced in rats by pentobarbital or γ-butyrolactone? Proc. 5th Int. Congress on Pharmacol., San Francisco, 1972.
24. Walters, J. R., R. H. Roth and G. K. Aghajanian. Dopaminergic neurons: similar biochemical and histochemical effects of γ-hydroxybutrate and acute lesions of the nigro-neostriatal pathway. *J. Pharmac. exp. Ther.* **186:** 630–639, 1973.
25. White, R. P. and F. E. Samson. Effects of fatty acid anions on electroencephalograms of unanesthetized rabbits. *Am. J. Physiol.* **186:** 271–274.
26. Winters, W. D. T. Ferrar-Allado, C. Guzman-Flores and M. Alcarez. The cataleptic state induced by ketamine: a review of the neuropharmacology of anesthesia. *Neuropharmacology.* **11:** 303–315, 1972.
27. Winters, W. D., K. Mori, C. E. Spooner and R. O. Bauer. The neurophysiology of anesthesia. *Anesthesiology* **28:** 65–79, 1967.