Pharmacology Biochemistry & Behavior, Vol. 3, pp. 957–960.

Enzyme Activity in Sleep and Sleep Deprivation

T. DEGUCHI[1], A. K. SINHA[2], W. C. DEMENT AND J. D. BARCHAS

Department of Psychiatry, Stanford University School of Medicine, Stanford, CA 94305

(Received 5 April 1974)

DEGUCHI, T., A. K. SINHA, W. C. DEMENT AND J. D. BARCHAS. *Enzyme activity in sleep and sleep deprivation.* PHARMAC. BIOCHEM. BEHAV. 3(6) 957–960, 1975. – Liver tyrosine transaminase activity is low during the day when the rats are mostly asleep and high during the night when they are awake. When wakefulness was imposed for 8 hr during daylight on the day of the experiment and the rats were allowed to sleep for the following 3 hr during darkness, the tyrosine transaminase activity became high during the day and low at night. That this reversal in enzyme activity is not mediated by the pituitary-adrenal axis is demonstrated by the fact that in adrenalectomized rats tyrosine transaminase activity increased during the day in the sleep deprived rats. However, in these rats the enzyme activity did not become low in the sleep-deprived-sleeping condition. Changes in tryptophan pyrrolase activity during sleep deprivation were demonstrated to be mediated by the pituitary-adrenal axis.

Sleep Sleep deprivation Tyrosine transaminase Tryptophan pyrrolase

THE purpose of this study was to investigate whether the activities of tyrosine transaminase and tryptophan pyrrolase are related to the sleep-wakefulness phenomenon and whether changes in the activity of these enzymes might alter catecholamine and serotonin levels in the brain. There are 2 reasons for such an investigation: (1) The activities of these 2 enzymes in the liver have been shown to vary tremendously over a 24 hr period [2, 10, 11, 14]. They show low activity during the daytime when the animals are mostly asleep and high activity during the night when they spend proportionately more time awake. However, it has not been demonstrated whether these changes in enzyme activity are related to the sleep-wakefulness phenomenon. (2) Since biogenic amines have found favor in recent years as candidates for a major role in the control of sleep [4,5], it seems appropriate to investigate the possibility that the two enzymes under investigation might affect brain amine content by depressing or activating the alternate metabolic pathways of tyrosine and tryptophan in the periphery.

METHOD

Animals

Wistar male rats, weighing 180–200 g, were obtained from Simonsen Laboratories, Gilroy, California, and maintained on Purina lab chow under controlled light (lights on from 7:00 a.m. to 7:00 p.m.) for several days before the experiment. Surgically-treated rats were used 6–7 and 13–14 days following adrenalectomy and hypophysectomy, respectively. The rats were divided into 3 groups:

Control group – Daytime sleep. Rats were housed 5 per cage and allowed to sleep ad lib. Groups of rats were sacrificed at 11:00 a.m., 3:00 p.m., 7:00 p.m. and 10:00 p.m.

Sleep-deprived Group – Daytime awake. Starting at 11:00 a.m. these rats were placed in a large plastic box (70 × 100 cm) and gently manipulated by hand to keep them awake. Groups of rats were sacrificed at 3:00 p.m., 7:00 p.m. and 10:00 p.m.

Sleep-deprived – Sleeping group, Daytime awake, Night sleep. Rats were deprived of sleep in the same manner as the second group from 11:00 a.m. to 7:00 p.m. After 8 hr of enforced wakefulness, the rats were allowed to sleep for 3 hrs. The rats in this group were sacrificed at 10:00 p.m.

Procedure

All rats were deprived of food from 11:00 a.m.; water was available at all times. Five rats were sacrificed in each time point, and experiments were repeated 2 or 3 times.

The rats were sacrificed by decapitation. Livers were removed quickly, chilled in ice, and homogenized in 3 volumes of 0.14 M KCl containing 0.02 M potassium phosphate buffer (pH 7.0). After centrifugation at 105,000 g for 30 min, the supernatant was used for enzyme assays. Tyrosine transaminase [3] and tryptophan pyrrolase [7] activity was measured by methods reported in the literature. Serotonin and norepinephrine were measured in the same sample [1]. For this purpose the cerebellum was discarded and the brain was divided into 2 parts: (1) pons and medulla, and (2) midbrain, basal ganglia and cerebral cortex.

[1]Current Address: Tokyo Metropolitan Institute for Neuroscience, 2–6 Musashidai, Fuchu – City, Tokyo, Japan.
[2]Send reprint request to A. K. Sinha, Department of Physiology, College of Medicine and Dentistry of New Jersey, Rutgers Medical School, Piscataway, NJ 08854.

Electroencephalogram (EEG) recordings were made using extradural stainless steel screws implanted on the skull over the cerebral cortical area. Intramuscular leads were inserted into the neck muscle for electromyogram (EMG) recordings. EEG and EMG of 3 implanted rats were recorded during the last hour of an 8 hr sleep deprivation period and the following 3 hr when the rats were allowed to sleep. The EEG and EMG records were scored, by established procedure [9], to determine if the rats were awake, asleep, or in rapid eye movement sleep during each successive 15 sec period.

RESULTS

Consolidation of Sleep Following 8 hr of Sleep Deprivation

Table 1 shows the percentage of time spent in wakefulness, non-rapid eye movement sleep and rapid eye movement sleep by 3 rats during the last hour of the 8 hr sleep deprivation period and during the following 3 hr when the rats were allowed to sleep. On the average, the rats were sleeping for about 5 percent of the time during the eighth hour of sleep deprivation. So we suspect that the rats in the so-called sleep deprived group were probably sleeping for 5–10 percent of the time and the rats in the sleep-deprived-sleeping group were awake for about 14 percent of the time. The latter fell asleep within a few minutes of the conclusion of the sleep deprivation period and the wakefulness periods and the rapid eye movement periods were approximately uniformly distributed over the 3 hr of sleeping time.

Tyrosine Transaminase and Tryptophan Pyrrolase in the Intact Rat

As shown in Fig. 1, tyrosine transaminase activity in control rats was low at 11:00 a.m. and 3:00 p.m., and then slightly increased at 7:00 p.m. At 10:00 p.m., the enzyme activity increased two-fold compared to the daytime level. This variation between day- and nighttime activity is comparable to that found by other investigators [2, 10, 14]. In sleep-deprived rats, tyrosine transaminase activity increased more than two-fold after 4 hr of sleep deprivation and remained at this level while the rats were kept awake. The enzyme activity was almost the same as the night level of control rats. In sleep-deprived-sleeping rats, tyrosine transaminase activity decreased rapidly to half, which is the same level as the daytime level of control rats. Rats usually sleep most of the daytime and awake around the onset of darkness [12,13]. These results, therefore, suggest that tyrosine transaminase activity changes in parallel with sleep-wakefulness cycle – low activity during sleep and high activity during wakefulness.

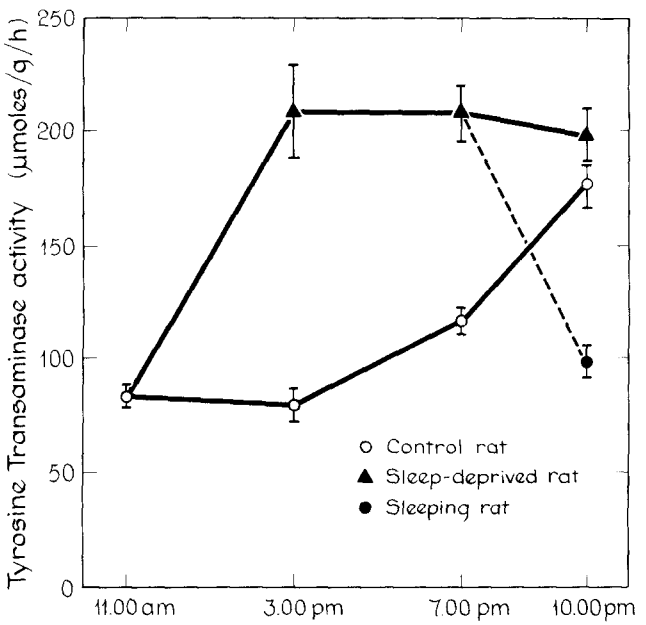

FIG. 1. Tyrosine transaminase activity in intact rats. Vertical lines represent standard errors of the means.

Tryptophan pyrrolase activity was measured in the same enzyme preparations, and the results are shown in Fig. 2.

TABLE 1

SLEEP PARAMETERS OF SLEEP DEPRIVED AND SLEEP-DEPRIVED-SLEEPING RATS

	During the Last Hour of 8 hr of Sleep Deprivation				During 3 hr of Sleep Following 8 hr of Sleep Deprivation			
Rat	Awake %	NREMS %	REMS %	Total Sleep %	Awake %	NREMS %	REMS %	Total Sleep %
1	97.0	3.0	0.0	3.0	15.1	70.3	14.6	84.9
2	95.4	4.6	0.0	4.6	8.4	65.6	26.1	91.6
3	93.8	6.2	0.0	6.2	17.1	71.2	11.7	82.9
Mean	95.4	4.6	0.0	4.6	13.5	69.0	17.5	86.5

EEG and EMG were recorded and scored as described in Methods. Numbers represent time spent in wakefulness, total sleep, non-rapid eye movement sleep (NREMS), and rapid eye movement sleep (REMS) as percent of total elapsed time.

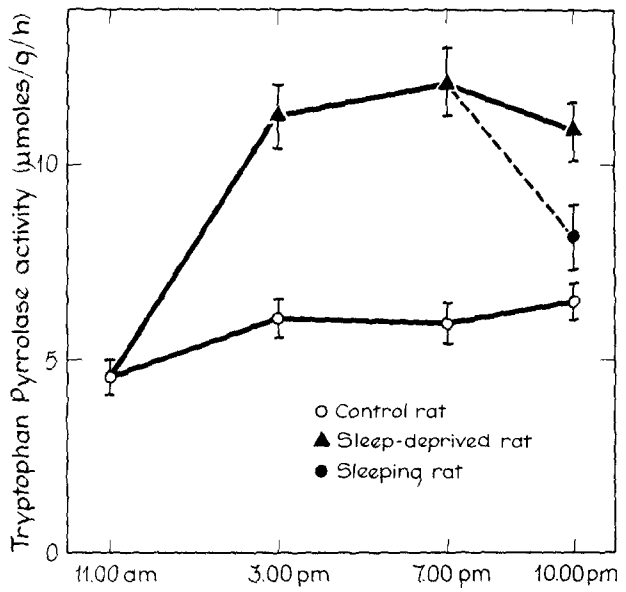

FIG. 2. Tryptophan pyrrolase in intact rats. Vertical lines represent standard errors of the means. All 3 groups of rats were sacrificed at 10:00 p.m. The sleep-deprived rats were kept awake between 11:00 a.m. and 10:00 p.m. The sleeping rats were sleep-deprived from 11:00 a.m. to 7:00 p.m. and then were allowed to sleep for 3 hours up to 10:00 p.m.

Control rats showed a small but significant increase at 10:00 p.m., compared to daytime levels. Four hours of sleep deprivation increased tryptophan pyrrolase activity almost two-fold, which remained at the elevated level while rats were kept awake. In sleep-deprived-sleeping rats, tryptophan pyrrolase activity decreased significantly from sleep-deprived rats, but was still higher than control rats at 10:00 p.m.

Tyrosine Transaminase and Tryptophan Pyrrolase in Adrenalectomized and in Hypophysectomized Rats

To check the possibility that the changes in these enzyme activities might be induced by adrenal or pituitary hormones [6, 8], the same type of experiments were designed with adrenalectomized or hypophysectomized rats. As shown in Fig. 3A, tyrosine transaminase activity was low in adrenalectomized rats. Although tyrosine transaminase activity showed a day-night variation, the change was reduced as compared to the change in intact rats. Sleep deprivation increased tyrosine transaminase activity by 20–45 percent. At 7:00 p.m., the enzyme activity was significantly increased in sleep-deprived rats ($p<0.05$). At 10:00 p.m., however, no significant difference was observed between the 3 groups. The sleep-deprived-sleeping rats did not show any decline in tyrosine transaminase activity, which was clearly observed in intact rats.

The results of experiments with hypophysectomized rats are shown in Fig. 3B. Control rats showed clear day-night changes, increasing two-fold at 10:00 p.m. At 3:00 p.m., sleep-deprived rats had significantly ($p<0.01$) higher enzyme activity than control rats. The enzyme activity of sleep-deprived rats was the same as the night level of control rats. Sleep-deprived-sleeping rats did not show any decrease in enzyme activity, after the rats were allowed to sleep.

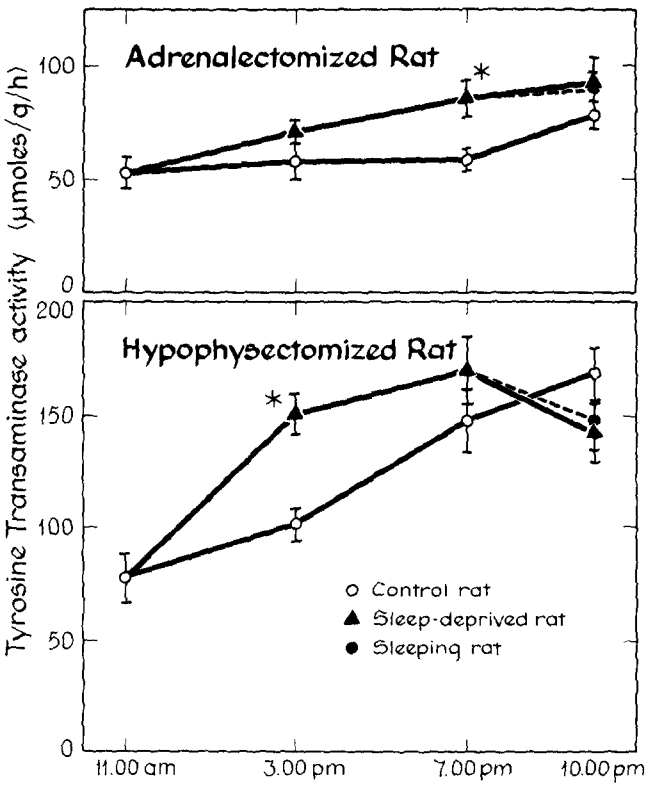

FIG. 3. Tyrosine transaminase activity in adrenalectomized or hypophysectomized rats. Vertical lines represent stand errors of the means. The asterisk indicates significantly different (by *t*-test) values from control rats.

These results show that sleep deprivation increased tyrosine transaminase activity in adrenalectomized or hypophysectomized rats. Although the increase was not as great as in the intact rats, some of the increase was statistically significant. On the other hand, the rapid decline of tyrosine transaminase activity during sleep was observed neither in adrenalectomized nor in hypophysectomized rats.

Following sleep deprivation tryptophan pyrrolase did not show any significant change in adrenalectomized or in hypophysectomized rats.

Serotonin and Norepinephrine Levels in Brain

In order to investigate if the changes of the hepatic enzyme measured above result in the changes of the serotonin or norepinephrine levels in the brain, serotonin, as well as norepinephrine, levels were measured and the results are shown in Table 2. Although there was a tendency for serotonin to be lower in sleeping rats, no statistically significant difference was observed in serotonin or norepinephrine levels in the brain of the three groups.

DISCUSSION

The results reported here suggest a possible relationship between an enzyme activity in a peripheral tissue and the sleep-wakefulness phenomenon. Tyrosine transaminase activity in intact rats increased in response to sleep deprivation and decreased in rats allowed to sleep. Sleep

TABLE 2

LEVELS OF 5-HT AND NOREPINEPHRINE IN BRAIN

	Pons and Medulla		Midbrain, Basal Ganglion and Cerebral Cortex	
	5-HT	Norepinephrine	5-HT	Norepinephrine
Control (7)	0.547 ± 0.041	0.601 ± 0.028	0.347 ± 0.041	0.290 ± 0.012
Sleep-deprived for 11 hr (7)	0.506 ± 0.018	0.582 ± 0.019	0.315 ± 0.033	0.298 ± 0.015
Sleeping for 3 hr (7)	0.518 ± 0.014	0.625 ± 0.016	0.292 ± 0.029	0.276 ± 0.009

Values are μg/g of tissue expressed as means ± SE. Seven animals were used in each group. All 3 groups were sacrificed at 10 p.m.

deprivation also increased tyrosine transaminase activity in operated rats, although the increase was less than that found in intact rats. The decrease in tyrosine transaminase activity during sleep-deprived-sleep was not observed in operated rats. This decrease might have been mediated by a permissive role of steroids or by catecholamines in the adrenal medulla. The results of our experiments with adrenalectomized and hypophysectomized rats indicate that changes in tryptophan pyrrolase activity during sleep deprivation is mediated by the pituitary-adrenal axis and probably is related to the stress of the sleep deprivation procedure rather than to the sleep-wakefulness phenomenon itself.

Of particular interest is the finding that the levels of serotonin and norepinephrine in the brain are essentially unchanged despite two-fold changes in tyrosine transaminase or tryptophan pyrrolase activities. These data would suggest that the levels of the potential neuroregulators are not controlled by the levels of the peripheral enzyme activities which metabolize the precursors, tyrosine and tryptophan. But our data do not allow us to draw any inferences regarding the synthesis or degradation rates of these neurotransmitters, which may be more relevant indices of their putative transmitter function.

ACKNOWLEDGEMENT

We would like to thank Mrs. Pam Angwin, Mrs. Elizabeth Erdelyi, and Mr. Humberto Garcia for their excellent technical assistance. Supported in part by NASA Grant NGR 05–020–168, and NIMH Grants MH 13,860 MH 13,259, and MH 8304. WCD holds Research Scientist Award MH 5804 from the NIMH. JDB holds Research Scientist Development Award MH 24,161 from the NIMH.

REFERENCES

1. Barchas, J., E. Erdelyi and P. Angwin. Simultaneous determination of indole- and catecholamines in tissues using a weak cation exchange resin. *Anayt. Biochem.* **50:** 1–17, 1972.
2. Black, I. B. and J. Axelrod. Regulation of the daily rhythm in tyrosine transaminase activity by environmental factors. *Proc. natn. Acad. Sci. U. S. A.* **61:** 1287–1291, 1968.
3. Diamondstone, T. E. Assay of tyrosine transaminase activity by conversion of p-hydroxyphenylpyruvate to p-hydroxybenzaldehyde. *Analty. Biochem.* **16:** 395–401, 1966.
4. Jouvet, M. The role of monoamines and actylcholine-containing neurones in the regulation of the sleep-waking cycle. *Rev. physiol. biochem. exptl. Pharmac.* **64:** 166–307, 1972.
5. Jouvet, M. Serotonin and sleep in the cat. In: *Serotonin and Behavior*, edited by J. Jarchas and E. Usdin. New York: Academic Press, 1973, pp. 385–400.
6. Knox, W. E. and V. H. Auerbach. The hormonal control of tryptophan peroxidase in the rat. *J. Biol. Chem.* **214:** 307–313, 1955.
7. Knox, W. E., M. M. Piras and K. Tokuyama. Tryptophan pyrrolase of liver. *J. Biol. Chem.* **241:** 297–303, 1966.
8. Lin, E. C. C. and W. E. Knox. Adaptation of the rat liver tyrosine-α-ketoglutarate transaminase. *Biochem. biophys. Acta* **26:** 85–88, 1957.
9. Morden, B., G. Mitchell and W. Dement. Selective REM sleep deprivation and compensation phenomena in the rat. *Brain Res.* **5:** 339–349, 1967.
10. Potter, V. R., R. A. Gebert, H. C. Pitot, C. Peraino, C. J. R. Lamar, S. Lesher and H. P. Morris. Systematic oscillations in metabolic activity in rat liver and in hepatomas. *Cancer Res.* **26:** 1547–1560, 1966.
11. Rapoport, M. I., R. D. Feigin, J. Bruton and W. R. Beisel. Circadian rhythm for tryptophan pyrrolase activity and its circulating substrate. *Science* **153:** 1642–1644, 1966.
12. Rechtschaffen, A., R. A. Lovell, D. X. Freedman, P. K. Whitehead and M. Aldrich. Effect of parachlorphenylalanine on sleep in rats. *Psychophysiology* **6:** 223, 1969.
13. Richter, C. P. Sleep and activity: Their relation to the 24-hour clock. *Res. Publs Ass. Res. nerv. ment. Dis.* **45:** 8–29, 1965.
14. Wurtman, R. J. and J. Axelrod. Daily rhythmic changes in tyrosine transaminase activity of the rat liver. *Proc. natn. Acad. Sci. U. S. A.* **57:** 1594–1598, 1967.