Pharmacology Biochemistry and Behavior, Vol. 1, pp. 117–119. ANKHO International Inc., 1973. Printed in the U.S.A.

BRIEF COMMUNICATION

Inhibition of Glycolytic Metabolism and Sleep-Waking States in Cats[1]

JAAK PANKSEPP[2], JOHN E. JALOWIEC, A. J. ZOLOVICK, WARREN C. STERN AND P. J. MORGANE

Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

(Received 27 December 1972)

PANKSEPP, J., J. E. JALOWIEC, A. J. ZOLOVICK, W. C. STERN AND P. J. MORGANE. *Inhibition of glycolytic metabolism and sleep-waking states in cats.* PHARMAC. BIOCHEM. BEHAV. 1(1) 117–119, 1973.–In cats, injections of 2-deoxy-D-glucose, a glucose antimetabolite, produces dose-dependent increases of slow wave sleep and decreases of REM sleep. Accordingly, variations of glycolytic metabolism may participate in the control of sleep-waking behavior.

Sleep REM 2-deoxy-D-glucose Glycolytic metabolism

THE NEUROCHEMICAL and neuroanatomical foundations of sleep-waking behavior have been extensively studied. By comparison, there is little information concerning the influence of energy metabolism on sleep-waking states. Since cerebral energy metabolism is critically dependent on glucose availability, states of vigilance should vary as a function of cerebral glycolysis. In the following study we modified glycolytic metabolism with intraperitoneal injections of 2-deoxy-D-glucose (2DG), a sugar which decreases intracellular glucose metabolism in brain and other tissues by competitive antagonism of the phosphohexose–isomerase reaction and a membrane transport system normally used by glucose [2], and have observed striking increases in slow wave sleep and decreases in rapid eye movement sleep. (REM).

METHOD

Electrophysiological indices of sleep-waking states were monitored in four mongrel, adult, female cats stereotaxically prepared with the traditional array of chronic sleep recording electrodes (cortex, hippocampus, lateral geniculate nucleus, neck muscle and eye muscle). Two weeks were allowed for postoperative recovery before the start of testing. Polygraphic recordings were carried out in an electrically shielded, dimly lit, sound attenuated cubicle. A flexible cable was attached to the electrode plug cemented to the skull and connected to a counter-weighted 15-lead slip-ring system. Recording was done on Grass Model 5 and 7 polygraphs at paper speeds of 2.5 to 3mm/sec. The records were hand scored in 10–12 sec epochs according to well-established criteria [14,15] into 5 categories. Briefly: (1) active awake was characterized by gross body movement artifacts; (2) quiet awake was characterized by a desynchronized waking EEG in the absence of REM indicators; (3) spindle sleep was characterized by intermittent bursts of spindle and slow wave activity interspersed with bursts of cortical desynchronization; (4) slow wave sleep was characterized by continuous electrocortical slow wave activity and complete bodily quiessence; and, (5) REM sleep was characterized by a desynchronized cortex, total neck atonia, rapid eye movements, lateral geniculate spikes and persistent hippocampal theta activity.

Polygraphic recordings of sleep-waking parameters were monitored for 8 hr immediately following intraperitoneal injections of 250, 500, and 750 mg/kg of 2DG, and were compared to baseline patterns of sleep-waking activity obtained by testing each animal for eight 8-hr sessions under no-drug conditions. After the 750 mg/kg dose of 2DG cats received extended 24 hr recording sessions, and these data were compared to 24 hr control sessions

[1] This research was supported by NIMH grants MH-02211, MH-10625 and MH-23945-01.
[2] Present Address: Department of Psychology, Bowling Green State University, Bowling Green, Ohio 43403.

following 750 mg/kg injections of d-glucose. The cats were observed visually once every hour of recording. No food or water were available during the 8-hr tests. During 24-hr test sessions, food and water were introduced into the test chambers after the first 8 hr of recording. At least a day was allowed between successive tests.

To verify that 2DG was producing its normal biochemical effects, blood samples (1.0--3.0 ml) were withdrawn from the cephalic veins of six additional female cats just prior to a 500 mg/kg injection of 2DG and one hour later. Plasma samples were anlayzed for glucose according to the ferricyanide method with a Technicon Autoanalyzer (Kane Medical Labs, Worcester, Mass.).

RESULTS

Highly consistent changes in electrophysiologically defined sleep-waking patterns were observed. The 2DG dramatically increased slow wave sleep and decreased REM sleep in dose-dependent fashion (Fig. 1, t=3.2, df=3, $p<0.05$ at 500 and 750 mg/kg conditions, hr 1--4). Waking time was also decreased, while spindle sleep was essentially unmodified. Though slow wave sleep was back to control levels during the second half of recording sessions, REM remained depressed (t =3.4, df=3, $p<0.05$ for 500 and 750 mg/kg conditions, hr 4--8).

Analysis of 24 hr sleep records after injection of 750 mg/kg 2DG (Fig. 2) indicated that REM sleep remained depressed for about 8 hr, whereupon there was a compensatory rebound of REM, yeilding no significant change in the duration of that state for the 24 hr period following administration. There was no parallel compensatory decrease of slow wave sleep, and the total duration of slow wave sleep was significantly higher for the 24 hr period following 2DG as compared to d-glucose administration (t=6.09, df=3, $p<0.01$).

Behavioral observations of animals indicated the presence of normal sleep postures during drug-induced periods of slow wave sleep. Supplementary observations of other animals indicated no gross impairments in arousability during 2DG action. All animals, woke when approached and responded to petting. In this regard, it should also be noted that the levels of 2DG employed in the present experiments are comparable to those customarily used to elicit feeding in rats and monkeys [13].

After injection of 500 mg/kg of 2DG, plasma glucose levels increased from basal levels of 83.5±5.7 mg% (mean ± SEM) to 295.2±33.9 mg%, indicating biochemical changes in cats similar to those elicited by 2DG in rats and monkeys [13].

DISCUSSION

Considerable caution has to be employed in interpreting the present results. No criteria have yet been established to separate physiological sleep from possible depressive states resembling sleep. At the present time, we can only assert that according to electrophysiological criteria were cats treated with 2DG obtaining more normal slow wave sleep than untreated animals. In any case, 2DG is, as far as we know, the only biochemical manipulation to date, aside from those which modify activity of putative neurotransmitters, capable of producing electrocortical synchrony resembling slow wave sleep.

Since the primary biochemical effect of 2DG is to

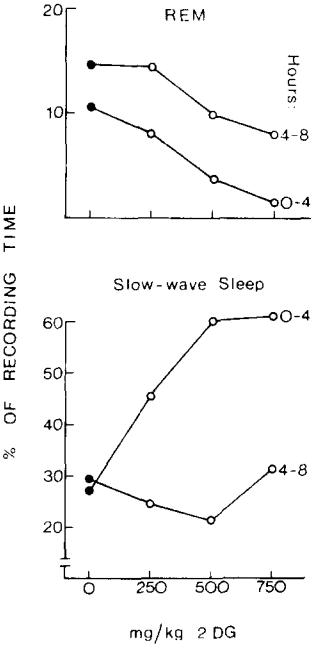

FIG. 1. Percentage of total recording time spent in REM and slow wave sleep during the first and second halves of 8 hr recording sessions as a function of 2DG dose.

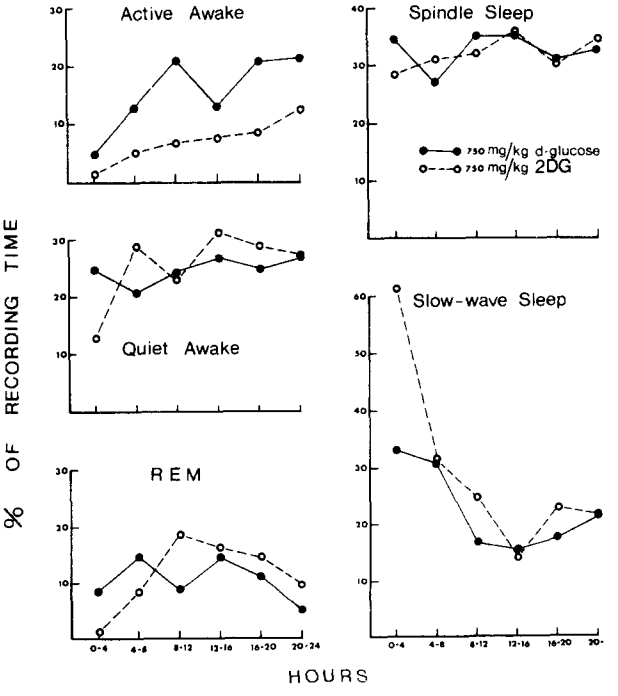

FIG. 2. Sleep-waking states after injections of 750 mg/kg of d-glucose and 2-deoxy-D-Glucose. The continuous 24 hr recording session is broken down into successive 4 hr blocks.

attenuate glucose catabolism in all tissues of the body, the present data suggest that inhibition of glycolysis is a sufficient condition for elicitation of electrocortical synchrony and somnolence. Accordingly, normal daily decreases in cerebral glycolysis may predispose sleep.

Several investigators have observed direct decrements of cerebral energy metabolism during sleep [3, 5, 12], but the failure to find a parallel overall decrease in cerebral oxygen consumption [7,8] has been taken to indicate the unimportance of fluctuations in cerebral metabolism in the normal appearance of sleep. Still, it seems certain that complex changes in brain metabolism do occur with sleep [11,16], and measures of overall cerebral metabolism may dilute shifts in regional glycolytic rates which may participate in the initiation of of sleep. A precedent for such a possibility has been established by studies of single unit activity in brain during sleep and waking: The overall stability of total neural activity during sleep and waking belies reliable shifts in distributions of firing neurons which do occur during these state shifts [4].

Further, it may be noted that several subcortical areas related to the organization of visceral activities---the area postrema [1,6], the nucleus of the solitary tract [10], the ventromedial hypothalamus [9] are all able to synchronize electrocortical activity. Since these areas coincide with or are closely related to weaknesses of the blood brain barrier, they may well have metabolic patterns distinct from the rest of the central nervous system. Perhaps the older physiological theory of sleep being at least partially attributable to decreases of cerebral metabolism has been prematurely discarded.

REFERENCES

1. Bronzino, J. D., P. J. Morgane and W. S. Stern. EEG synchronization following application of serotonin to area postrema. *Am. J. Physiol.* **233:** 376--383, 1972.
2. Brown, J. Effects of 2-deoxyglucose on carbohydrate metabolism: review of the literature and studies in the rat. *Metabolism* **11:** 1098--112, 1962.
3. Cocks, J. A. Changes in concentration of lactic acid in rat and hamster brain during natural sleep. *Nature* **215:** 1399--1400, 1967.
4. Evarts, E. V. Unit activity in sleep and wakefulness. In: *The Neurosciences A Study Program,* edited by G. C. Quarton, T. Melnechuck and F. O. Schmitt. New York: Rockefeller U. Press, 1967, pp. 545--556.
5. Jana, H. Energy metabolism in hypnotic trance and sleep. *J. appl. Physiol.* **20:** 308--310, 1965.
6. Koella, W. P. The mode and locus of action of serotonin in its effects on the recruiting responses and the EEG of cats. *Am. J. Physiol.* **211:** 926--934, 1966.
7. Lubbers, D. W., D. Ingvar, E. Betz, H. Fable, M. Kessler and F. W. Schmahl. Sauerstoffverbrauch der Grosshirnrinde in Schlaf- und Wachzustand beim Hund. *Pflugers Arch. ges. Physiol.* **281:** 58, 1964.
8. Mangold, R., L. Sokoloff, E. Conner, J. Kleinerman, P. -O. G. Therman and S. S. Kety. The effects of sleep and lack of sleep on cerebral circulation and metabolism of normal young men. *J. clin. Invest.* **34:** 1092--1100, 1955.
9. Morgane, P. J. The function of the limbic and rhinic forebrain-limbic midbrain system and reticular formation in the regulation of food and water intake. *Ann. N.Y. Acad. Sci.* **157:** 806--848, 1969.
10. Moruzzi, G. Synchronizing influences of the brain stem and the inhibitory mechanisms underlying the production of sleep by sensory stimulation. *Electroenceph. clin. Neurophysiol. Suppl.* **13:** 231--256, 1960.
11. Reich, P., S. J. Geyer and M. L. Karnovsky. Metabolism of brain during sleep and wakefulness. *J. Neurochem.* **19:** 487--492, 1972.
12. Richter, D. and R. M. C. Dawason. Brain metabolism in emotional excitement and in sleep. *Am. J. Physiol.* **154:** 73--79, 1948.
13. Smith, G. P. and A. N. Epstein. Increased feeding in response to decreased glucose utilization in the rat and monkey. *Am. J. Physiol.* **217:** 1083--1087, 1969.
14. Sterman, M. B., T. Knauss, M. D. Lehman and E. D. Clemente. Circadian sleep and waking patterns in the laboratory cat. *Electroenceph. clin. Neurophysiol.* **19:** 509--517, 1965.
15. Ursin, R. The two stages of slow wave sleep in the cat and their relation to REM sleep. *Brain Res.* **11:** 347--356, 1968.
16. Von den Noort, S. and K. Brine. Effect of sleep on brain labile phosphates and metabolic rate. *Am. J. Physiol.* **218:** 1434--1439, 1970.