95. *Revaluation of Humoral Factors in Sleep Mechanism*

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(Communicated by Yasuji KATSUKI, M. J. A., Sept. 12, 1980)

The importance of humoral factors in sleep mechanisms has been increasingly recognized. The monoamine theory of Matsumoto^{1),2)} and Jouvet^{1),3)} proposes the participation of serotonin (5-HT) in slow wave sleep (SS) and noradrenaline (NA) in paradoxical sleep (PS). However, based upon our observation,⁴⁾ pairs of parabiotic rats display significantly higher synchronization rates of PS than control pairs of rats who are attached by skin only. This result suggests the preferential participation of humoral factors in PS. In contrast, there are the observations of completely independent sleep patterns in human Siamese twins jointed at the skulls⁵⁾ and in the dog with an extra head transplanted into its circulation.⁶⁾ But in the former case the pathway of humoral substances between the twins could not be demonstrated, and in the latter case their survival time was short (108 hr) and their hypnogram was not normal. On the other hand, recently, several findings showing the existence of humoral substances determining the development of the sleep and wakefulness states were reported.⁷⁾⁻⁹⁾

Now the humoral theory in sleep mechanism should be revalued. As previously reported, in order to examine a possible relationship between sleep states and its mechanism, effects of inhibitors of protein synthesis on sleep were studied in rats implanted with chronic electrodes. Intraventricular injection of cycloheximide (2.5 mg/kg) suppressed PS for 4–14 hr, but no effect was observed on SS during 24 hr.¹⁰⁾ Already Oswald¹¹⁾ suggested increased protein synthesis in the brain during PS rebound after drug administration in man, and several workers^{12),13)} also reported that inhibitors of protein synthesis suppressed PS in animals. These reports and our result clearly indicate that cycloheximide (CX) has the suppressive effect on PS.

On the other hand, there is no agreement concerning the relation between the appearance of SS and protein synthesis. For example, subcutaneously injected CX significantly increased SS in mice.¹⁴⁾ And chloramphenicol¹⁵⁾ and anisomycin¹⁶⁾ showed no effects on SS in mice and in rats respectively. The present study was designed to clarify

the effect of CX on SS, using 14 male Wistar rats, and the administration time was shifted and SS was divided into light sleep (LS) and deep sleep (DS) for the further analysis, according to EEG patterns as follows: LS, 100–200 μ V and 2–8 cps with regular respiration, DS, 200–400 μ V and 1–13 cps with regular respiration.

Effects of cycloheximide on LS and DS. Looking on the divided LS and DS as shown in Table I and Fig. 1, under the normal conditions (dotted part) it is apparent that the day-night variations of LS is not so obvious as that of DS and the amount of DS is significantly larger in the light period than in the dark period. After the administration of CX, DS was significantly decreased in the light

Table I. Effects of intraventricular injection of CX (2.5 mg/kg) on LS and DS in every six hours, when the administration time was shifted

		06:00–12:00	12:00–18:00	18:00–0:00	00:00–06:00
LS	Baseline	12.61 $\pm$ 5.30	13.14 $\pm$ 3.88	10.50 $\pm$ 1.88	10.20 $\pm$ 3.86
	Experiment	16.69 $\pm$ 6.45*	16.14 $\pm$ 4.39*	17.76 $\pm$ 4.95***	15.36 $\pm$ 5.33**
DS	Baseline	39.25 $\pm$ 10.05	36.07 $\pm$ 8.22	21.12 $\pm$ 4.73	25.25 $\pm$ 7.23
	Experiment	29.68 $\pm$ 8.27**	29.51 $\pm$ 9.11*	23.58 $\pm$ 7.18	22.63 $\pm$ 8.40

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. With matched-paired t-test.

Fig. 1. Effects of intraventricular injection of CX on LS (left) and DS (right) in rats every six hours, when the administration time was shifted. Dotted parts, baseline; shaded parts, drug injected. 18-0-6 indicates the dark period and 6-12-18 indicates the light period. The value (%) on each concentric circle means the amount of LS or DS per recording time of 6 hr. A, B, C and D indicate the beginning of each experiment, in which CX was injected at 00:00, 06:00, 12:00 and 18:00, respectively; i.e. A indicates the amount of LS or DS during the 6 hr from 00:00 to 06:00 when CX was injected at 00:00 and in the baseline recording. Subsequent data for case A during each 6 hr lie in every first quarter; and so on, for B, C, and D.

period and LS was significantly increased in all cases. From the result it may be concluded that CX has the suppressive effect on DS, taking the greater part of SS.

Already Quay¹⁷⁾ has reported that the 5-HT concentration in the frontal cortex, hypothalamus and lateral lower brain-stem of rats showed rhythmic changes, and its rhythm seems to coincide with the diurnal variations of DS amount in our observation. From these results, DS is thought to be a principal element of SS in the circadian rhythm of sleep in rats, having interaction with 5-HT.

Only in the group given CX at 18:00, the suppressive effect of CX on DS during first 6 hr (from 18:00 to 00:00) was weak and not only LS but also DS was increased, although the change in DS was not statistically significant. The exceptional data of this case may be explained by assuming that some factor, might be protein synthesizing activity, which competes against the action of CX to inhibit protein synthesis, predominates in rats at about 18:00.

Explanation of suppression of PS and DS by cycloheximide. Based upon the monoamine theory of sleep mechanism, we examined whether or not the suppression of PS by CX was due to inhibition of the enzymes of NA synthesis, injecting DOPA or NA to CX-treated rats. From the results¹⁰⁾ that the suppression of PS by CX was not eliminated by DOPA or NA, the suppression may not be caused by depletion of NA and this explanation is supported by the observation that CX does not decrease the endogeneous level of NA.¹⁸⁾

Concerning the relation between CX and 5-HT, Grahame-Smith has suggested that CX does not affect the synthesis of brain 5-HT even with doses larger than that used in our experiment.¹⁹⁾ Considering his report and our results, CX seems to suppress DS without inhibition on protein synthesis concerning 5-HT synthesis.

Thus it may be suggested that CX suppresses DS and PS by acting upon utilizing process of 5-HT and NA in the brain and not upon synthetic process of them. Consequently, it may be concluded that humoral factors participate preferentially in DS and PS. It is also inferred that 5-HT is involved not in the total SS mechanism but only in DS.

Induction of LS and conclusion. Concerning LS, it may be induced principally by a neural factor from wakefulness to sleep-onset period. As previously reported,²⁰⁾ we have found that the skin temperature of the hand was gradually elevated in sleep-onset period in both infants and adults. The peripheral arteries in the hand skin are exclusively innervated by the sympathetic nerves.^{21), 22)} From these facts and results we have suggested that SS (exactly LS) may be induced by suppressing sympathetic activity in the waking state.

In order to verify the suggested hypothesis, inhibitory electrical stimulation was given to the ventromedial nucleus of the hypothalamus (ergotropic, or sympathetic center) in 6 cats using low frequency repetitive pulse of low voltage (1.0 V, 0.5 msec, 14-1 Hz, 2-5 min). Sleep could be induced a few seconds after the stimulus onset and it continued after its cessation. Slow waves appeared in the EEG, heart rate decreased drastically, and respiration became slower and regular, and the cat lay down and curled up. Difference in the heart rate during 20 sec period before and after the stimulation was significant ($P < 0.001$).²³⁾

It should be noted that this effect of stimulus was observable only during the moderate vigilance state of the cat. It was not induced when the animal was feeding or grooming at a state of higher vigilance. (We think this is a physiological character of sleep, since sleep is able to be deprived by man's will, accompanying sympathetic excitation. If the stimulation is effective at any vigilance level, induced state may be anaesthetic.) If the stimuli were applied through the cranial bone, sleep was similarly induced. But stimulation of the lateral nucleus of the hypothalamus (trophotropic, or parasympathetic center) did not induce sleep with the same parameters. These results indicated that LS was induced by a relative increase in parasympathetic activity due to a suppression in the sympathetic activity.

By the administration of CX, DS was suppressed but LS was not and increased. It shows that neural mechanism inducing LS was not inhibited by CX and neuro-biochemical reaction concerning transfer from LS to DS was inhibited.

Considering these results, as postulated above, participation of humoral factors in sleep mechanism is thought to be restricted in DS and PS, and neural factor in LS. Accordingly, sleep activity may be arranged with each participating factor as follows, schematized in Fig. 2: (1) Initiating phase (from wakefulness to LS)—suppression

Fig. 2. Diagram of sleep phase and mechanism, divided into initiating, maintaining and awakening phase, with each participating factor.

of sympathetic activity, (2) maintaining phase (from DS to the end of PS)—serotonin and noradrenaline utilizing process, (3) awakening phase (from the end of PS to wakefulness)—excitation of sympathetic activity.

In the future, neural and biochemical interaction between each phase should be further investigated.

Acknowledgement. This study was supported by a grant-in-aid for Special Project Research "The Integrative Control Functions of the Brain (No. 410811)" from the Ministry of Education, Science and Culture of Japan.

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