Suppression by Cyproheptadine of Human Growth Hormone and Cortisol Secretion during Sleep

KAZUO CHIHARA, YUZURU KATO, KIYOSHI MAEDA, SHIGERU MATSUKURA, and HIROO IMURA

From the Third Division, Department of Medicine and Department of Neuropsychiatry, Kobe University School of Medicine, Kobe, Japan

ABSTRACT The effect of cyproheptadine on plasma growth hormone and cortisol levels was studied in seven male volunteers with polygraphic sleep monitoring.

Sleep-related growth hormone release was completely inhibited in three of the seven normal subjects by the intravenous infusion of cyproheptadine (5 mg) which was started at the onset of sleep. In the other four, growth hormone release during sleep was significantly decreased or delayed by cyproheptadine when the drug infusion was started at 7:00 p.m., 1–2 h before the onset of sleep.

The usual increase in plasma cortisol in the early morning was completely suppressed in all five subjects given cyproheptadine infusions from 4:00 to 7:00 a.m.

The intravenous infusion of cyproheptadine increased slow wave sleep, although the time from sleep onset to the first occurrence of slow wave sleep was not affected. In contrast, rapid eye movement sleep was significantly decreased by cyproheptadine.

These results suggest that cyproheptadine inhibits growth hormone and ACTH secretion during sleep in man, possibly by antagonizing serotoninergic mechanisms although other actions of the drug are not ruled out.

INTRODUCTION

Accumulating evidence in recent years suggests that serotoninergic mechanisms are implicated in the regulation of both growth hormone (GH)[1] and ACTH in laboratory animals (1–5). In our previous studies we observed that oral administration of 5-hydroxytryptophan, a precursor of serotonin, caused an increase in plasma GH, ACTH, and cortisol in normal subjects (6). These results suggest that serotoninergic mechanisms play a role in the release of GH and ACTH in man.

Cyproheptadine is known to be a potent serotonin antagonist, although it has additional actions such as antihistaminic and anticholinergic effects (7). Our subsequent studies have shown that cyproheptadine inhibits plasma GH responses to 5-hydroxytryptophan, arginine, or L-dihydroxyphenylalanine (8, 9). It has also been demonstrated in man that not only GH and cortisol release induced by hypoglycemia but also plasma GH response to exercise are significantly blunted by pretreatment with cyproheptadine (10–12).

The present study was designed to investigate the effect of cyproheptadine on the nocturnal rise of plasma GH and cortisol levels in man.

METHODS

Seven male volunteers, aged 19–23, were used throughout the experiment. They were non-obese (less than 10% above ideal body weight) and apparently normal in endocrine and autonomic nerve function. None of them took any drugs regularly. They slept normally for several nights before the day of the experiment and were not allowed to nap in the daytime.

They lay down in bed in our sleep laboratory at 8:00 p.m. for study I and at 4:00 p.m. for study II, and were kept recumbent till the end of the experiment. Electroencephalograms, electrooculograms, electromyograms, and electrocardiograms were recorded by standard techniques and sleep was scored as stage 1, 2, 3, 4, and stage of rapid eye movement (REM) according to the criteria of Rechtschaffen and Kales (13).

An indwelling needle (18 gauge) was inserted into an antecubital vein under local anesthesia and connected to an infusion apparatus. Another indwelling needle was inserted distally into another vein of the same arm.

Blood was drawn every 20 min through the latter needle into heparinized syringes. Plasma samples were immediately separated and stored at −20°C until subsequent measure-

Received for publication 7 July 1975 and in revised form 29 January 1976.

[1] *Abbreviations used in this paper:* GH, growth hormone; REM, rapid eye movement; and SWS, slow wave sleep.

ment of plasma GH and cortisol. Plasma GH was measured by double antibody radioimmunoassay as previously described (14) with highly purified human GH (NIH HS 1863) as the standard, and plasma cortisol by a competitive protein-binding assay (15).

Study I. Seven subjects participated in this study, but two of them completed only the first and second night experiments. During the first night, physiological saline solution was infused at a rate of 1 ml/min throughout the night. This night served as the first base-line night. During the second night, 5 mg of cyproheptadine dissolved in 180 ml of saline was infused intravenously for 3 h after the onset of sleep, which was defined as the first entry into stage 2 sleep (13), and was followed by saline solution. During the third night, cyproheptadine solution was infused from 4:00 to 7:00 a.m. after the physiological saline infusion which was started before the onset of sleep. During the fourth night, physiological saline was infused throughout the night. The fourth night study served as the second base-line night.

Study II. The infusion of cyproheptadine was begun at 7:00 p.m. and finished at 10:00 p.m. in four of the seven subjects who had participated in study I. The third base-line night consisted of studies in which subjects were infused with saline alone, starting at 7:00 p.m. Two of the four complained of drowsiness during the infusion of cyproheptadine, but were not allowed to fall sleep until 8:00 p.m. when the lights were turned off.

Statistical analysis was performed by Student's *t* test.

RESULTS

Effect of cyproheptadine on GH release during sleep periods. A significant increase in plasma GH during early sleep periods was observed during the first and second base-line nights in all subjects studied. The peak values of plasma GH in these subjects varied from 17.0 to 51.0 ng/ml. However, the pattern of GH release during early sleep periods in each subject was very similar on the two base-line nights (Fig. 1).

Polygraphic records of the first base-line night showed that stage 3 sleep occurred 5–19 min after the onset of sleep with a mean (±SEM) of 11±2 min, and that the first increase in plasma GH (more than 5 ng/ml) was

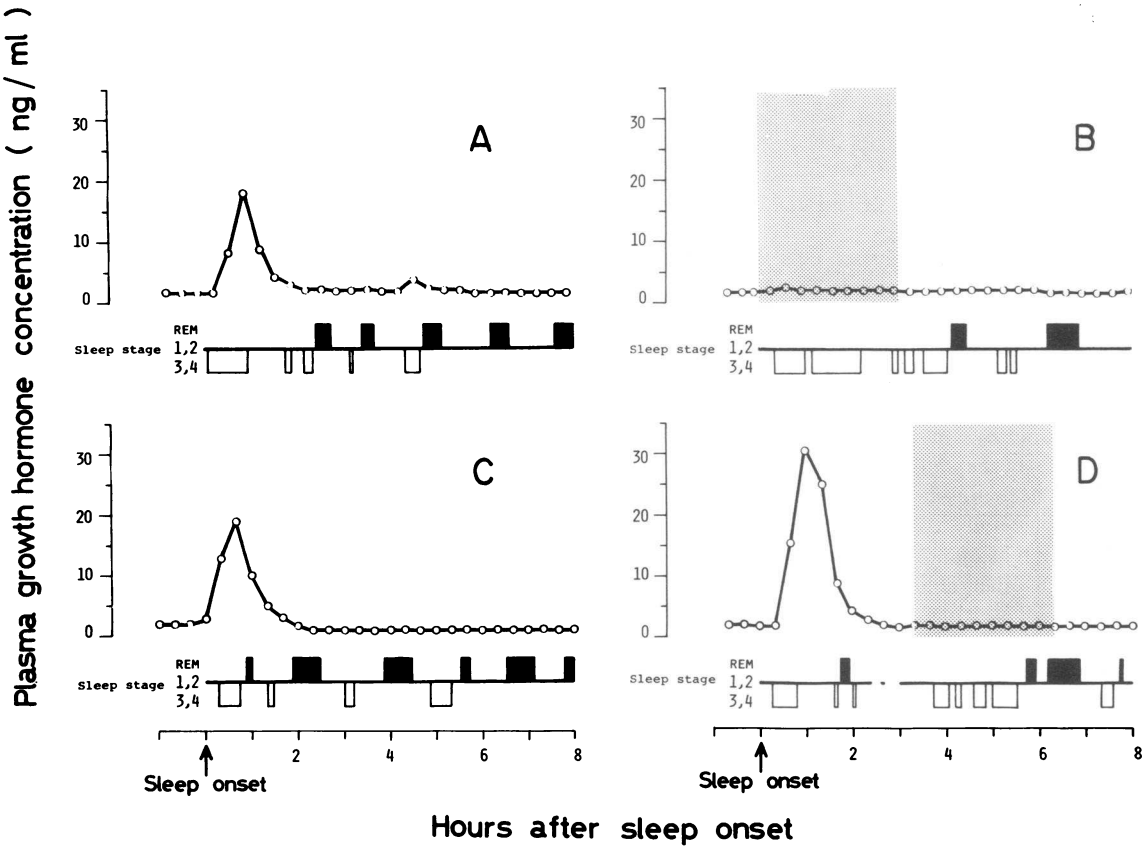

FIGURE 1 Plasma GH concentration after onset of sleep (entry into stage 2) during base-line nights and nights of cyproheptadine infusion in subject H. I. The sleep histogram is shown beneath each panel: Open histograms (□) show slow wave stages 3 and 4, closed histograms (■) REM stage, and solid line (—) stages 1 and 2. Breaks in lines represent waking periods. Shaded areas indicate periods of cyproheptadine infusion. (A), First base-line night; (B), Night with cyproheptadine infused after sleep onset; (C), Second base-line night; (D), Night with cyproheptadine infused from 4:00 a.m.

noted 25–35 min after the onset of sleep with a mean (±SEM) of 29±2 min. Results of the second base-line night experiment did not differ significantly from those of the first base-line night study, as shown in Table I. During the two base-line nights, the first appearance of slow wave sleep (SWS) was closely related with the initial GH increment, although the occurrence of SWS slightly but consistently preceded the first GH rise.

TABLE I

Plasma GH Levels and Occurrence of SWS in Seven Normal Subjects during Control Nights (a,c) and Nights with Cyproheptadine Infusion (b,d)

Subject	Time from sleep onset: First occurrence of sleep stage 3	Time from sleep onset: First elevation of plasma GH	Peak value of plasma GH after the first elevation
	min		*ng/ml*
(*a*) First base-line night			
H. I.	5	34	17.6
T. S.	7	25	21.0
T. T.	11	25	20.0
H. N.	19	26	36.0
K. K.	11	35	28.0
N. M.	4	34	51.0
H. F.	18	25	17.0
Mean±SEM	11±2	29±2	27.2±4.7
(*b*) Night with cyproheptadine infused after sleep onset			
H. I.	17	No peak*	No peak
T. S.	6	No peak	No peak
T. T.	8	No peak	No peak
H. N.	11	15	67.0
K. K.	9	17	28.5
N. M.	7	14	56.0
H. F.	6	16	28.0
Mean±SEM	9±1§	16±1‡	44.9±10.0‡§
(*c*) Second base-line night			
H. I.	16	23	17.2
T. S.	8	45	7.7
T. T.	22	30	20.5
H. N.	13	22	34.0
K. K.	23	31	11.2
Mean±SEM	16±3	30±4	18.1±4.6
(*d*) Night with cyproheptadine infused from 4:00 a.m.			
H. I.	14	41	30.5
T. S.	7	38	7.2
T. T.	19	32	40.0
H. N.	14	24	40.5
K. K.	11	35	22.7
Mean±SEM	13±2§	34±3	28.2±6.2§

* Plasma GH levels did not exceed 5 ng/ml.
‡ Subjects with no peak were excluded.
§ vs. control night, $P > 0.05$.

During the night when cyproheptadine was infused after the onset of sleep, the time from sleep onset to the first occurrence of SWS (mean±SEM: 9±1 min) did not differ significantly from that seen on the base-line nights, but GH release during early sleep showed two different patterns. In one (subjects H. I., T. S., and T. T.) the plasma GH increment noted early during base-line sleep was completely inhibited by cyproheptadine infusion, as shown in Fig. 1. In the other (subjects H. N., K. K., N. M., and H. F.), GH release was not suppressed by cyproheptadine infusion, and plasma GH peaks ranged from 28.0 to 67.0 ng/ml 14–17 min after sleep onset (Figs. 2, 3). However, when cyproheptadine was infused from 7:00 to 10:00 p.m. in these four subjects, the plasma GH rise was either completely absent or significantly delayed and occurred 130–193 min after sleep onset (Fig. 3 and Table II). The mean levels of plasma GH during the first 100 min period after sleep onset were significantly lower ($P < 0.005$) than those on the third base-line night (Fig. 4). The interval between sleep onset and the first occurrence of SWS, 10±3 min (Mean±SEM), did not differ from that (14±2 min) recorded on the third base-line night.

GH release during sleep was not affected by cyproheptadine infused from 4:00 to 7:00 a.m. in any of the five subjects examined.

Effect of cyproheptadine on plasma cortisol levels during sleep periods. Plasma cortisol concentrations during the base-line nights exhibited a progressive increase in the early morning, starting 3–5 h after the onset of sleep (i.e. at 4:00 a.m.) (Fig. 5). When cyproheptadine was infused intravenously from 4:00 to 7:00 a.m., cortisol release in these subjects was completely inhibited during the infusion, as shown in Fig. 6.

The mean cortisol concentrations while cyproheptadine was being infused from 4:00 to 7:00 a.m. were significantly ($P < 0.005$) lower than those during the same periods on the second base-line night (Fig. 6), which were slightly lower than those on the first base-line night.

Effect of cyproheptadine on sleep EEG stages. As shown in Table III, the percentage of time spent in each sleep stage was not significantly different between the first and second base-line nights, which were considered to be within normal limits in this laboratory.

On the night when cyproheptadine was infused after sleep onset, total sleep time was not significantly different from that on the base-line nights. However, SWS (sleep stage 3 and 4) was significantly increased ($P < 0.025$), whereas REM sleep was significantly decreased ($P < 0.005$) compared with the base-line nights. Total REM sleep time was also significantly decreased ($P < 0.05$) during the night when cyproheptadine was infused from 4:00 to 7:00 a.m. On the night when cyprohepta-

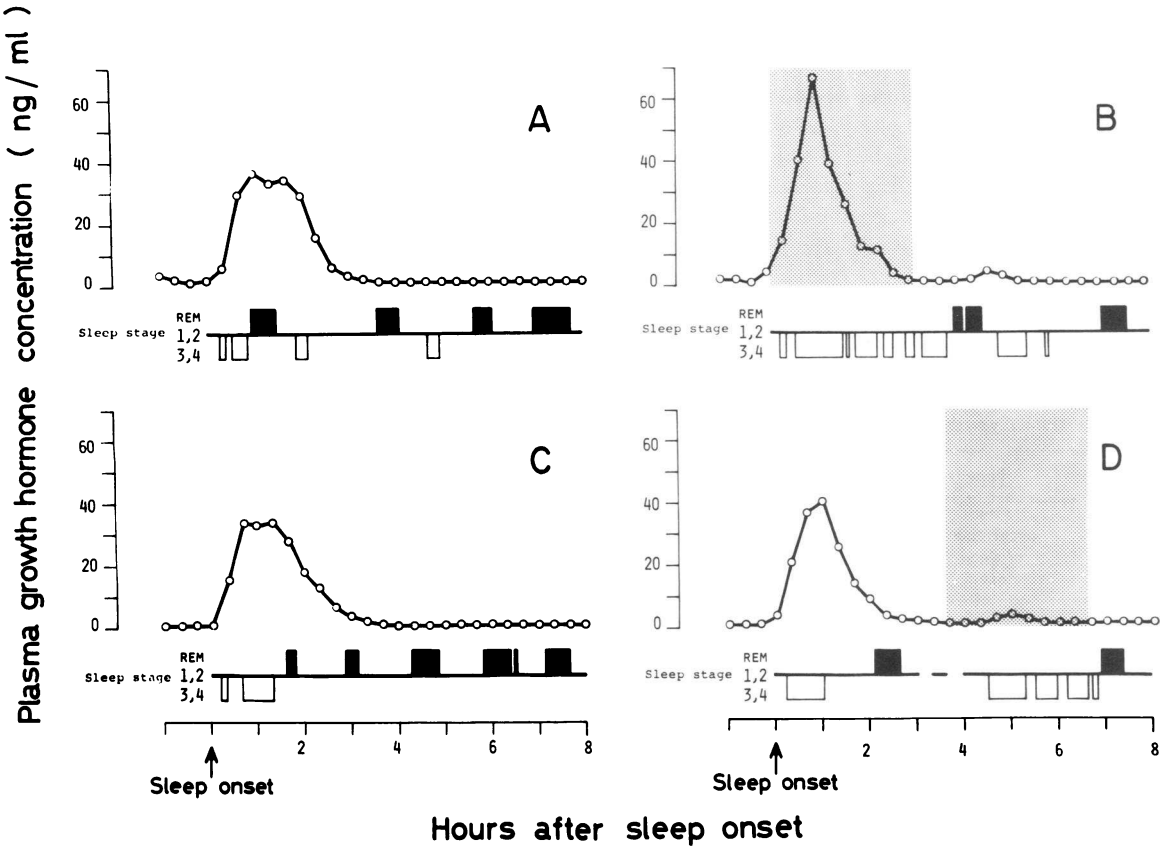

FIGURE 2 Plasma GH concentrations during base-line nights and nights of cyproheptadine infusion in subject H. N. Legends for sleep histogram and shaded areas are the same as in Fig. 1. (A), First base-line night; (B), Night with cyproheptadine infused after sleep onset; (C), Second base-line night; (D), Night with cyproheptadine infused from 4:00 a.m.

dine was infused from 7:00 to 10:00 p.m. (study II), the percentage of time spent in SWS and REM sleep was comparable to that on the night when cyproheptadine was infused after the onset of sleep.

DISCUSSION

It is well-known that plasma GH increases during the early phase of sleep with a peak occurring within the first 90 min of sleep (16–19). The lack of correlation between nocturnal GH release and plasma glucose, FFA, or insulin levels suggests that sleep-related GH release is not controlled by systemic metabolic substrates but by central nervous system rhythm (16, 20). Although GH secretion is influenced by various monoamines, brain catecholamines probably do not play a major role in sleep-induced GH release, since phentolamine, propranolol, chlorpromazine, and L-dihydroxyphenylalanine do not significantly alter the GH secretory pattern during sleep periods (16, 21, 22).

The present experiments demonstrate that the intravenous infusion of cyproheptadine suppresses GH release during early sleep periods in man. Cyproheptadine completely blocked GH release during sleep in three of the seven subjects given the drug by intravenous infusion after the onset of sleep, although it was ineffective in the remaining four subjects. Since the shortness from the onset of drug infusion to the first occurrence of SWS seemed to be responsible for the failure of drug effect, we infused cyproheptadine from 7:00 p.m. (1 h before lights were turned off) in the four unresponsive subjects. In this case, the plasma GH rises during sleep were either completely inhibited or significantly delayed, as compared with those on the third base-line night.

Parker et al. (23) noted that sleep-induced GH release is consistently suppressed during somatostatin infusion after sleep onset. Their observation may be explained by the direct action of somatostatin on the pituitary (24).

Different from somatostatin, cyproheptadine infusion is not always effective in suppressing the GH rise during sleep when it is started from sleep onset, but effective when started during presleep periods. The need of a latent time for the action of cyproheptadine sug-

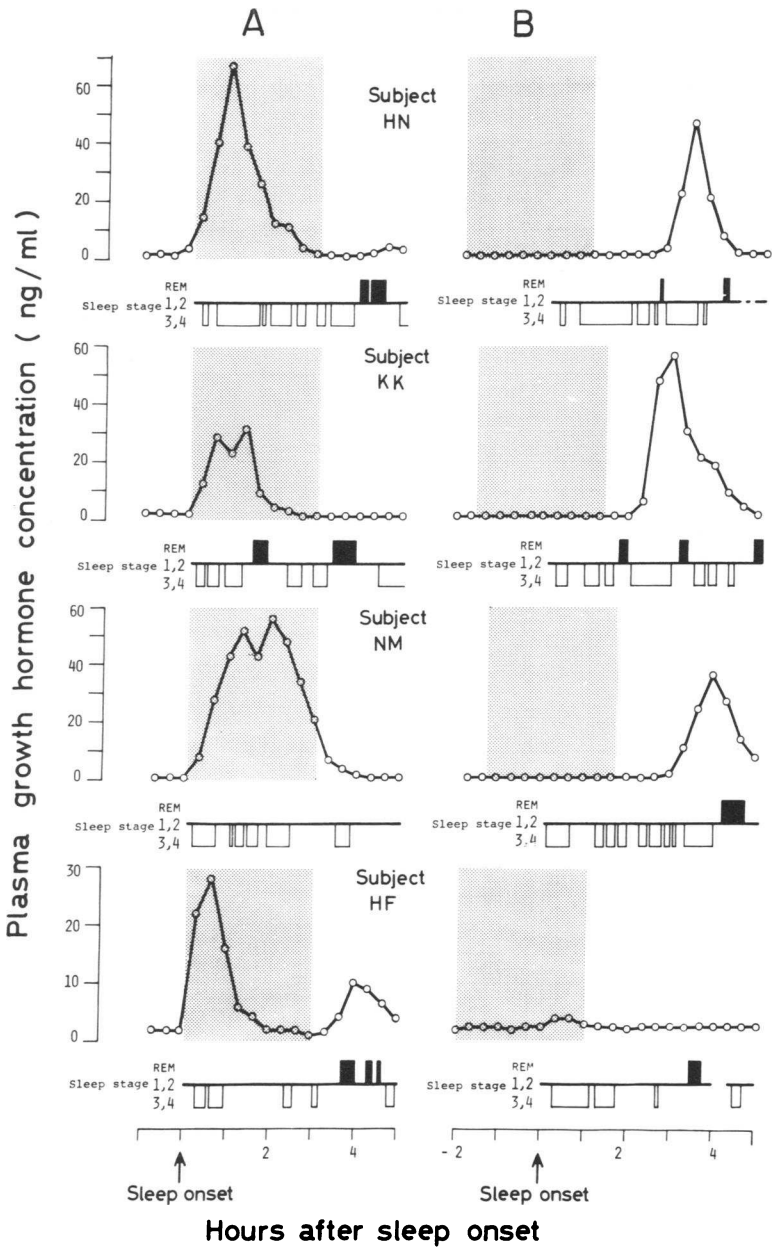

FIGURE 3 Effect of cyproheptadine infused at different times on plasma GH concentrations during early sleep periods in four subjects. A shows plasma GH values and the sleep pattern of each subject when cyproheptadine was infused after the onset of sleep, and B, when cyproheptadine was infused at 7:00 p.m. Legends for sleep histogram and shaded areas are the same as in Fig. 1.

gests that the drug inhibits the initiation of the neural trigger inducing GH release during early sleep periods at the suprahypophyseal level. However, the neural mechanisms inducing GH secretion and sleep seem to be controlled in different ways, since no difference was noted in the time from sleep onset to the first occurrence of SWS between those who responded to cyproheptadine and those who did not.

We have observed that plasma cortisol rises, possibly caused by ACTH release, in the early morning were completely inhibited by cyproheptadine infused from 4:00 to 7:00 a.m. Since plasma cortisol response to

TABLE II

Plasma GH Levels and Occurrence of SWS in Four Normal Subjects during Control Night and Night with Cyproheptadine Infused from 7:00 to 10:00 p.m.

Subject	Time from sleep onset to: First occurrence of sleep stage 3	Time from sleep onset to: First elevation of plasma GH	Peak value of plasma GH after the first elevation
	min		*ng/ml*
(*e*) Third base-line night			
H. N.	14	29	28.5
K. K.	15	14	17.5
N. M.	8	30	40.0
H. F.	19	17	30.0
Mean±SEM	14±2	23±4	29.0±4.6
(*f*) Night with cyproheptadine infused from 7:00 p.m.			
H. N.	10	179	46.7
K. K.	7	130	56.0
N. M.	5	193	36.5
H. F.	17	No peak*	No peak
Mean±SEM	10±3§	163±19‡‖	46.4±5.6‡§

* Plasma GH levels did not exceed 5 ng/ml.
‡ Subject with no peak was excluded.
Statistical significance vs. control night: § $P > 0.05$, ‖ $P < 0.005$.

ACTH was not altered in subjects receiving cyproheptadine,[2] the suppressive effect of cyproheptadine on the rise of plasma cortisol in the early morning could not be due to the direct action of this drug on the adrenal. These results agree with the observation of Krieger and Rizzo (25) that cyproheptadine, cinaserine, and parachloroamphetamine inhibit the daily rise in plasma corticosteroids in cats. It appears, therefore, that cyproheptadine inhibits the diurnal fluctuation of pituitary-adrenal function both in man and animals.

[2] S. Matsukura, K. Chihara, Y. Kato, K. Maeda, and H. Imura. Unpublished observation.

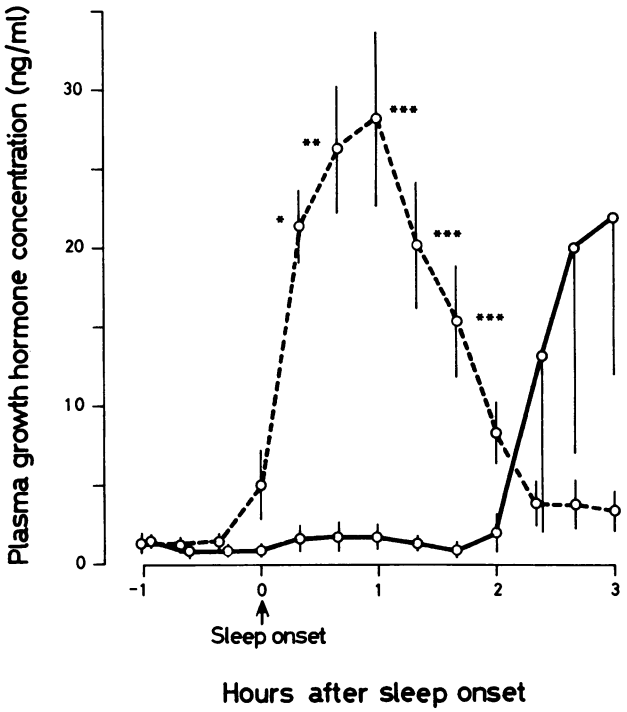

FIGURE 4 Effect of cyproheptadine infusion started during presleep period (7:00–10:00 p.m.) on sleep-induced GH rises in four normal subjects. The broken and solid lines show plasma GH values during the third base-line night (○- - -○) and during the night when cyproheptadine was infused from 7:00 to 10:00 p.m., respectively (○——○). Mean±SEM are shown. Statistical differences in plasma GH levels between these two nights are shown by asterisks: (*) $P < 0.005$, (**) $P < 0.01$, (***) $P < 0.025$. Plasma GH levels were analyzed considering the nearest point of sampling to the onset of sleep as time "0" after sleep onset.

TABLE III

Effect of Intravenous Infusion of Cyproheptadine on Sleep Stages

	Total sleep time	Percentage of sleep stage: Awake	1	2	3	4	REM	Total SWS time	Total REM sleep time
	min							*min*	*min*
First base-line night (7)‡	476±23*	1.7±0.8	5.1±1.5	54.2±1.5	8.1±0.8	8.6±1.8	22.2±0.9	88±9	106±6
Night with cyproheptadine infused after sleep onset (7)	495±17	1.5±0.9	3.2±1.0	50.2±1.8	15.6±2.3¶	15.6±1.6¶	13.9±0.7§	156±16§	69±5§
Night with cyproheptadine infused from 4:00 a.m. (5)	592±11‖	2.1±1.3	4.2±1.0	56.6±3.7	12.8±2.6	11.5±1.1	12.9±1.5‖	143±19¶	72±12**
Second base-line night (5)	487±31	2.0±0.6	4.9±1.5	56.1±2.6	6.7±1.2	6.4±1.9	25.7±2.1	61±9	127±17

* Values are shown as mean±SEM.
‡ Numbers of subjects are shown in parentheses.
Statistical significance vs. control nights: § $P < 0.005$, ‖ $P < 0.01$, ¶ $P < 0.025$, ** $P < 0.05$.

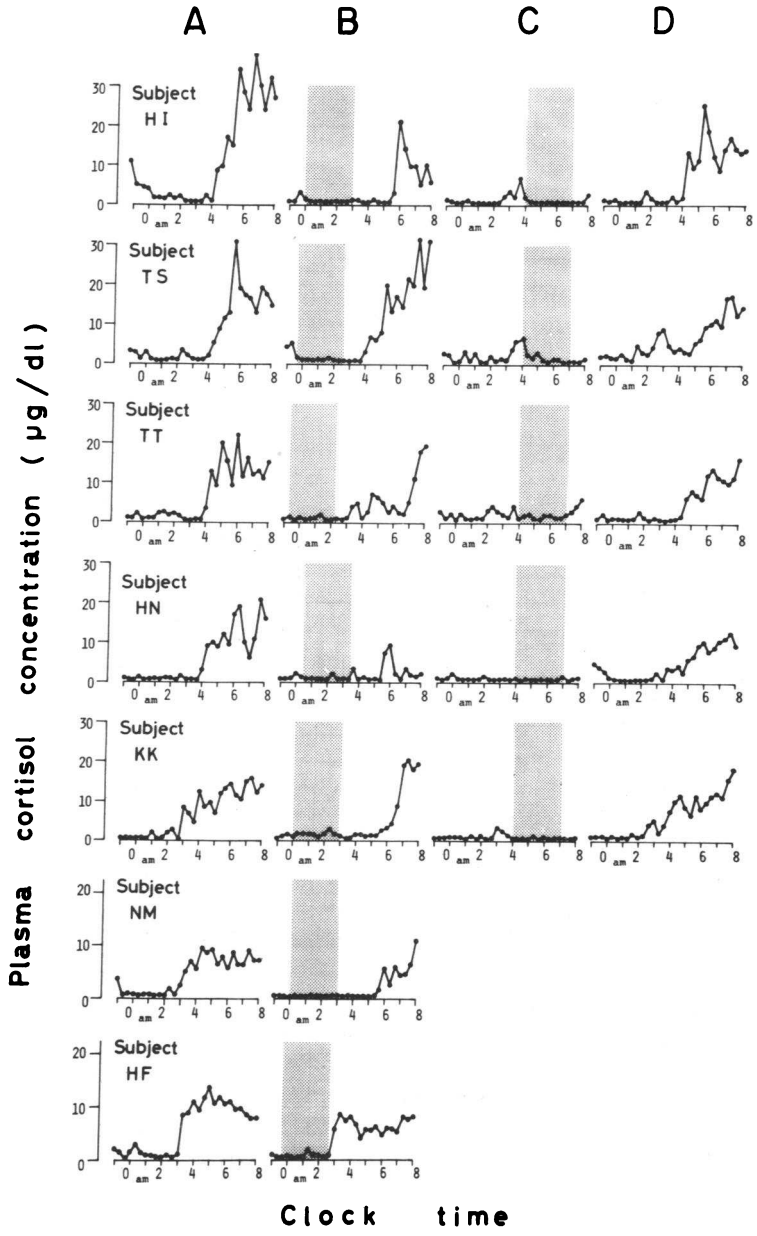

FIGURE 5 Plasma cortisol values in seven normal subjects with and without cyproheptadine infusion. A shows plasma cortisol concentrations plotted against clock time during the first base-line night, B during the night when cyproheptadine was infused after the onset of sleep, C during the night when cyproheptadine was infused from 4:00 to 7:00 a.m., and D during the second base-line night. Shaded areas indicate periods of cyproheptadine infusion.

In contrast with these observations, Plonk et al. (11) reported that base-line concentrations of serum cortisol in the morning were not changed by the oral administration of cyproheptadine, although serum cortisol response to insulin-induced hypoglycemia was blocked by this treatment. The discrepancy between our results and Plonk's may be explained by the different route, dose, and timing of the administration of cyproheptadine. Van Riezen (26) observed that cyproheptadine in a dose-dependent fashion blocked all effects supposed to be induced via central indole-sensitive receptors in mice. It has been reported that the GH release stimulated by 5-hydroxytryptophan, a serotonin precursor, is inhibited by cyproheptadine in man (6).

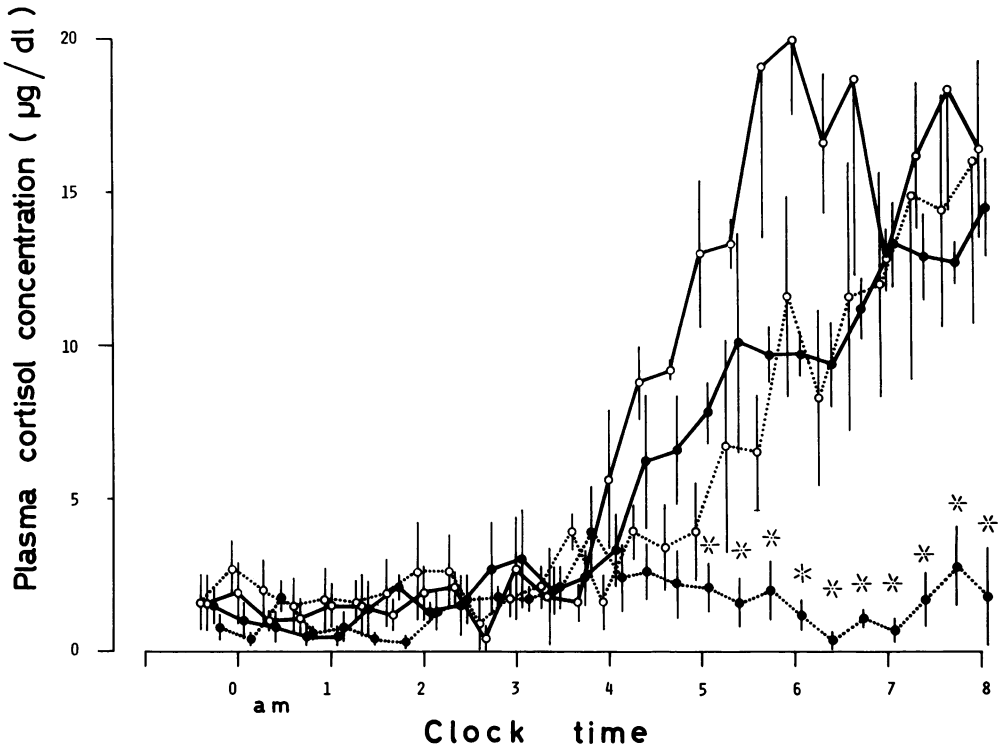

FIGURE 6 Effect of cyproheptadine on circadian rises in plasma cortisol during early morning periods in five normal subjects. The broken lines show plasma cortisol values during the night when cyproheptadine was infused for 3 h after the onset of sleep (○- - -○) or from 4:00 to 7:00 a.m. (●- - -●). The continuous lines show plasma cortisol values during either the first (○——○) or the second (●——●) base-line night. Mean±SEM are shown. Statistical differences in plasma cortisol levels between the second base-line night and the night when cyproheptadine was infused from 4:00 to 7:00 a.m. are shown by asterisks: (*) $P < 0.005$.

All these results may indicate that cyproheptadine inhibits GH and ACTH release by antagonizing brain serotoninergic mechanisms controlling secretion of these hormones. It has been known, however, that cyproheptadine, in addition to its antiserotonin activity, has antihistamine effects and very mild anticholinergic effects (7). Therefore, the possibility that the observed changes in GH and ACTH secretion are related to other actions of the drug than antiserotonin effect cannot be ruled out completely.

After this manuscript was submitted for publication, Mendelson et al. (27) reported that methysergide, a blocker of serotonin, rather enhanced sleep-related GH secretion in man. They administered methysergide (2 mg) orally every 6 h for 48 h with the final dose given at 6 a.m. on the day when sleep studies were performed. They also reported that methysergide inhibited insulin-induced GH release when the drug was given 30 min before the injection of insulin. The discrepancy between their results and ours may be explained by the different route and timing of the administration of the drug.

Polygraphic sleep monitoring on the night when cyproheptadine was infused during early sleep periods showed an apparent increase of SWS accompanied by a decrease of REM sleep. A prominent enhancement of SWS during the intravenous infusion of cyproheptadine is in agreement with the observation by Chakrabarty et al. (28) that the intravenous injection of cyproheptadine caused an increase of high voltage slow waves in both hypothalamic and cortical areas in cats. Recently, Krieger et al. (29) have reported that an increase in SWS was recognized in one subject with Cushing's disease receiving chronic cyproheptadine therapy.

Extensive studies in cats by Jouvet (30) have indicated that SWS may be initiated by a central serotoninergic mechanism. However, Dement (31) proposed a hypothesis derived from their animal experiments that serotonin regulates the ponto-geniculo-occipital activity, the driving force of REM sleep rather than a controller of SWS under normal circumstances. It has been reported that the administration of parachlorophenylalanine, an inhibitor of tryptophane hydroxylase, in patients

with carcinoid tumors caused a significant reduction of REM sleep without essential changes of SWS (32), and that 5-hydroxytryptophan prolonged REM sleep with a slight decrease of SWS in man (33, 34). Therefore, changes of sleep stages obtained by cyproheptadine in our studies may be explained, at least partly, by its antagonism to the central serotoninergic system. Alternatively, the effect of cyproheptadine on sleep patterns may be explained by an antihistaminic property of the drug to inhibit monoamine oxidase activity (35), since monoamine oxidase inhibitors cause irregular or transient enhancement of SWS with suppression of REM sleep (36). However, several investigators (37, 38) have suggested that these effects of monoamine oxidase inhibitors on sleep patterns might be related to brain serotonin changes.

It may be of interest to point out that cyproheptadine suppressed sleep-related GH release in spite of the enhancement of SWS. The sleep-related GH release is known to be closely related to SWS under normal circumstances (16–19). The apparent dissociation between the GH rise and SWS after the administration of cyproheptadine suggests that the subcortical mechanisms originating in the brain stem may act independently on cortical and hypothalamic areas under certain circumstances.

ACKNOWLEDGMENTS

We are indebted to the National Pituitary Agency and National Institute of Arthritis, Metabolism, and Digestive Diseases for providing highly purified human GH (NIH HS 1863) and Nippon Merk-Banyu Co. Tokyo for supplying cyproheptadine.

We also wish to thank Dr. Yoshikatsu Nakai and Dr. Noboru Sakamoto for encouragement and advice, and Miss Kikuko Nanba for excellent technical assistance.

This paper was supported in part by grants from the Ministry of Education and the Ministry of Welfare, Japan.

REFERENCES

1. Collu, R., F. Fraschini, P. Visconti, and L. Martini. 1972. Adrenergic and serotoninergic control of growth hormone secretion in adult male rats. *Endocrinology.* **90**: 1231–1237.
2. Naumenko, E. V., and R. Yu. Ilyychenok. 1967. Central action of serotonin on the hypophyseal-adrenal cortex system. *Bull. Exp. Biol. Med. (Engl. Transl. Byull, Eksp. Biol. Med.)* **64**: 978–980.
3. Naumenko, E. V. 1968. Hypothalamic chemoreactive structures and the regulation of pituitary-adrenal function. Effects of local injections of norepinephrine, carbachol and serotonin into the brain of guinea pigs with intact brain and after mesencephalic transection. *Brain Res.* **11**: 1–10.
4. Krieger, H. P., and D. T. Krieger. 1970. Chemical stimulation of the brain: effect on adrenal corticoid release. *Am. J. Physiol.* **218**: 1632–1641.
5. Smythe, G. A., and L. Lazarus. 1973. Growth hormone regulation by melatonin and serotonin. *Nature (Lond.).* **244**: 230–231.
6. Imura, H., Y. Nakai, and T. Yoshimi. 1973. Effect of 5-hydroxy-tryptophan (5-HTP) on growth hormone and ACTH release in man. *J. Clin. Endocrinol. Metab.* **36**: 204–206.
7. Stone, C. A., H. C. Wenger, C. T. Ludden, J. M. Stavorski, and C. A. Ross. 1961. Antiserotonin-antihistaminic properties of cyproheptadine. *J. Pharmacol. Exp. Ther.* **131**: 73–84.
8. Nakai, Y., H. Imura, H. Sakurai, H. Kurahachi, and T. Yoshimi. 1974. Effect of cyproheptadine on human growth hormone secretion. *J. Clin. Endocrinol. Metab.* **38**: 446–449.
9. Nakai, Y., and H. Imura. 1975. Suppressive effect of cyproheptadine on L-DOPA-induced growth hormone release in man. *Endocrinol Jpn.* **22**: 357–360.
10. Bivens, C. H., H. E. Lebovitz, and J. M. Feldman. 1973. Inhibition of hypoglycemia-induced growth hormone secretion by the serotonin antagonists cyproheptadine and methysergide. *N. Engl. J. Med.* **289**: 236–239.
11. Plonk, J. W., C. H. Bivens, and J. M. Feldman. 1974. Inhibition of hypoglycemia-induced cortisol secretion by the serotonin antagonist cyproheptadine. *J. Clin. Endocrinol. Metab.* **38**: 836–840.
12. Smythe, G. A., and L. Lazarus. 1974. Suppression of human growth hormone secretion by melatonin and cyproheptadine. *J. Clin. Invest.* **54**: 116–121.
13. Rechtschaffen, A., and A. Kales. 1968. USPHS Manual of Standardized Terminology, Techniques and Scoring Systems for Sleep Stages of Human Subjects. U. S. Government Printing Office, Washington, D. C.
14. Maeda, K., Y. Kato, S. Ohgo, K. Chihara, Y. Yoshimoto, N. Yamaguchi, S. Kuromaru, and H. Imura. 1975. Growth hormone and prolactin release after injection of thyrotropin releasing hormone in patients with depression. *J. Clin. Endocrinol. Metab.* **40**: 501–505.
15. Murphy, B. E. P. 1967. Some studies of the protein binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. *J. Clin. Endocrinol. Metab.* **27**: 973–980.
16. Takahashi, Y., D. M. Kipnis, and W. H. Daughaday. 1968. Growth hormone secretion during sleep. *J. Clin. Invest.* **47**: 2079–2090.
17. Honda, Y., K. Takahashi, S. Takahashi, K. Azumi, M. Irie, M. Sakuma, T. Tsushima, and K. Shizume. 1969. Growth hormone secretion during nocturnal sleep in normal subjects. *J. Clin. Endocrinol. Metab.* **29**: 20–29.
18. Sassin, J. F., D. C. Parker, J. W. Mace, R. W. Gotlin, L. C. Johnson, and L. G. Rossman. 1969. Human growth hormone release: relation to slow-wave sleep and sleep-waking cycles. *Science (Wash. D. C.).* **165**: 513–515.
19. Parker, D. C., J. F. Sassin, J. W. Mace, R. W. Gotlin, and L. G. Rossman. 1969. Human growth hormone release during sleep: electroencephalographic correlation. *J. Clin. Endocrinol. Metab.* **29**: 871–874.
20. Quabbe, H. J., E. Schilling, and H. Helge. 1966. Pattern of growth hormone secretion during a 24-hour fast in normal adults. *J. Clin. Endocrinol. Metab.* **26**: 1173–1177.
21. Lucke, C., and S. M. Glick. 1971. Experimental modification of the sleep-induced peak of growth hormone secretion. *J. Clin. Endocrinol. Metab.* **32**: 729–736.
22. Chihara, K., Y. Kato, K. Maeda, and H. Imura. 1976. Suppressive effect of L-DOPA on human prolactin release during sleep. *Acta Endocrinol.* **81**: 19–27.
23. Parker, D. C., L. G. Rossman, T. M. Siler, J. Rivier, S. S. C. Yen, and R. Guillemin. 1974. Inhibition of the

sleep-related peak in physiologic human growth hormone release by somatostatin. *J. Clin. Endocrinol. Metab.* **38**: 496–499.
24. Brazeau, P., W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier, and R. Guillemin. 1973. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science (Wash. D. C.)*. **179**: 77–79.
25. Krieger, D. T., and F. Rizzo. 1969. Serotonin mediation of circadian periodicity of plasma 17-hydroxycorticosteroids. *Am. J. Physiol.* **217**: 1703–1707.
26. Van Riezen, H. 1972. Different central effects of the 5-HT antagonists mianserine and cyproheptadine. *Arch. Int. Pharmacodyn. Ther.* **198**: 256–269.
27. Mendelson, W. B., L. S. Jacobs, J. D. Reichman, E. Othmer, P. E. Cryer, B. Trivedi, and W. H. Daughaday. 1975. Methysergide: Suppression of sleep-related prolactin secretion and enhancement of sleep-related growth hormone secretion. *J. Clin. Invest.* **56**: 690–697.
28. Chakrabarty, A. S., R. V. Pillai, B. K. Anand, and B. Singh. 1967. Effect of cyproheptadine on the electrical activity of the hypothalamic feeding centres. *Brain Res.* **6**: 561–569.
29. Krieger, D. T., L. Amorosa, and F. Linick. 1975. Cyproheptadine-induced remission of Cushing's disease. *N. Engl. J. Med.* **293**: 893–896.
30. Jouvet, M. 1972. The role of monoamines and acetylcholine containing neurons in the regulation of the sleep-waking cycle. *In Ergebnisse der Physiologie* (Reviews of Physiology). Neurophysiology and neurochemistry of sleep and wakefulness. **64**: 166–307.
31. Dement, W. C. 1969. The biological role of REM sleep. *In* Sleep, Physiology and Pathology. A Symposium, A. Kales, editor. J. B. Lippincott Co., Philadelphia. pp. 245–265.
32. Wyatt, R. J., K. Engelman, D. Kupfer, J. Scott, A. Sjoerdsma, and F. Snyder. 1969. Effect of parachlorophenylalanine on sleep in man. *Electroencephalogr. Clin. Neurophysiol.* **27**: 529–532.
33. Wyatt, R. J., V Zarcone, K. Engelman, W. C. Dement, F. Snyder, and A. Sjoerdsma. 1971. Effect of 5-hydroxytryptophan on the sleep of normal human subjects. *Electroencephalogr. Clin. Neurophysiol.* **30**: 505–509.
34. Mandell, M. P., A. J. Mandell, and A. Jacobson. 1964. Biochemical and neurophysiological studies of paradoxical sleep. *Recent. Adv. Biol. Psychiatr.* **7**: 115–122.
35. Tickner, A. 1951. Inhibition of amine oxidase by antihistamine compounds and related drugs. *Br. J. Pharmacol.* **6**: 606–610.
36. Dunleavy, D. L. F., V. Brezinova, I. Oswald, A. W. Maclean, and M. Tinker. 1972. Changes during weeks in effects of tricyclic drugs on the human sleeping brain. *Br. J. Psychiatr.* **120**: 663–672.
37. Jouvet, M. 1969. Neurophysiological and biochemical mechanism of sleep. *In* Sleep, Physiology and Pathology. A Symposium. A. Kales, editor. J. B. Lippincott Co., Philadelphia, pp. 89–100.
38. Hartmann, E. L. 1968. Some studies in biochemistry of dreaming sleep. *In* Proceeding of IV World Congress of Psychiatry, Madrid. Part 4. Exerpta Medica International Congress Series 150. Exerpta Medica, Amsterdam. p. 3100–3102.