Methysergide

SUPPRESSION OF SLEEP-RELATED PROLACTIN SECRETION AND ENHANCEMENT OF SLEEP-RELATED GROWTH HORMONE SECRETION

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ABSTRACT Methysergide, a clinically-used blocker of serotonin receptors, was administered to 10 normal young men at a dose of 2 mg every 6 h for 48 h. After drug treatment, serum levels of growth hormone during sleep were 41.9% higher than placebo values ($P < 0.001$). In contrast, drug treatment was associated with a 36.4% decrease in stimulated growth hormone secretion during insulin tolerance testing ($P < 0.01$). These opposite effects of methysergide suggest that different mechanisms are responsible for sleep-related and insulin-induced growth hormone secretion. Accordingly, data obtained with pharmacologic stimuli may lead to erroneous inferences regarding physiologic growth hormone control mechanisms. Administration of methysergide profoundly suppressed sleep-related prolactin secretion; overall nocturnal mean prolactin fell by 70.3% from 4.30±0.19 to 1.28±0.06 ng/ml ($P < 0.0001$).

It appears that serotonin may be a significant modulating neurotransmitter for the control of growth hormone secretion, limiting sleep-related release, and enhancing insulin-induced release. It seems likely from these data that the role of serotonin in the control of prolactin secretion is relatively more important, since serotonin receptor blockade dramatically reduced sleep-related prolactin secretion.

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INTRODUCTION

There is evidence that both prolactin (PRL)[1] and growth hormone (GH) have nocturnal secretory patterns related to the electroencephalographic sleep stages. In the former, there appear to be a series of cycles whose nadirs occur during rapid eye movement (REM) sleep (1); in the latter, there is a peak occurring during the first 90 min of sleep, lasting from 1.5 to 3.5 h and related to slow wave sleep (2). There is evidence that adrenergic and serotonergic systems are involved in acute daytime release of both hormones (3–5). On the other hand, there are no data available as to whether drugs active on the adrenergic and serotonergic systems influence sleep-related secretion of PRL or GH. In an effort to evaluate further the role of serotonergic systems in this area, we have measured plasma PRL and GH during the sleep of normal subjects who have been pretreated with the clinicaly-used serotonin receptor blocker, methysergide (6). In addition, an insulin tolerance test (ITT) was performed. This was done to confirm an earlier observation that methysergide may decrease insulin-induced GH secretion (4) and to compare observed changes after insulin with those occurring during sleep.

METHODS

Subjects were 10 male paid volunteers, ages 21–30, with no history of major mental illness and no personal or family history of diabetes mellitus. Their height/weight ratios were between 2.3 and 3.2 cm/kg (7). They were

[1] *Abbreviations used in this paper:* GH, growth hormone; ITT, insulin tolerance test; PRL, prolactin; REM, rapid eye movement.

screened by a medical history, physical examination, complete blood count, and blood chemistry profile. After detailed verbal and written explanation of the study, written informed consent was obtained.

The study was designed in a double-blind crossover manner. After an adjustment night in the laboratory, each subject spent drug and placebo nights which were 2 wk apart. The sequence in which drug and placebo were given was randomized. Before drug nights, subjects received methysergide orally, 2 mg every 6 h for nine doses. The final dose was at 6 a.m. of the drug night, 30 min before the ITT. Placebo tablets were given using the same drug schedule.

On the study nights, a unipolar electroencephalogram (EEG), horizontal electro-oculogram, and submental electromyogram were recorded from 10 p.m. until 6 a.m. Recordings were done on a Grass Model 7 polygraph (Grass Instrument Co., Quincy, Mass.) with a paper speed of 15 mm/s calibrated for 50 μV to produce a 10-mm deflection. Recordings were read blindly using sleep stage criteria of Rechtschaffen and Kales (8). During the night, 5-cm^3 samples of blood were taken every 20 min from an indwelling venous catheter for GH and PRL analysis. The catheter was kept open by slow infusion of one-half normal saline containing 3,000 U of heparin per liter. Total intake was up to 500 cm^3 of this solution.

At approximately 6:30 a.m., the patient was given 0.1 U/kg of regular crystalline pork insulin IV; 5 cm^3 venous blood samples for GH analysis were drawn every 15 min for 2 h and 5-cm^3 samples for blood glucose were drawn every 30 min. All specimens for GH and PRL assay were allowed to clot; the serum was promptly separated and stored at -18°C.

Determinations of GH and PRL in the subjects' serum samples were performed by radioimmunoassay (9, 10). The antisera, standards and tracer, and current details of the GH method have been recently described (11). The usual sensitivity of the GH assay was 0.25 ng/ml of serum and 0.5 ng/ml of the PRL assay. Blood glucose determinations were done by the Clinical Chemistry Laboratory of Barnes Hospital using a glucose oxidase technique. Hormonal data during sleep were processed by an analysis of variance derived from the Statistical Analysis System computer package (12). For the purposes of this analysis, the data were divided into three time periods, hours 1 and 2, hours 3 and 4, and hours 5–8 after the start of sleep. The data were subjected to a logarithmic$_{10}$ transformation to normalize them (13), and partial sums of squares were employed because of the unbalanced, nonorthogonal design. This analysis allowed separate examination of the contributions to the total variance of differences between subjects, the effects of time period, sleep stages and drug treatment, and the interactions between treatment and time period and between treatment and sleep stage (see Tables I and IV).

As usual, the GH values after the ITT exhibited great variability in level from subject to subject and also in time of onset of rise and time of peak increment. Therefore, these data were analyzed by a two-way analysis of variance so that all the data could be employed appropriately in the determination of a possible drug effect. To minimize the problem of nonindependence of sequential data points in a secretory episode, an Analysis of Variance (ANOVA) table was constructed by subtracting the GH value at each time during the methysergide ITT from the corresponding GH value for the same time and from the same subject during the placebo ITT. Thus, a table of differences was constructed in which the 10 columns represented the 10 subjects and the 9 rows represented the sampling times during ITT (0, 15, 30, 45, 60, 75, 90, 105, and 120 min). In addition to avoiding the problem of nonindependence of multiple time points, this approach also permits the determination of whether or not the two GH curves (see Fig. 2) deviate from parallelism with each other. Such a deviation is indicated if the effect of time is significant in the ANOVA, since the differences between the two curves are the data base. The effect of methysergide treatment is determined by posing the question whether or not the overall mean of these data is significantly different from zero. If so, the treatment is indicated to have been effective; if the mean is not different from zero, treatment has not affected the GH response to ITT.

RESULTS

Sleep. As reported in detail elsewhere (14), methysergide significantly diminished the proportion of total sleep time spent in REM sleep (12.4 vs. 19.3%, $P < 0.025$) without significantly altering total sleep time or sleep latency.

GH. Changes throughout the night are shown graphically in Fig. 1A. As expected, there was a peak of secretion during the early part of the night. Table I is an analysis of variance table for a regression analysis using treatment, sleep stage, and time of night as classification variables. Partial sums of squares (controlling for all other independent variables) are reported. A large variation between individuals was noted. Drug treatment resulted in a small but significant increase in sleep-related GH serum levels over the entire night from a mean of 1.84±0.17 ng/ml on placebo to 2.61±0.23 ng/ml on drug nights ($P < 0.001$; Table II). When the analysis was limited to the period of active GH secretion (the first 2 h), this difference was larger (3.12±0.49 on placebo vs. 5.73±0.74 ng/ml on methysergide; $P < 0.01$). Similarly, there was an increase in peak GH levels from 7.86±1.35 ng/ml on placebo to

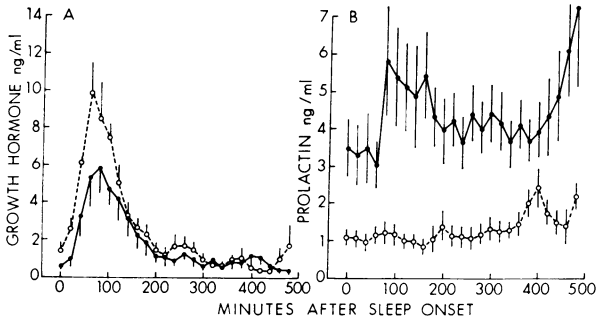

FIGURE 1 Effect of methysergide on sleep-related GH and PRL secretion. Methysergide-treated subjects (dotted line) had increased GH secretion, particularly in first 2 h of sleep, compared to placebo group (solid line). PRL secretion throughout night was significantly decreased by methysergide. Lines on either side of mean values represent 1 SEM.

TABLE I

Analysis of Variance: Sleep-Related GH Secretion

Source	df	Partial sum of squares	*F* Value	Probe > *F*
Subjects	9	6.47798599	8.24480	0.0001
Drug treatment	1	0.96555228	11.06009	0.0010
Time period	2	11.63188115	66.61970	0.0001
Treatment, time period	2	0.60032804	3.43828	0.0320
Sleep stage	5	1.46441414	3.35488	0.0058
Treatment, sleep stage	5	0.45077355	1.03269	0.3983
Error	421	36.75355825		
Total	445	62.76310612		

10.80±1.39 ng/ml on methysergide. There was a significant time-treatment interaction ($P < 0.03$). Serum values were highest during the first 2 h of sleep with a progressive decrease during the rest of the night. Time period alone was related to levels of GH ($P < 0.0001$). There was no interaction between treatment and sleep stage.

The relation of sleep stage to GH levels was determined by comparing each stage against REM. There was a significant ($P < 0.001$) inverse relationship between the waking state and GH levels. Stages 3 and 4 were directly related to GH levels ($P < 0.01$ and $P < 0.04$ respectively).

In contrast to sleep-related secretion, GH response to insulin was decreased by methysergide (Fig. 2). Thus, the area under the curve (GH concentration on ordinate, time on abscissa) was 859.9±277.8 ng–min on placebo, but was only 547.4±208.5 ng–min on methysergide. Similarly during the ITT the rise in GH on methysergide was 10.29±4.58 ng/ml compared with 15.02±5.17 ng/ml on placebo. These comparisons fell short of statistical significance, but the difference in plasma levels of GH on placebo and drug was significant at the $P < 0.01$ level when analyzed by a two-way analysis of variance as described in detail above and shown in Table III.

PRL. Significance levels and technical details of the analysis of variance are shown in Table IV and mean values appear in Table V. As with GH secretion, there was large variation between subjects. Drug treatment produced a highly significant decrease in sleep-related PRL secretion from a mean of 4.30±0.19 ng/ml on placebo to 1.28±0.06 ng/ml on methysergide ($P < 0.0001$); Fig. 1B). Similarly, peak PRL secretion was also suppressed on methysergide from 8.51± 1.12 to 2.64 ± 0.45 ng/ml ($P < 0.001$). Time period had a significant effect ($P < 0.003$); a large increase in PRL secretion was noted after about 7 h of sleep, particularly on placebo nights (Fig. 1). The sleep stages themselves were not related to PRL levels.

TABLE II

Effect of Methysergide on Sleep-Related GH Secretion

	Hours 1 and 2		Hours 3 and 4		Hours 5–8		Total (sleep stages)
	Methysergide	Placebo	Methysergide	Placebo	Methysergide	Placebo	
Intermittent waking	0.94±0 (1)	0.72±0.19 (2)	3.55±2.25 (2)	0.39±0.10 (7)	0.83±0.15 (5)	0.55±0.30 (4)	0.88±0.26 (21)
Stage 1	0.50±0 (1)	0.29±0.17 (2)	2.80±0.10 (2)	5.65±2.45 (2)	1.36±0.50 (10)	1.45±0.48 (9)	1.72±0.38 (26)
Stage 2	6.68±1.37 (20)	2.97±0.58 (24)	2.96±0.43 (31)	3.98±0.97 (17)	1.08±0.16 (74)	0.83±0.12 (62)	2.18±0.21 (228)
Stage 3	5.29±1.22 (13)	3.62±2.04 (4)	5.68±1.42 (9)	1.90±0.10 (2)	1.45±0.35 (10)	1.09±0.39 (10)	3.41±0.53 (48)
Stage 4	5.48±1.14 (12)	3.40±0.99 (17)	11.70±0 (1)	3.62±0.78 (13)	0.42±0.04 (5)	1.92±0.68 (6)	3.63±0.50 (54)
REM	— (0)	10.80±0 (1)	1.43±0.65 (3)	1.87±0.88 (9)	0.96±0.26 (22)	0.54±0.09 (34)	1.03±0.21 (69)
Total (treatment)	5.73±0.74 (47)	3.12±0.49 (50)	3.57±0.45 (48)	2.99±0.46 (50)	1.08±0.12 (126)	0.86±0.09 (125)	
Total (time)	4.39±0.46 (97)		3.27±0.32 (98)		0.97±0.07 (251)		

Values in ng/ml±SEM; parentheses refer to number of samples which were obtained during the indicated sleep stage and time period.

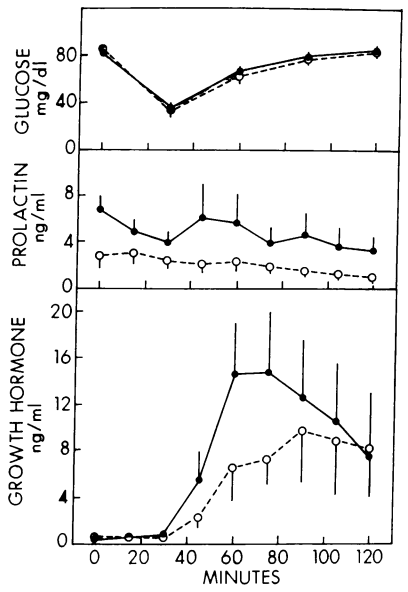

FIGURE 2 Effect of methysergide on glucose and GH and PRL secretion during ITT. Methysergide (dotted line) was related to decreased GH secretion during an ITT; PRL levels were lower after methysergide and did not change after insulin. 1 SEM is indicated on either side of the mean by the vertical lines.

Because of a previous report that peaks of PRL were associated with episodes of non-REM sleep (1), we also examined this relationship. First we duplicated the analytic method of this previous study by lining up PRL values using the end of REM sleep periods as reference points. A one-way analysis of variance of the data arranged in this manner did not show any difference between values during REM and subsequent non-REM sleep periods. In a second analysis, we defined a peak as a PRL level ≥ 1 SD above the mean serum concentration of 4.30±2.79 ng/ml (SD). There was no difference in the relative number of peaks in REM and non-REM sleep on placebo nights. A similar analysis was technically unsatisfactory on methysergide nights because of the marked suppression of PRL and resultant decreased precision near the detection limit of the assay.

The mean increase in PRL during the ITT after methysergide was 1.32±0.56 ng/ml compared to 2.96± 2.27 ng/ml after placebo (Fig. 2). These increments were not significantly different (two-tail paired *t*-test); in addition, neither "increase" represented a significant alteration from basal values.

DISCUSSION

As in the case of all clinical drug studies, interpretation of data must be tempered with the reservation that the observed effects may be due to other actions of the drug employed than the one presumed. This is clearly true in our study as well. It should be noted, however, that methysergide has relatively few other reported actions which cannot be accounted for by serotonergic blockade. The only other reported actions are weak vasoconstrictor and oxytocic properties (15, 16).

It has been suggested that some central actions of methysergide may not be accounted for by its anti-serotonin properties. Haigler and Aghajanian (17) have reported that microiontophoretically administered methysergide does not reverse the suppression of firing of single cells normally produced by serotonin in such areas as the raphe nuclei, ventral lateral geniculate, optic tectum, and amygdala of rats. They conclude that agents shown to be serotonin antagonists in certain conditions cannot be assumed to have such effects under all circumstances. Although this caution is certainly in order, it should be noted that Segal and Bloom (18) have observed that methysergide blocks serotonin inhibitory responses in pyramidal cells of the hippocampus. Methysergide has been shown to enter the brain (19), inhibit the central toxic action of 5-hydroxytryptophan (20), and block uptake of labeled serotonin in in vitro "nerve-ending membrane" fractions of tissue from the hypothalamus, basal ganglia, and gray areas of the mesencephalon (21). Hence, the mechanism of the central action of methysergide remains uncertain. Our data showing marked suppression of sleep-related PRL secretion, enhancement of sleep re-

TABLE III
Analysis of Variance: GH during ITT

Source	df	Sum of squares	*F*	*P*
Time	8	463.51	0.83	NS
Subject	9	7,309.78	11.67	$P < 0.001$
Treatment	1	582.47	8.37	$P < 0.01$
Error	72	5,010.69		
Total	90	13,366.45		

TABLE IV
Analysis of Variance: Sleep-Related PRL Secretion

Source	df	Partial SS	*F* Value	Prob > *F*
Subjects	9	13.98006659	50.22074	0.0001
Drug treatment	1	8.48880731	274.44987	0.0001
Time period	2	0.37953320	6.13530	0.0028
Treatment, time period	2	0.26920926	4.35187	0.0133
Sleep stage	5	0.28481790	1.84168	0.1027
Treatment, sleep stage	5	0.12346547	0.79835	0.5530
Error	421	13.02164158		
Total	445	49.16091220		

SS, sum of squares.

TABLE V

Effect of Methysergide on Sleep-Related PRL Secretion

	Hours 1 and 2		Hours 3 and 4		Hours 5–8		Total (sleep stages)
	Methysergide	Placebo	Methysergide	Placebo	Methysergide	Placebo	
Intermittent waking	1.60±0 (1)	2.35±0.45 (2)	0.25±0.15 (2)	9.78±2.38 (7)	2.50±0.75 (5)	5.68±2.18 (4)	5.26±1.16 (21)
Stage 1	0.80±0 (1)	3.95±0.05 (2)	2.25±2.15 (2)	3.50±1.20 (2)	1.80±0.32 (10)	4.01±0.63 (9)	2.86±0.35 (26)
Stage 2	1.04±0.18 (20)	3.90±0.51 (24)	0.96±0.14 (31)	4.23±0.58 (17)	1.28±0.11 (74)	4.72±0.30 (62)	2.65±0.16 (228)
Stage 3	1.26±0.26 (13)	2.43±1.59 (4)	0.97±0.21 (9)	1.70±0.10 (2)	1.16±0.25 (10)	4.21±0.83 (10)	1.91±0.29 (48)
Stage 4	1.24±0.16 (12)	3.08±0.61 (17)	1.50±0 (1)	5.20±0.80 (13)	2.08±0.17 (5)	6.17±1.10 (6)	3.40±0.37 (54)
REM	— (0)	5.70±0 (1)	0.73±0.20 (3)	2.83±0.60 (9)	1.50±0.17 (22)	3.52±0.34 (34)	2.70±0.63 (69)
Total (treatment)	1.16±0.11 (47)	3.48±0.35 (50)	0.98±0.13 (48)	4.88±0.53 (50)	1.43±0.09 (126)	4.40±0.21 (125)	
Total (time)	2.35±0.22 (97)		2.97±0.34 (98)		2.91±0.15 (251)		

Values in ng/ml±SEM; parentheses refer to number of samples which were obtained during the indicated sleep stage and time period.

lated GH secretion, and suppression of GH response to ITT are compatible with either type of mechanism.

Our findings regarding the relation of GH levels to the sleep stages confirm those of Takahashi, Kipnis, and Daughaday (2) who found that GH secretion is related to stages 3 and 4. The question of a possible relation of PRL to the sleep stages is less clear. Sassin, Frantz, Weitzman, and Kapen (22), who studied PRL over 24 h in three men and three nulliparous women, found no readily observable relationship of peaks to sleep stages. There was, however, no specific analysis made of this possible relationship. Parker, Rossman, and Vanderlaan (1), studying 14 normal males, also found no relationship between PRL rises or peaks and specific sleep stages. They did, however, find peaks of PRL in overall non-REM periods after REM sleep. Our study confirms these two reports insofar as we also found no relation between plasma PRL levels and specific sleep stages. When we used the method of analysis of the latter study on our data, we did not confirm the finding of a rise in PRL after the end of REM sleep.

When comparing the difference in results between our study and that of Parker et al. (1), two differences in experimental approach may be relevant. First of all, our subjects were 21–30-yr old, whereas theirs were 12–24-yr old, 43% of whom were pubertal. Secondly, our subjects were studied on placebos for one night each, whereas they presented the data from 58 nights of recordings taken from 14 subjects. Since differences between individual subjects are clearly a significant element in studies of this type (see Table IV), the use of certain subjects on multiple nights (up to 11) in their study could clearly have an important influence on results obtained.

Although there is no shortage of data available on the possible role of monoamines in the control of secretion of GH in response to insulin, there has been little data available on control of sleep-related secretion. Takahashi et al. (2) noted that administration of imipramine resulted in decreased GH secretion. Since imipramine has been reported to have several actions in the central nervous system, including adrenergic and anticholinergic effects (23), it is difficult to draw conclusions regarding the mechanism of its action on GH secretion. Although it is possible that the effects of methysergide we have observed might be mediated by nonserotonergic mechanisms, we favor the interpretation that serotonin plays some role in hypothalamic control of sleep-related GH secretion, possibly in an inhibitory manner. It seems clear, of course, that other neurohumors play a role in this system, possibly in a balanced manner in which several neurohumors have differing effects.

There is some evidence that GH response to insulin is related to both adrenergic and serotonergic systems. It has been reported to be increased by beta blockade and decreased by alpha blockade (24) and to be decreased by norepinephrine depletors (3). Administration of L-dopa, a norepinephrine precursor, is related to increased GH levels in normal humans (25, 26) and in Parkinsonian patients (27). Evidence for serotonergic involvement comes from the work of Gordon and Meldrum (28). They showed that in the rat insulin-induced hypoglycemia resulted in an increase in hypothalamic serotonin. Following this up, Bivens, Lebovit, and Feldman (4) showed that the insulin-induced secretion of GH in humans was inhibited by the serotonin antagonists cyproheptadine and methysergide. Cyproheptadine was also shown, by Smythe and Lazarus, to inhibit stimulated GH secretion (29).

Our finding that methysergide decreased GH secretion during the ITT seems compatible with both studies. Melatonin, a derivative of serotonin, was also shown to inhibit insulin-induced GH secretion by the latter authors. Whether this inhibition is due to its putative role as a central serotonin antagonist (30) or to other actions remains nuclear.

The decreased GH response to insulin after methysergide could be the reciprocal result of the previous increased release during sleep. This might be expected if there were exhaustion of a critical releasable pool of GH. This seems unlikely for several reasons. First, normal subjects respond with increased GH secretion to separate stimuli presented 1 h apart (31) or even 30 min apart (32). Secondly, we have shown that the amount of GH secretion during morning naps is unrelated to the amount of GH secretion the previous night (33). Finally, analysis of the data of the present study shows no correlation between the increase in sleep-related GH release after methysergide and the decrease in insulin-stimulated release in the individual subjects.

An important implication of our data is that GH response to different stimuli may be controlled by different mechanisms. There is some precedence for this concept. Blackard and Heidingsfelder (24), as described above, found that GH secretion in response to insulin was increased by beta blockade and decreased by alpha blockade; in contrast, methylamphetamine-induced secretion has been reported to be enhanced by both alpha and beta adrenergic blockers (34). Similarly, GH response to the same adrenergic stimulus may differ in normal and pathological states. Administration of L-dopa has been reported to increase GH in normal humans (25), but decreases GH in acromegalic patients (35). Our data on methysergide may imply, once again, that GH secretion in response to the same agent may vary in different conditions; in this case, it is increased during sleep and decreased in response to insulin.

It should be noted that unlike pharmacologic stimuli to GH secretion (e.g., insulin, methylamphetamine) sleep-induced secretion is a physiologic state. One might speculate that in some patients with idiopathic hypopituitarism, pharmacologic measures of GH secretion might be normal, whereas physiologic sleep-induced secretion might be deficient.

The role of biogenic amines in sleep-related GH and PRL secretion is incompletely understood. Our data with methysergide imply that serotonin has a role in this regulation. The role of serotonin in PRL secretion may be relatively more important than in the case of GH: treatment with methysergide accounted for 40% of the variance in sleep-related PRL secretion. Once again, it seems likely that control of PRL secretion is probably due to the combined effects of more than one neurohumor.

Release of PRL in response to pharmacologic stimuli is reviewed by Meites (5). There is fairly good evidence that catecholamines (which stimulate release of GH) inhibit release of PRL (5). Similarily, increased release of PRL inhibiting factor from the hypothalamus results from giving drugs that increase hypothalamic catecholamine activity (36). A recent report presents data strongly suggesting that PRL inhibiting factor is, in fact, dopamine (37). Studies on the serotonergic system are less clear. Although Coppola (38) and Talwalker, Ratner, and Meites (39) were unable to find a role for serotonin in PRL release, Kamberi, Mical, and Porter (36) demonstrated that intraventricular injection of serotonin resulted in increased plasma PRL in rats, as did intraperitoneal injection of 5-hydroxytryptophan. Perhaps consistent with the possibility that a serotonergic system is involved in pharmacologic PRL release are drug studies which show that ergot derivatives such as ergocornine, ergonovine, and lysergic acid diethylamide inhibit PRL release in several species (40). There is some evidence that this is due to both a direct effect on the anterior pituitary and an effect on hypothalamic release of PRL inhibiting factor (5). Methysergide, of course, shares actions with other ergot derivatives and thus might be expected to inhibit PRL release. This is supported by the findings of MacIndoe and Turkington (41). They observed that intravenous infusion of L-tryptophan was associated with a rise in serum PRL in humans, and that this effect was decreased by pretreatment with methysergide in the two subjects so tested. Similarly, Kato, Nakai, Imura, Chihara, and Ohgo (42) reported that oral 5-hydroxytryptophan increased daytime secretion of PRL in nor-

mal humans, and that this effect was blunted by intravenous infusion of the serotonin blocker cyproheptadine. These studies seem compatible with our data on the effect of methysergide on sleep-related PRL secretion. Although we have not studied the effect of cyproheptadine on sleep-related PRL secretion, the evidence cited above, together with the relative pharmacologic specificity of methysergide, suggest that the inhibition of nocturnal PRL secretion is probably related to serotonin blockade. However, the possibility that the methysergide effect we have observed is, in part, due to a direct action on the pituitary or is due to non-serotonergic mechanisms is not totally excluded by our data.

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