Reductions in circulating anabolic hormones induced by sustained sleep deprivation in rats

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Everson, Carol A., and William R. Crowley. Reductions in circulating anabolic hormones induced by sustained sleep deprivation in rats. Am J Physiol Endocrinol Metab 286: E1060–E1070, 2004.—The main systemic disorders resulting from prolonged sleep deprivation in laboratory animals are a negative energy balance, low circulating thyroid hormones, and host defense impairments. Low thyroid hormones previously have been found caused by altered regulation at the level of the hypothalamus with possible pituitary involvement. The present studies investigated the effects of sleep deprivation on other major anabolic hormonal systems. Plasma growth hormone (GH) concentrations and major secretory bursts were characterized. Insulin-like growth factor I (IGF-I) was evaluated as an integrative marker of peripheral GH effector activity. Prolactin (PRL) was assessed by basal concentrations and by stimulating the pituitary with exogenous thyrotropin-releasing hormone. Leptin was studied for its linkage to metabolic signs of sleep loss and its correspondence to altered neuroendocrine regulation in other disease states. Last, plasma corticosterone was measured to investigate the degree of hypothalamic-pituitary-adrenal activation. Sleep deprivation was produced by the disk-over-water method, a well-established means of selective deprivation of sleep and noninterference with normal waking behaviors. Hormone concentrations were determined in sham comparisons and at intervals during baseline and experimental periods lasting at least 15 days in partially and totally sleep-deprived rats. The results indicate that high-amplitude pulses of GH were nearly abolished and that concentrations of GH, IGF-I, PRL, and leptin all were suppressed by sleep deprivation. Corticosterone concentration was relatively unaffected. Features of these results, such as low GH and low IGF-I, indicate failed negative feedback and point to hypothalamic mechanisms as containing the foci responsible for peripheral signs.

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SLEEP IS CONSIDERED CRITICAL for the maintenance of health (2, 14, 49b, 77a) and support of life (18, 35, 54). Some of the clinical states associated with abnormal sleep include pulmonary and cardiovascular disorders, cerebrovascular disease, thrombotic disease, epilepsy, arthritis, mood disorders, chronic pain, and shortened life span (reviewed in Refs. 39 and 49a). The physical consequences of sleep deprivation have not been localized. Evidence from studies in laboratory animals suggest that multitropic factors in immune and hormonal systems contribute to the absence of localization.

The three principal consequences of prolonged sleep deprivation in the rat are a progressively deepening negative energy balance (8, 18, 23), reduced thyroid hormone concentrations (8, 19, 20), and abnormal control of microorganisms (22). Sleep-deprived rats become strikingly and progressively hyperphagic but do not gain weight. The negative energy balance is not due to malabsorption of calories (8) or diabetes (18) but may be a metabolic response to infectious processes. Abnormal control of microorganisms was discovered by the detection of live, pathogenic bacteria in normally sterile body tissues (16, 22). This deficiency in the host defense system is the earliest clinically and biologically significant sign so far discovered, occurring within five experimental days when the outward appearance of the animal is healthy and metabolic changes are not as marked as they later become. The third principal feature, hypothyroxinemia, is manifested by a progressive decline in both thyroxine (T4) and triiodothyronine (T3), with T4 eventually reaching levels below assay detection in some cases (20). After an average of 3 wk, these and other signs result in advanced morbidity (scrawny and feeble appearance, decreased food intake from peak consumption) and hypothermia (8, 18), primary bacteremia with poor or absent inflammatory responses (16), and subsequent lethality (53).

Recent findings on the alterations in thyroid hormones in sleep-deprived rats point to the brain as the essential site of sleep deprivation effects. A progressive decline in plasma T4 in sleep-deprived rats is not accompanied by an increase in plasma thyroid-stimulating hormone (TSH), whereas low plasma T4 is normally a potent stimulant for pituitary TSH secretion (71). Challenge tests of TSH release from the pituitary by exogenous administration of the hypothalamic peptide thyrotropin-releasing hormone (TRH) revealed normal stimulated release of TSH and an appropriate increase in circulating T4 and T3 (20). Examination of the paraventricular nucleus (PVN) of the hypothalamus revealed an eventual tripling of TRH transcript levels in most experimental animals, suggesting a compensatory response to low T4 presumably through increased negative feedback to the hypothalamus. Even so, TSH failed to respond to increased TRH biosynthesis (19). These data suggest that TRH stimulation of TSH is inhibited and that one locus of sleep deprivation’s effects is TRH translation or release by the hypothalamus.

Relatively little is known regarding other neuroendocrine consequences of sustained sleep deprivation and whether there is broad pituitary or hypothalamic involvement. One of the objectives of the present series of experiments was to determine whether chronic sleep deprivation also affects the secretion of growth hormone (GH) and prolactin (PRL). Alteration in their pleiotropic functions could provide future avenues of...
discovery for how sleep deprivation specifically results in health consequences. Linkage of GH and PRL secretion to non-rapid-eye-movement (NREM) sleep has been repeatedly demonstrated in humans. Plasma peak GH concentration generally coincides with sleep onset and is delayed if sleep is delayed. Smaller GH peaks generally coincide with subsequent slow-wave sleep phases (52, 56, 64, 73). PRL concentrations are elevated during sleep, even if sleep is delayed (74). Short-term sleep deprivation of 24–36 h blunts the amplitude of major GH secretory periods during that time (10, 11), whereas sleep after sleep deprivation is associated with a return of normal amplitudes (11, 59, 73). Similarly, GH pulses in rats are abolished during short-term sleep deprivation of 3 h (34). Whether abolishment would persist beyond 3 h and whether this is an acute reaction are unknown. The proximal hypothalamic mechanisms regulating GH secretion by the pituitary are growth hormone-releasing hormone (GHRH) and somatostatin (somatotropin release-inhibiting factor; SRIF), which operate in a reciprocal manner. Previous studies have shown that short-term sleep deprivation of 6–8 h can reduce the content of GHRH in the hypothalamus (26) and result in increased GHRH mRNA in the PVN and increased SRIF mRNA in the arcuate nucleus of the hypothalamus (69). Whether these changes are important to hypophysioportal stimulation of GH secretion is unknown, whereas hypotheses exist for their role in the regulation of sleep behavior (26). Furthermore, GHRH binding and receptor mRNA levels are decreased by 50% in the hypothalamus of rats sleep deprived for 8 h, whereas pituitary levels remain unchanged. These latter findings have been interpreted as indicating downregulation of receptors in response to GHRH release (27). In addition to its role in lactation, PRL is a major anabolic hormone and cytokine (5). Short-term sleep deprivation and sleep fragmentation in humans are associated with lower nocturnal PRL levels than when sleep occurs (74, 80) and is reduced by 24 and 72 h, but not by 48 h, with lower nocturnal PRL levels than when sleep occurs (74, 80). Similarly, GH pulses in rats are abolished during short-term sleep deprivation of 3 h (34). Whether abolishment would persist beyond 3 h and whether this is an acute reaction are unknown. The proximal hypothalamic mechanisms regulating GH secretion by the pituitary are growth hormone-releasing hormone (GHRH) and somatostatin (somatotropin release-inhibiting factor; SRIF), which operate in a reciprocal manner. Previous studies have shown that short-term sleep deprivation of 6–8 h can reduce the content of GHRH in the hypothalamus (26) and result in increased GHRH mRNA in the PVN and increased SRIF mRNA in the arcuate nucleus of the hypothalamus (69). Whether these changes are important to hypophysioportal stimulation of GH secretion is unknown, whereas hypotheses exist for their role in the regulation of sleep behavior (26). Furthermore, GHRH binding and receptor mRNA levels are decreased by 50% in the hypothalamus of rats sleep deprived for 8 h, whereas pituitary levels remain unchanged. These latter findings have been interpreted as indicating downregulation of receptors in response to GHRH release (27). In addition to its role in lactation, PRL is a major anabolic hormone and cytokine (5). Short-term sleep deprivation and sleep fragmentation in humans are associated with lower nocturnal PRL levels than when sleep occurs (74, 77). PRL is considered to be sleep promoting in laboratory animals (50, 80) and is reduced by 24 and 72 h, but not by 48 h, of sleep deprivation combined (i.e., confounded) with constant forced locomotion (33). Deficiency of PRL results in grossly reduced immunocompetency in laboratory animals (49), to which sleep-deprived rats share a resemblance.

It is also unknown at present whether sustained sleep deprivation affects the secretion of the adipocyte-derived hormone leptin, which acts on the hypothalamus. Leptin has endocrine actions and pleiotropic effects that include a major role in the regulation of energy balance (reviewed in Refs. 24, 25, and 31). Several lines of evidence suggest a link between this hormone and different pathophysiological changes resulting from sleep deprivation. For example, decreased leptin concentrations occur with decreased fat reserve and impairments of immunity (reviewed in Ref. 25), which are consequences of sleep deprivation. On the other hand, increases in leptin secretion are associated with increased sympathetic activation (58), elevated metabolic rate (45), and decreased body weight (12), which are also produced by prolonged sleep deprivation (reviewed in Ref. 17). Leptin also increases in response to exposure to bacterial cell components (reviewed in Ref. 30), which would be an expected event resulting from bacterial translocation and efforts to control posttranslocation survival in sleep-deprived animals (22). In humans, substantially reduced leptin concentrations have been found in narcoleptic patients, who have abnormal sleep architecture (36, 60), and mildly reduced leptin concentrations have been found in normal subjects under conditions of acute sleep reduction (62).

The objective of the present studies was to determine whether chronic sleep deprivation changes the regulation of GH, PRL, and leptin, and, if so, the nature of the alterations. The effects of sleep deprivation were determined on concentrations of insulin-like growth factor I (IGF-I), because it is the principal physiological mediator of many GH actions, and on concentrations of corticosterone, which in high levels is associated with endocrine abnormalities in other disease states (reviewed in Ref. 44). To this end, we employed a well-established procedure for producing sleep deprivation (9) and examined its effects on hormonal secretory patterns at intervals during the first two-thirds of the typical survival period for rats, at times before advanced morbidity. The present results indicate abnormally low concentrations of GH, IGF-I, PRL, and leptin, but appropriately increased PRL release when stimulated. These results provide evidence of altered hormonal regulation at the level of the hypothalamus and implicate suppression of anabolic hormones among the important health consequences of sleep deprivation.

MATERIALS AND METHODS

Animals and surgical procedures. All procedures were carried out in accordance with the National Institutes of Health guidelines on the care and use of animals and an approved animal study protocol. Normal male Sprague-Dawley rats aged 25 ± 2 (SD) wk, weighing 559 ± 130 (SD) g at the time of surgery were obtained from Harlan Laboratories (Frederick, MD, and Indianapolis, IN). All surgical procedures, including catheter insertions, were performed during a single episode of anesthesia to avoid confounding effects of anesthesia and surgery during the sleep deprivation period.

Rats were anesthetized with ketamine hydrochloride (100 mg/kg ip), xylazine hydrochloride (2.4 mg/kg im), and atropine sulfate (0.1 mg/kg im). Supplemental doses of ketamine hydrochloride (10 mg/kg ip) were provided as needed. Surgery included implantation of cortical (EEG) and muscle electrodes for electrophysiographic determination of sleep stages and wakefulness, as described previously (9). To enable repeated sampling of blood in freely moving animals, a venous catheter was inserted in the external jugular and advanced to the right atrium, as described previously (9, 20, 23). The catheter was sutured to neck muscles and threaded subcutaneously to exit the scalp at the back of the head, where it was anchored to an electrode head plug assembly. Catheter tubing was extended up along a 45-cm EEG recording cable attached to a swivel commutator and counterbalanced boom assembly, which permitted daily blood sampling without handling the animal. Catheters were flushed daily with heparinized saline containing a small amount of ampicillin (1.25% wt/vol). Beginning 4 days after surgery, 75 units of streptokinase in 150 μl of heparinized saline were infused (1 ml/min) to fill the catheter lumen after procedures to reduce the occurrence of fibrin clot blockages. Rats were permitted at least 7 days of recovery from surgery before study.

The rats to be sleep deprived or serve as a yoked comparison were housed in the apparatus described below. After the week allotted for recovery, rats were fed ad libitum a balanced purified diet that was isocaloric to normal and augmented with protein (40.7% protein, 44.7% carbohydrate, 4.3% fat, and 10.3% minerals, fiber, and vitamins; 3.8 cal/g metabolizable energy), as used in previous studies for comparison (19, 20, 23). Food and water intake and body weight were recorded daily. Special food tubes, each with a waste receptacle, afforded accurate measurement of food consumption (9). Ambient light was constant to diminish the influence of circadian rhythm on factors under study (15). Ambient temperature was kept constant at...
28°C, within the thermonutral zone for rats (63), by means of thermostatically controlled heat lamps.

Procedure for producing sleep deprivation and yoked controls. The experimental apparatus and procedures used in the present experiments are those of the Bergmann-Rechtschaffen method (9) with subsequent minor modifications. The method, which has been validated for its selectivity of sleep deprivation (8, 9, 18, 23, 53), features a benign arousal stimulus, freedom of movement, and comparison conditions of partial or full sleep opportunities, described in more detail and illustrated elsewhere (9, 23). Briefly, two animals are housed under identical experimental conditions on each side of a large, round platform (46 cm diameter) divided by a Plexiglas wall. One rat is designated for sleep deprivation, and the other rat is designated as a yoked animal to be subjected to the same experimental variables, yet allowed opportunities to sleep. Beneath and around the platform is shallow water that serves as a soft boundary. The rats always avoid the water and stay on the platform where they can eat, sleep, and explore normally. Recording of EEG variables under the rats’ freely moving conditions is accomplished by connecting the long recording cable attached to the head electrode assembly to a 360° commutator and then to a counterbalanced boom assembly.

During a 7-day period of basal measurements, the platform is rotated one time per hour for 6 s to acquaint the rats with platform movement. The method produces a consistent amount of sleep reduction under the rats’ movement. The method produces a consistent amount of sleep reduction in our studies (16, 18, 21, 23). Under baseline conditions, sleep occurs during an average of 53% of the time. The sleep comprises 48% NREM sleep (comparable to human stages 1–4) and 6% paradoxical sleep (PS; also known as rapid eye movement sleep). After the baseline period, the experimental period begins. Detection of sleep onset in the sleep-deprived rat, by programmed microprocessing of amplitude changes in cortical EEG, cortical theta, and electromyographic variables, results in 6 s of forced locomotion for both rats, which is sufficient to produce arousal. This is repeated upon each subsequent sleep onset in the sleep-deprived rat, resulting in wakefulness sustained over 90% of total time. The other 10% of time consists mostly of transitional sleep and fragmented high-amplitude NREM sleep. PS comprises <1% of total time.

The yoked rat may sleep when the platform is stationary because the sleep-deprived rat is engaged in behaviors other than trying to sleep. Yoked animals are awake 58% of the time and obtain NREM and PS sleep during 38 and 3% of total time, respectively. Each yoked animal is frequently awakened when asleep because sleep normally occupies 50% of the time, and the yoked rat is paired to a sleep-deprived rat that may fall asleep, thus triggering platform rotation. Yoked rats therefore are partially sleep deprived and usually exhibit several signs in the same direction as do sleep-deprived rats but not to the same degree (e.g., Ref. 18). The yoked animals are considered important in showing that severe morbidity and death in sleep-deprived rats are not due to the experimental conditions extraneous to sleep loss, because these were matched for both groups (54). In addition, the yoked rats are viewed as comprising part of a dose–response curve, wherein partial sleep deprivation is intermediate to no sleep loss and total sleep loss.

Eight sets of experiments, each one on a pair of sleep-deprived and yoked rats, were carried out for determination of plasma IGF-I, leptin, and corticosterone and changes in food intake and body weight. In a subset of four of these experiments, serial blood sampling protocols were followed for determination of basal and stimulated PRL secretion and for the characteristics of pulsatile GH secretion. The duration of each experiment was a 4- to 6-wk period that included at least 7 days for recovery from surgery, 7 days of baseline conditions, and 15 or more experimental days. In addition, there were operated animals that were intended to serve as back-up animals in the apparatus during the postsurgery recovery period should a catheter become nonpatent in one of the first two rats. Five of these animals were not used for that purpose and continued under study as home cage animals for determinations of plasma IGF-I, leptin, corticosteroids, and changes in food intake and body weight. The home cage control animals were subjected to the same daily procedures as the rats housed in the apparatus, except that they were housed in cylindrical cages (30 cm OD: 30 cm high) and permitted uninterrupted sleep.

General procedure for sampling blood and for measurement of blood hormone concentrations. Blood specimens were collected, starting at the same time of day, according to a rotating schedule that permitted the determination of factors under study at intervals across baseline and experimental periods. IGF-I, leptin, and corticosterone were determined one or more times during each consecutive 4-day experimental period, whereas pulsatile GH and basal and stimulated PRL release were determined weekly. Briefly, a small amount of blood was aspirated to clear the catheter of clots, and an initial 100 units of heparin in 0.1 ml of vehicle were infused. After 2 min, 0.1–0.15 ml of heparin and whole blood were withdrawn and discarded. A blood sample was drawn and placed in a microcentrifuge tube on ice. The volume drawn was replaced by an equivalent volume of heparinized saline injected through the catheter to minimize hypovolemia. Specimens were centrifuged cold (8,000 g for 10 min), and the plasma was pipetted into chilled microcentrifuge tubes for subsequent storage at ~80°C. To help prevent anemia, the red blood cells (RBCs) were resuspended with heparinized saline, injected through the plasma and flushed through with heparinized saline. This general protocol applied to plasma samples stored for determination of IGF-I, leptin, and corticosterone, and to the elaborated protocols described below.

Determination of major GH secretory periods. Basal pulsatile GH was assessed in the subset of four sleep-deprived and yoked pairs by means of blood collection from each animal every 20 min across a 6-h period (57, 65). Previous studies have determined that the GH secretory pattern is not affected by constant illumination (65). The protocol for determination of GH secretory pulses in each sleep-deprived and yoked rat was conducted one time during its 7-day baseline period and at 6- to 7-day intervals during the experimental period. The total amount of blood drawn from each rat was 0.7 ml/h with volume and RBC replacement given throughout the 6-h period. Blood was not collected during the day after sampling for GH secretory episodes.

Determination of basal PRL secretory periods and stimulated PRL release. PRL secretion exhibits low-amplitude pulsatility (40, 42), and therefore measurements integrated over time better reflect basal PRL secretion than do singular determinations. Blood was sampled six times at 10-min intervals over a 50-min period, based on data by Lopez et al. (42) and Lafuente et al. (40) that a PRL pulse lasts 16–25 min and would be expected to occur every 40 min. The protocol was conducted in the same subset of experiments in which major GH secretory periods were determined, but on different days during the baseline period and one time per week during the experimental period. The total volume of blood drawn under this protocol was 1.8 ml, with volume and RBC replacement. PRL challenge tests by intravenous TRH bolus injection were completed one time during the baseline period and one time per week during the experimental period in the experimental subgroup. Blood was sampled immediately before and at 10, 20, and 40 min after intravenous administration of TRH (10 µg/kg), based on previous studies (40, 48). The total volume of blood drawn was 2.1 ml, with volume and RBC replacement.

Hormone assays in plasma. GH and PRL antisera and reference hormone were provided by Dr. A. F. Parlow (National Hormone and Pituatory Program, National Institute of Diabetes and Digestive and Kidney Diseases). The low detection limit of the GH assay was 2.4 pg/ml, and the high detection limit was 466–583 pg/ml. The low and high sensitivities for PRL were 0.6 and 16 ng/ml, respectively. The intra-assay coefficients of variation (CVs) for GH and PRL were <10%. Determinations of basal plasma IGF-I, leptin, and corticosterone were performed using commercial IGF-I and enzyme immunoassay kits. The sensitivity range for IGF-I was 127–4714 ng/ml (Diagnostic Systems Laboratories, Webster, TX). The intra- and interassay CVs were both <4%. The low and high detection limits for
leptin were 0.5 and 50 ng/ml (Linco Research, St. Charles, MO), and the intra- and interassay CVs were <4 and 6%, respectively. The low and high detection limits for corticosterone were 20 and 2,000 ng/ml (Diagnostic Systems Laboratories), and the intra- and interassay CVs were <5 and 6%, respectively. Determinations were made in duplicate. Values that fell below or above the range of assay detection were set equal to the lower or upper limit of sensitivity, respectively.

Data analysis. Apart from the baseline period, the data for each rat during the experimental period were truncated at 16 days if the experimental conditions had been continued longer. This permitted values for IGF-I, leptin, corticosterone, food intake, and changes in body weight to be partitioned into four consecutive 4-day intervals (i.e., bins). The data were averaged across each 4-day interval for each rat and then averaged within intervals of each experimental group. These data were compared by means of an unbalanced, two-way repeated-measures ANOVA. One yoked subject was eliminated from study because the electrode assembly became detached from the skull, resulting in an imbalance in the number of subjects per group. Occasionally, there were missing values within a subject across time, typically the result of indwelling catheter nonpatency on the day of blood collection for a particular factor or insufficient specimen volume for analysis. Missing values for a given 4-day bin were replaced by interpolation or extrapolation if they were isolated instances. Interpolations and extrapolations were limited to two (1 yoked and 1 home cage) data points for each leptin and IGF-I, and one data point for corticosterone. Subject values were excluded in toto from a given analysis if two or more 4-day values were missing. Exclusions included one yoked rat in the determination of leptin and IGF-I; one sleep-deprived rat in the determination of food intake; and one animal of each sleep-deprived, yoked, and home cage groups in the analysis of corticosterone. For these analyses, statistical significance was considered at \( P < 0.05 \). Post hoc comparisons were performed by means of Student’s \( t \)-tests. Such comparisons included analysis of changes in a group between particular time points, such as between baseline and a specific 4-day interval, or between groups at a particular time point. Paired \( t \)-tests were used when possible, but comparison of unpaired means was performed on those occasions when the data could not be paired because of exclusion of a subject. Nonsignificant statistical differences are designated as not significant. Variance is given as the mean ± SE unless specifically noted as mean ± SD.

Characteristics of GH pulsatile secretion were analyzed with the ULTRA statistical algorithm to separate small peaks from background variation, according to guidelines described by Van Cauter (72). In ULTRA, the CV of the assay is used to determine whether increments or decrements of chronological data points are within the range of expected variation. The number of CVs used determines the threshold for significant change; typically, two to three CVs are applied (72). In this way, increments or decrements in hormonal concentration may be considered part of a pulse if the values are above a criterion threshold for assay variability, thus limiting false-positive pulses. In our analysis, we used three times the CV of the radioactive counts per minute, which ranged from 4.4 to 5.8%, as the constant in the algorithm signifying a significant change from previous and subsequent values across all values within that particular assay. Characterization of GH and PRL secretion were based on the following parameters given by Lafuente et al. (40): 1) minimum and maximum values observed; 2) integrative value (average value of the samples collected during the period of observation); 3) absolute amplitude (difference in concentration between peak and minimum values); and 4) relative amplitude (ratio of absolute amplitude to the minimum value). Pulses of GH detected by ULTRA additionally were assessed for frequency in those instances in which two peaks were captured during the 6-h sampling period. Parameters characterizing PRL and GH concentrations were compared a priori by Student’s \( t \)-test, and \( P < 0.05 \) was considered statistically significant.

RESULTS

Physiological variables. Sleep-deprived rats became hyperphagia (Fig. 1A), eventually consuming nearly two times the basal amounts of food [group, sleep deprived vs. yoked: \( F(1,12) = 6.4, P < 0.05 \); group × time, sleep deprived vs. home cage: \( F(3,30) = 5.5, P < 0.01 \)]. Yoked animals also exhibited increased food consumption over time, by 40–50% [group × time interaction, yoked vs. home cage control: \( F(3,30) = 8.8, P < 0.01 \)]. Despite increased food intake, sleep-deprived rats showed evidence of weight loss relative to the yoked rats [group × time, \( F(3,39) = 6.2, P < 0.01 \)] and, especially, to the home cage controls [\( F(3,33) = 44.5, P < 0.01 \)], the latter having gained weight without an increase in food intake (Fig. 1B). Body weight losses were only 2–5% in sleep-deprived rats during the first 8 days of observation, but eventually body weight had dropped by 10% during days 13–16.

Basal GH and IGF-I concentrations. Basal GH secretion was determined by analysis of blood specimens collected every 20 min during 6-h periods in four sleep-deprived and four yoked animals during baseline, four sleep-deprived and three yoked animals during days 4–6, and four sleep-deprived and two yoked animals during days 11–14 of the experimental period. The different group numbers reflect the varying success in maintaining catheter patency for 30 days or more after surgery. Only 5 of the 399 data points comprising the secretory GH profiles analyzed were missing values replaced by interpolation.

Fig. 1. Mean percentage change (means ± SE) from individual baseline values in food intake (A) and body weight (B) for sleep-deprived (food intake, \( n = 7 \); body weight, \( n = 8 \)) yoked (\( n = 7 \)), and operated home cage control (\( n = 5 \)) rats during 4-day intervals of the experimental period.
Analysis of GH secretory periods revealed near abolishment of major GH peaks in sleep-deprived rats. Representative profiles of the GH secretory periods during the baseline and experimental period are shown for a yoked rat and a sleep-deprived rat in Fig. 2. During the baseline period, high-amplitude GH peaks, i.e., >300 ng/ml or >50% of the maximal values measured, were detected in each of the eight animals, and occasional intermediate peaks (i.e., 150–300 ng/ml) additionally were detected in a few animals. Of the pulses detected during baseline, 60% were considered large pulses (>300 ng/ml), 8% were considered intermediate, and 32% were considered small (<150 ng/ml). During the experimental period in sleep-deprived rats, no high-amplitude pulses and only one intermediate-amplitude pulse in a single individual were observed. The remaining 94% of pulses were considered small. In contrast, large pulses continued to be observed in all yoked subjects at each time point and comprised 52% of pulses. Overall, the frequency of pulses determined using ULTRA, regardless of amplitude, was not appreciably different among conditions. Per 6-h period of study, there were 3.6 ± 1.6 (SD) pulses under baseline conditions compared with 3.0 ± 1.0 (SD) in yoked rats and 3.4 ± 1.1 (SD) in sleep-deprived rats.

Four of eight animals displayed more than one high-amplitude GH pulse during the 6-h time frame of blood sampling under baseline conditions before sleep deprivation and thus allowed calculation of the frequency of major GH secretory episodes for these animals. During baseline, the peak-to-peak interpulse interval of high-amplitude GH episodes for the five animals was 3.1 ± 0.2 (SD) h (n = 4), and during the experimental period in yoked animals it was 3.2 ± 0.7 (SD) h (n = 2), which is considered equivalent to previous investigations in other animal models (57, 65). A comparison could not be made in sleep-deprived animals because high-amplitude pulses uniformly were abolished during the experimental period.

The average GH value for all determinations during the 6-h sampling period is an integrative measurement of GH secretion (Fig. 3). Sleep-deprived animals displayed a significant decrease in GH concentration during the experimental period compared with the baseline period [week 1: paired t(3) = 12.3, P < 0.001; week 2: paired t(3) = 10.0, P < 0.005] and compared with the yoked animals [week 1: unpaired t(3) = 9.2, P < 0.001; week 2: unpaired t(4) = 5.6, P < 0.005]. The decrease from baseline GH concentrations by week 1 was 71% in sleep-deprived animals and 38% in the yoked animals.
which, for yoked animals, was not statistically different from baseline.

The significant decrease in plasma IGF-I concentrations in sleep-deprived rats, as shown in Fig. 4, is consistent with the absence of high-amplitude pulses and overall suppression of GH concentration. The concentrations of plasma IGF-I were significantly lower in sleep-deprived rats compared with yoked rats \( F(1,11) = 15.8, P < 0.01 \) and home cage rats \( F(1,11) = 32.4, P < 0.01 \). Yoked animals as a group did not show suppression of IGF-I over time, except insofar as the IGF-I concentrations were not elevated as in the home cage animals [percent change from baseline, yoked vs. home cage: \( F(1,11) = 12.8, P < 0.01 \)], for which sleep had been permitted ad libitum and for which there was a positive correlation between IGF-I concentration and body weight \( (r = 0.74, P < 0.001) \).

**Basal and stimulated PRL concentrations.** Basal PRL secretion was determined in the same animal subset during baseline, week 1 (i.e., days 4–6), and week 2 (days 11–14) of the experimental period by sampling blood six times during a 50-min period. Four animals comprised the results of each group except for the last time period, when three animals comprised the yoked group, due to nonpatency of one catheter. The resulting baseline values (±SD) for the 8 animals were comparable to those of previous reports for male rats (40, 42): minimum, 0.77 ± 0.83; maximum, 2.14 ± 1.73; average, 1.26 ± 1.03 in ng/ml. During weeks 1 and 2 of the experimental period, sleep-deprived rats showed a significant decrease from baseline in the average PRL concentration compared with yoked rats (weeks 1 and 2, 1-tailed, paired \( t \)-tests = −2.4 and −3.8, respectively, \( P < 0.05 \); Fig. 5). By week 2, sleep-deprived animals also displayed a significant decrease in the maximum PRL value, expressed as a percentage change from baseline, by −57.7 ± 18.8% (mean ± SD) compared with +152.4 ± 77.4% (mean ± SD) in yoked animals [paired \( t(3) = 2.6, 1 \)-tailed, \( P < 0.05 \)]. Also, by week 2, sleep-deprived animals showed a significant percentage decrease from baseline in the absolute PRL amplitude by −49.9 ± 38.5% (mean ± SD) compared with +16.4 ± 34.6% (mean ± SD) in yoked animals [paired \( t(2) = 2.9, 1 \)-tailed, \( P < 0.05 \)]. The decreases in these PRL parameters suggest diminished secretion with lower amplitude during sleep deprivation.

Stimulated release of PRL by TRH administration was assessed by measurement of PRL in plasma collected before and at 10, 20, and 40 min after TRH administration. Four animals comprised the results of each group except for the last time period, when two animals comprised the yoked group because of nonpatency of two catheters. The data for the yoked subjects during week 2 are insufficient for statistical tests but are shown for comparison. The absolute and relative amplitudes from time 0 to peak concentration did not differ in sleep-deprived rats compared with baseline or yoked animals. The relative amplitude is shown in Fig. 6A. The average value of PRL during the 40 min after TRH stimulation was significantly changed from baseline by −56 ± 25% (mean ± SD) during week 1 [paired \( t(3) = 3.0, P < 0.05 \)] and −51 ± 16% (mean ± SD) during week 2 [paired \( t(3) = 3.9, P < 0.025 \); Fig. 6B]. The average value of PRL in yoked rats after TRH stimulation showed a strong tendency toward decreased amounts compared with baseline values. Two yoked animals showed higher post-TRH average PRL values during week 1 compared with their individual baseline values, which did not occur in any of the sleep-deprived animals studied.

**Plasma leptin and corticosterone concentrations.** Leptin concentrations were suppressed early and continuously in sleep-deprived and yoked animals compared with operated home cage controls [percent change, group × time interactions: sleep-deprived vs. home cage, \( F(3,33) = 3.6, P < 0.025 \); yoked vs. home cage, \( F(3,27) = 3.0, P < 0.05 \); Fig. 7], and

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*Fig. 5. Mean percentage change (±SE) from individual baseline values in the prolactin average value across 50-min observation periods for sleep-deprived \( (n = 4) \) and yoked \( (n = 4) \) during baseline and during week 1, \( n = 3 \) during week 2 rats during weeks 1 and 2 of the experimental period. *Statistical significance between groups.*

*Fig. 4. Change in plasma IGF-I, expressed as percent change in individual values from a grouped baseline (means ± SE), in sleep-deprived \( (n = 8) \), yoked \( (n = 6) \), and operated home cage \( (n = 5) \) rats during baseline and consecutive 4-day intervals during the experimental period. Group differences (between sleep-deprived and yoked, sleep-deprived and home cage, and yoked and home cage) were all statistically significant by 2-way repeated-measures ANOVA.*
sleep-deprived and yoked animals did not differ from each other. The average leptin value across the 4-day intervals for sleep-deprived and yoked animals was 1.2 ± 0.07 (SE) ng/ml. Operated comparison animals showed evidence of increased leptin concentrations that correspond to postsurgery weight gain and increasing adiposity (r = 0.61, P < 0.001). No significant differences in plasma corticosterone concentrations were found by group or by the interaction of group by time. The absolute concentrations of corticosterone during baseline and consecutive 4-day intervals of the experimental period are shown in Fig. 8.

**DISCUSSION**

The major findings of the present studies are that prolonged sleep deprivation in rats produced profound decreases in circulating GH, IGF-I, PRL, and leptin. The suppressions occurred within the early phase of chronic sleep deprivation, a time when the overt appearance of the animals was relatively healthy. Reduced secretion of these hormones was sustained throughout the experimental period and occurred in conjunction with weight loss and dramatically increased food consumption, the latter of which has been reported previously (18, 23). Considered together with the prior demonstration from this laboratory that sleep deprivation produces hypothyroxinemia of central origin (20), the present results indicate that sleep
deprivation results in, and sustains, a wide range of neuroendocrine disruptions. The nature of these disruptions points to altered central regulation at the level of the hypothalamus.

GH secretion was assessed by measurement of major GH secretory periods in a subgroup of sleep-deprived and yoked comparisons studied under intensive blood sampling protocols and under freely moving conditions. Sleep-deprived animals showed diminished circulating GH and a prevailing absence of major secretory GH bursts that had been observed during their baseline periods. IGF-I, considered the main effector molecule of GH secretion in many tissues, was also consistently suppressed in sleep-deprived rats by 31 to 42% throughout each 4-day interval of observation, concomitant with robust food intake. Measurement of circulating IGF-I levels in well-nourished subjects is believed to reflect 24-h secretion of GH from the pituitary gland (reviewed in Ref. 43). Yoked animals under partial sleep deprivation conditions showed a strong tendency for an overall decrease from baseline GH concentration but showed evidence of relatively well-preserved major GH secretory periods of high-amplitude pulses that were relatively unchanged in duration or frequency. Taken together, these results provide evidence of diminished GH availability and effector activity in the periphery of totally sleep-deprived rats.

PRL and GH share 40% homology, and GH can substitute for PRL deficiency to some extent (reviewed in Ref. 7). Sleep-deprived animals also showed a large reduction of 52% in basal circulating PRL during the first few days of sleep deprivation and maintenance of suppressed concentrations during the experimental period. By comparison, yoked animals under partial sleep deprivation were variously affected; individual instances sometimes were of increased PRL concentration. Challenge tests of pituitary secretion of PRL by TRH administration yielded an initial PRL response that was proportional to normal in both sleep-deprived and yoked rats. However, the total amount of poststimulation PRL secreted over 40 min was significantly less in sleep-deprived rats than during the baseline period, reflecting diminished basal concentrations. Yoked animals under conditions of partial sleep deprivation tended to show the same direction of change in post-TRH PRL concentrations as did the totally sleep-deprived rats.

Both experimental groups of sleep-deprived rats and partially sleep-deprived yoked rats showed significant reductions in circulating leptin concentrations. This finding has effectively ruled out hyperleptinemia as a plausible mediator of sleep deprivation signs, such as activation of the sympathetic nervous system, increased energy expenditure, loss of weight, and suppression of IGF-I. Rather, plasma levels of leptin appeared to reflect energy stores that became inadequate, eventually manifested by the animal’s increased food consumption and failure to gain weight. Leptin is now considered a critical peripheral hormonal signal of the status of body energy stores that show increases in SRIF mRNA in the arcuate nucleus during short-term sleep deprivation.

Further studies will be necessary to characterize the mechanisms underlying suppression of GH, PRL, IGF-I, and leptin elucidated by the present studies, and inappropriately low TSH, as shown previously (19, 20). However, several lines of evidence suggest the hypothalamus is the primary locus affected by sleep deprivation, with possible secondary pituitary involvement. For example, under normal conditions, a decrease in IGF-I is associated with an increase in GH release via a long negative feedback loop to the brain (reviewed in Refs. 47 and 55). The specific feedback mechanisms of IGF-I to GH mechanisms in the hypothalamus are not well elucidated but are believed to involve increased inhibitory tone by SRIF or reduced GHRH (reviewed in Ref. 68), which normally operate in a reciprocal manner to regulate GH secretion. For reduced IGF-I negative feedback to the hypothalamus to fail to increase GH would suggest increased SRIF tone and/or decreased GHRH. Evidence consistent with this framework is a finding by Gardi et al. (26) that short-term sleep deprivation in rats reduces amounts of GHRH and tends to increase amounts of SRIF in the hypothalamus, and findings by Toppila et al. (69) that show increases in SRIF mRNA in the arcuate nucleus during short-term sleep deprivation.

PRL is different from other pituitary hormones in that it has no target organs to supply feedback hormones. The primary influence on PRL secretion is inhibition by the hypothalamus. In the present study, the absolute concentration of PRL was diminished in the sleep-deprived rats, yet exogenous stimulation by the hypothalamic peptide TRH led to a PRL response that was of comparable relative amplitude to baseline and to comparison conditions, suggesting that PRL synthesis and storage is not the primary reason for low circulating PRL. This finding is consistent with previous studies that showed an appropriate pituitary response of TSH release after TRH administration (20) and points to insufficient stimulation of the pituitary during sleep deprivation in a manner that would have resulted in maintenance of normal peripheral PRL concentrations.
It is possible that the decrease in circulating leptin, consequent to sleep deprivation-induced negative energy balance, contributes to the reductions in GH and PRL secretion and the inappropriately low TSH concentrations associated with hypothryoxinemia. Leptin exerts negative feedback directly on the hypothalamus (reviewed in Refs. 25 and 31). Low circulating leptin under conditions of a negative energy balance or induced by experimental means is a potent signal for increased mRNA expression, secretion of neuropeptide Y, and agouti-related peptide in the hypothalamus, and reduced signaling of α-melanocyte-stimulating hormone and its precursor proopiomelanocortin (reviewed in Ref. 25). These changes are relevant to determining mediation of increased food intake and the causal direction between altered regulation of neuroendocrine peptides and development of a negative energy balance. Diminished leptin is associated with abolition of GH secretory peaks, and administration of leptin is associated with restoration of those peaks (66) by affecting GHRH and SRIF (78). Similarly, decreased leptin resulting from food deprivation is associated with decreased circulating thyroid hormones and PRL, and administration of leptin under these conditions reverses both of these hormonal changes (1, 28, 41, 79). However, the present results suggest that leptin may not be the necessary and sufficient factor for reduction of these hormones, since partially sleep-deprived rats showed equivalent reductions in leptin secretion but less interference with pituitary hormone secretion. A growing body of evidence indicates central integration of leptin signaling and central control over leptin concentrations.

The hormonal changes in sleep-deprived rats may be viewed either as dysfunctional or as adaptive responses. On the one hand, there is abundant evidence that diseases with a central locus, such as hypothalamic dysfunction, can produce peripheral effects and changes in neuroendocrine regulation (reviewed in Ref. 47). On the other hand, low leptin and suppression of anabolic hormones may be viewed as adaptive responses to a negative energy balance, as evidenced by the progressive food intake and body weight changes in sleep-deprived rats. Suggestions that the central effects of sleep deprivation should be considered dysfunctional rather than adaptive come from experiments that contrast sleep-deprived and starved rats. Quite opposite to starved rats, declines of T4 and T3 in sleep-deprived rats are consistent with metabolic activation and not with downregulation. The ratio of T3 to T4 is dramatically increased (8), indicating a strong preference for the most metabolically active form of thyroid hormone without an increase in reverse T3 in a metabolically inactive pathway. Also, in contrast to starvation, sleep deprivation is associated with a 100-fold increase in the T4-to-T3 conversion enzyme 5’-deiodinase type II in brown adipose tissue (3), which is consistent with experimental hypothyroidism (13, 61), further suggesting that central changes in regulation of anabolic hormones produce a state in which peripheral mechanisms show activities consistent with compensation.

The clinical implications of suppression of these hormones are based on their many pleiotropic actions. Deficiency in PRL, induced by hypothryoxinemia, is associated with normocytic anemia, grossly reduced immunocompetence, decreased body weight, and several other abnormalities (49) shared by sleep-deprived animals. GH has widespread effects on the growth and metabolism of most tissues, including by increasing DNA, RNA, protein synthesis, and mitosis (reviewed in Ref. 43). Suppression of leptin would be expected to extend beyond the effects on energy balance to include numerous effects. For example, leptin-deficient (ob/ob) mice show many endocrine and metabolic abnormalities, including decreased body temperature and immune defects (reviewed in Ref. 25). Decreases in IGF-I would be expected to diminish some or many of its effector functions that include autocrine and paracrine growth factor mediation of mitosis, differentiation, chemotaxis, and apoptosis in many tissues (reviewed in Ref. 43). Administration of IGF-I during burn injury improves body weight and gut mucosal weight, stimulates mucosal DNA and protein content, and reduces the incidence of bacterial translocation (32). The endocrine and immune systems are intricately linked, and the hormonal changes may be contributing to decreased resistance to infectious disease in this animal model. Deficiencies in GH, IGF-I, PRL, thyroid hormones, and leptin induced by sleep deprivation affect health and provide one explanation for the absence of localization of specific sleep deprivation effects. Low basal PRL and lack of reciprocal relationships between IGF-I and GH, and between leptin and corticosterone, point to the hypothalamus as a locus for sleep deprivation effects, where key factors interact before presentation of integrated signals to the pituitary.

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