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Absence of Detectable Melatonin and Preservation of Cortisol and Thyrotropin Rhythms in Tetraplegia*

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ABSTRACT

The human circadian timing system regulates the temporal organization of several endocrine functions, including the production of melatonin (via a neural pathway that includes the spinal cord), TSH, and cortisol. In traumatic spinal cord injury, afferent and efferent circuits that influence the basal production of these hormones may be disrupted. We studied five subjects with chronic spinal cord injury (three tetraplegic and two paraplegic, all neurologically complete injuries) under stringent conditions in which the underlying circadian rhythmicity of these hormones could be examined. Melatonin production was absent in the three tetraplegic subjects with injury to their lower cervical spinal cord and was of normal amplitude and timing in the two paraplegic subjects with injury to their upper thoracic spinal cord. The amplitude and the timing of TSH and cortisol rhythms were robust in the paraplegics and in the tetraplegics. Our results indicate that neurologically complete cervical spinal injury results in the complete loss of pineal melatonin production and that neither the loss of melatonin nor the loss of spinal afferent information disrupts the rhythmicity of cortisol or TSH secretion. (*J Clin Endocrinol Metab* **85:** 2189–2196, 2000)

THE HUMAN circadian pacemaker, located in the paired hypothalamic suprachiasmatic nuclei (SCN), regulates the timing and amplitude of several endocrinological functions, including cortisol, melatonin, and TSH production, via a neural or neurohumoral circuit (Fig. 1). Cortisol, controlled by neurohumoral signaling in the hypothalamus-pituitary-adrenal axis, is a pulsatile hormone secreted with daily rhythmicity, such that its nadir occurs a few hours before bedtime, and it peaks just after waketime (1–3). Similarly, TSH is controlled by neurohumoral signaling in the hypothalamus-pituitary-thyroid axis, such that it is secreted with a daily sinusoidal pattern, having an onset several hours before bedtime (1, 4, 5). Peak concentrations of TSH can be observed during conditions of sleep deprivation, but are suppressed by sleep (5). Melatonin is typically produced in a single nightly episode, with onset occurring just before bedtime and offset occurring soon after waketime (6–8). Unlike TSH and cortisol, melatonin is thought to be influenced by the SCN primarily through an efferent neural pathway passing through the brain and spinal cord, with innervation of the pineal gland by the superior cervical ganglia (9, 10). In addition, it has been hypothesized that parasympathetic nerves and nerves that arise from the pretectum, which circumvent the spinal cord, innervate the pineal and may be able to significantly influence its production of melatonin (9, 11).

Neurologically complete spinal cord injury (SCI) would interrupt sympathetic hypothalamic, but not parasympathetic or pretectal, signaling to the pineal gland if the level of the injury were above the nerve roots that innervate the superior cervical ganglia. It has been hypothesized that such disruption abolishes rhythmic melatonin production (12, 13). However, it is unclear whether nonrhythmic pineal production of melatonin continues in the absence of innervation from the superior cervical ganglion (12, 13). SCI also disrupts afferent signaling to the hypothalamus from somatic nerves below the level of the lesion. Such disruption may indirectly affect the secretion patterns of TSH and cortisol. Several studies on SCI have presented conflicting evidence on such changes in both the amplitude and temporal organization of cortisol and TSH (12–30). Furthermore, none of these studies examined the temporal organization of these endocrine functions under the carefully controlled environmental and behavioral conditions necessary to assess endogenous circadian rhythmicity. It was, therefore, our intent to examine the endogenous circadian patterns of melatonin, cortisol, and TSH in SCI subjects under conditions that would enable the assessment of basal circadian hormone timing and amplitude.

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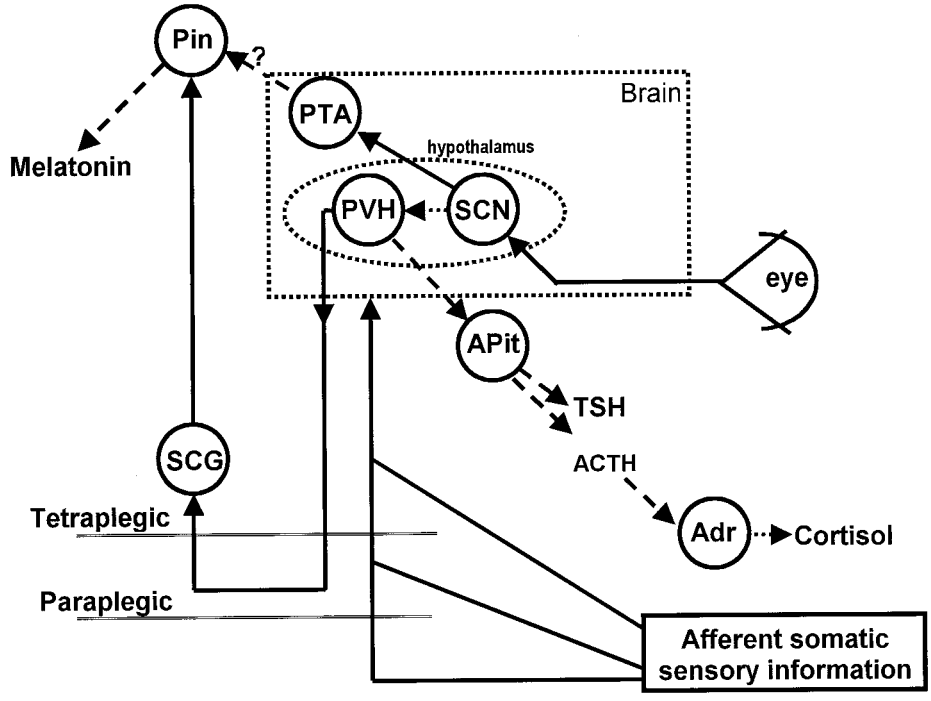

FIG. 1. Diagram of the putative pathways involved in the generation of circadian rhythmicity in TSH, cortisol, and melatonin. The pathways that would be interrupted by neurologically complete injury to the lower cervical (tetraplegic) and upper thoracic (paraplegic) spinal cord are also shown. In tetraplegia, peripheral sympathetic innervation of the pineal gland would be abolished, whereas in paraplegia, peripheral sympathetic innervation of the pineal gland would remain intact. In both paraplegia and tetraplegia, hypothalamic control of TSH and cortisol would remain intact, but most afferent somatic sensory information would not reach the brain. Adr, Adrenal cortex; APit, anterior pituitary; Pin, pineal; PTA, pretectal area; PVH, paraventricular nucleus of the hypothalamus; SCG, superior cervical ganglion.

TABLE 1. SCI patients' characteristics

Subject code	Age	Level of SCI	Years since SCI	Medications
1765	32	T4A	4.7	None
1826	31	T5A	6.2	None
1836	42	C6A	18.5	Valium 5 mg TID, Baclofen 10 mg TID
18F3	29	C6/C7A	7.7	Valium 10 mg TID, Baclofen 25 mg QID, Methenamine Hippurate 0.5 g QID
18H6	27	C4A	9.7	None

Subjects and Methods

Subjects

We studied five male, chronic SCI subjects (see Table 1 for characteristics) in a 4-day in-patient study. The level of the spinal cord injury was determined by neurological examination. Three of these subjects had neurologically complete (Frankel A) cervical injuries, and two had similarly complete thoracic injuries. Besides the neurological damage to their spinal cords, each subject was physically and mentally healthy, as determined by history and physical examination, psychological questionnaires (Beck Depression Inventory, Minnesota Multiphasic Personality Inventory II) and interview with a psychologist, electrocardiogram, blood and urine chemistries, and chest radiographs. In no case was brain damage or extended loss of consciousness associated with the SCI. All subjects gave their written informed consent to participate in the protocol, which was approved by the Brigham and Women's Hospital human research committee. Except as noted in Table 1, all subjects were drug free at the time of study, including prescription, nonprescription, and over the counter medications, as well as caffeine, nicotine, and alcohol, as confirmed by urine toxicology on the first day of the in-patient study. During the week before the study, subjects maintained a regular sleep/wake schedule, as confirmed by wrist actigraphy and self-report. Subjects entered the laboratory on the morning of day 1.

In-patient study design

For the duration of the 4-day in-patient study, subjects remained individually in rooms free from time cues (*i.e.* sound attenuation, no windows, no radio, no television, no information sources as to the time of day) and had regular contact with the technical and nursing staff. Day 1 was spent in ordinary indoor room light (~150 lux) relaxing, watching videos, and orienting to the laboratory and testing procedures. Subjects were scheduled for bed in total darkness (<0.03 lux) for 8 h, with the timing of their laboratory sleep episode established by averaging the bed times and wake times during the week before entry and scheduling the average midpoint of their at-home nightly sleep episode to be the midpoint of their scheduled sleep in the laboratory. Upon awakening on day 2, subjects began 46 h of enforced wakefulness in a constant semirecumbent posture referred to as a constant routine (31–33). The purpose of this procedure was to eliminate or hold constant variables that might otherwise mask the output of the circadian timing system. In short, subjects remained in bed throughout the constant routine, with the lower half of the bed horizontal and the head of the bed elevated to approximately 35°. Lights were kept dim (<10 lux), and room temperature remained stable, as did the number of blankets that covered the subject. Meals and fluids were given in equal, hourly aliquots, such that each subject received the same amount of fluid, nutrition, and calories during any 24-h period of the constant routine as they would have on a baseline day. A staff member was continuously present in the room with the subject during the constant routine to ensure compliance with the protocol. To reduce the risk of pressure sores, every 2 h subjects were allowed to change the side of their body on which they were lying. Furthermore, if necessary, bowel care was performed in bed during the first 5 h of the constant routine, and any medications were given at regularly scheduled intervals, as indicated in Table 1.

During h 42–44 of the constant routine, subjects were exposed to brighter (>600 lux) light, after which time they returned to the dim light condition for the final 2 h. Light exposure of this intensity has been previously shown to evoke an acute decrease in otherwise elevated nocturnal plasma melatonin concentrations in able-bodied subjects (34, 35). It has been shown that photic suppression of plasma melatonin is likely to indicate an intact eye to SCN to pineal pathway in humans (36). After the 46-h constant routine, subjects were allotted an 8-h sleep episode in darkness, after which time they were medically examined and cleared for discharge.

Physiological monitoring

Blood was collected from an indwelling, iv forearm catheter one to three times each hour, beginning on the afternoon of day 1 or upon awakening on day 2. The plasma was collected, frozen, and later assayed

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for melatonin (RIA; sensitivity of 5 pg/mL; intra- and interassay coefficients of variation, 8% and 13%, respectively; DiagnosTech, Osceola WI) (37, 38), cortisol (chemiluminescent assay; sensitivity, 0.26 μg/dL; intra- and interassay coefficients of variation, 7% and 10%, respectively), and TSH (chemiluminescent assay; sensitivity, 0.006 μIU/mL; intra- and interassay coefficients of variation, 17% and 5%, respectively). Body and skin temperature, urine volume and content, cardiovascular function, electroencephalography, and neurobehavioral performance test data were also collected during the protocol, although they are not discussed herein.

Additional comparison groups

Two historical groups of able-bodied controls, both previously studied in our laboratory, were used for endocrinological comparisons. One group, previously reported by Allan and Czeisler (5), provided normative data against which to compare the TSH rhythm in the SCI subjects (n = 14). Differences between the current experimental protocol and that of this comparison group were that the latter had 2 baseline days before the constant routine instead of 1, and the constant routine had an ambient illumination of about 150 lux. The second comparison group (n = 24), reported by Zeitzer (35), provides normative data against which to compare the rhythms of melatonin and cortisol. Differences between the current experimental protocol and that of this comparison group are that the latter had 3 baseline days before the constant routine instead of 1 and also had 2 weeks of a regular sleep/wake history before study entry instead of 1 week. Identical assays were performed on the samples collected from control and experimental groups.

Hormone analyses

For TSH, melatonin, and cortisol, up to three parameters were calculated for each subject: 24-h average, circadian amplitude, and circadian timing (phase). For each of the three hormones, the 24-h average was defined as the average of the values between h 5 and 29 of the constant routine. The first 5 h were excluded so as to reduce any influence of sleep or the small postural change (from supine to semirecumbent) that occurred at the beginning of the constant routine. The circadian amplitude and phase of cortisol were estimated by fitting a sine wave, with a period of 24 h, to data from h 5–42 of the constant routine. The sine wave that best fit the data was calculated using a nonlinear least squares fitting analysis based upon the Levenberg-Marquardt method (CurveExpert, version 1.34; D Hyams, Starkville, MS). The peak of the fitted sine wave was used as the marker of the phase of the rhythm, and half the distance between the peak and the trough of the fitted sine wave was used as the measure of rhythm amplitude. The circadian phase and amplitude of the TSH rhythm were estimated by fitting a sine wave, with a period of 24 h, to data from h 5–29 of the constant routine so as to reduce the residual effects of the prior sleep episode and the ongoing sleep deprivation on TSH secretion (5). The onset of TSH secretion was used as the marker of the phase of the rhythm and was determined by calculating the time at which the fitted curve rose to 25% of twice the amplitude (nadir + 25% of the peak to trough value) (39). Half the distance between the peak and the trough of the sine wave was used as a measurement of circadian amplitude. The circadian phase of melatonin was determined by calculating the midpoint between the time at which plasma melatonin concentrations first rose above the 24-h average and the time at which the plasma melatonin concentration first dropped below the 24-h average (midpoint of the upward and downward mean crossings) (35, 40, 41).

The suppression of plasma melatonin in response to the 2-h bright light exposure during h 42–44 of the constant routine was calculated as:

$$\%\text{Suppression} = \left(\frac{AUC_{t=18-20} - AUC_{t=42-44}}{AUC_{t=18-20}}\right) \times 100$$

in which AUC is the area under the plasma melatonin profile for the cumulative hours of the constant routine indicated by the subscript t, as calculated by the trapezoidal method. The suppression of plasma cortisol in response to the same 2-h bright light exposure was calculated using the same formula.

It has been previously demonstrated that the timings of physiological variables influenced by the SCN have typical phase relationships with the sleep/wake and dark/light schedule to which an individual is exposed (1–3, 5, 8, 42, 43). As such, to determine whether relationships were normal in individuals with SCI, we calculated the phase angle (ψ) between typical bedtime and TSH onset as well as that between typical waketime and both melatonin midpoint and cortisol maximum. The normalcy of these phase angles as well as the hormone amplitude data were evaluated by averaging across the subjects in the comparison groups, calculating a SD, and determining the 95% confidence intervals, then comparing the individual SCI subjects to these intervals.

Results

Melatonin

Plasma melatonin concentrations in the tetraplegic subjects were near or below the lower limit of assay sensitivity, thereby exhibiting no observable rhythmicity and precluding calculation of a 24-h average, melatonin phase, and melatonin suppression in these three subjects (Fig. 2). In contrast, both paraplegic subjects (T4A and T5A) exhibited normal melatonin rhythmicity. The 24-h average of plasma melatonin concentration and phase angle between the midpoint of the melatonin peak and typical bedtime in both paraplegic subjects were within the 95% confidence limits of the able-bodied comparison subjects (*i.e.* normal) (Table 2). Also, in these two paraplegic subjects the plasma melatonin concentration was suppressed by the 2 h of nocturnal light exposure at the end of the constant routine (T4A, 62.5% suppressed; T5A, 42.9% suppressed), consistent with the response observed in able-bodied subjects (34, 35). Such a response indicates an intact sympathetic innervation of the pineal gland.

Cortisol

In contrast to melatonin, rhythmic production of cortisol appeared intact in each of the five SCI subjects (Fig. 3). The average 24-h plasma concentration profile and circadian amplitude of all five SCI subjects were within the 95% confidence limits of the able-bodied comparison subjects (Table 2). The phase angle between the fitted maximum of cortisol secretion and typical waketime was normal in four of the five SCI subjects (Table 2). Plasma cortisol concentrations during the 2 h of brighter nocturnal light exposure at the end of the constant routine appeared to decline in four of the five SCI subjects, although more subjects would be required to determine the reliability of this observation.

TSH

Results from the analysis of TSH were similar to those of cortisol (Fig. 4). The average 24-h concentration in four of the five SCI subjects was normal (Table 2). However, though the average 24-h concentration in only one of the SCI subjects was beyond the 95% confidence limits of the able-bodied comparison subjects, the other four SCI subjects were on the low end of the normal range of TSH secretion. The phase angle between TSH onset and typical bedtime was normal in four of the five SCI subjects, with the same subject who displayed an abnormally early cortisol phase also having an abnormally early TSH phase (Table 2). The circadian amplitude was normal in four of the five SCI subjects, although a different subject had a lower circadian amplitude than the subject with the significantly low 24-h average (Table 2). As with the able-bodied subjects, there were no consistent re-

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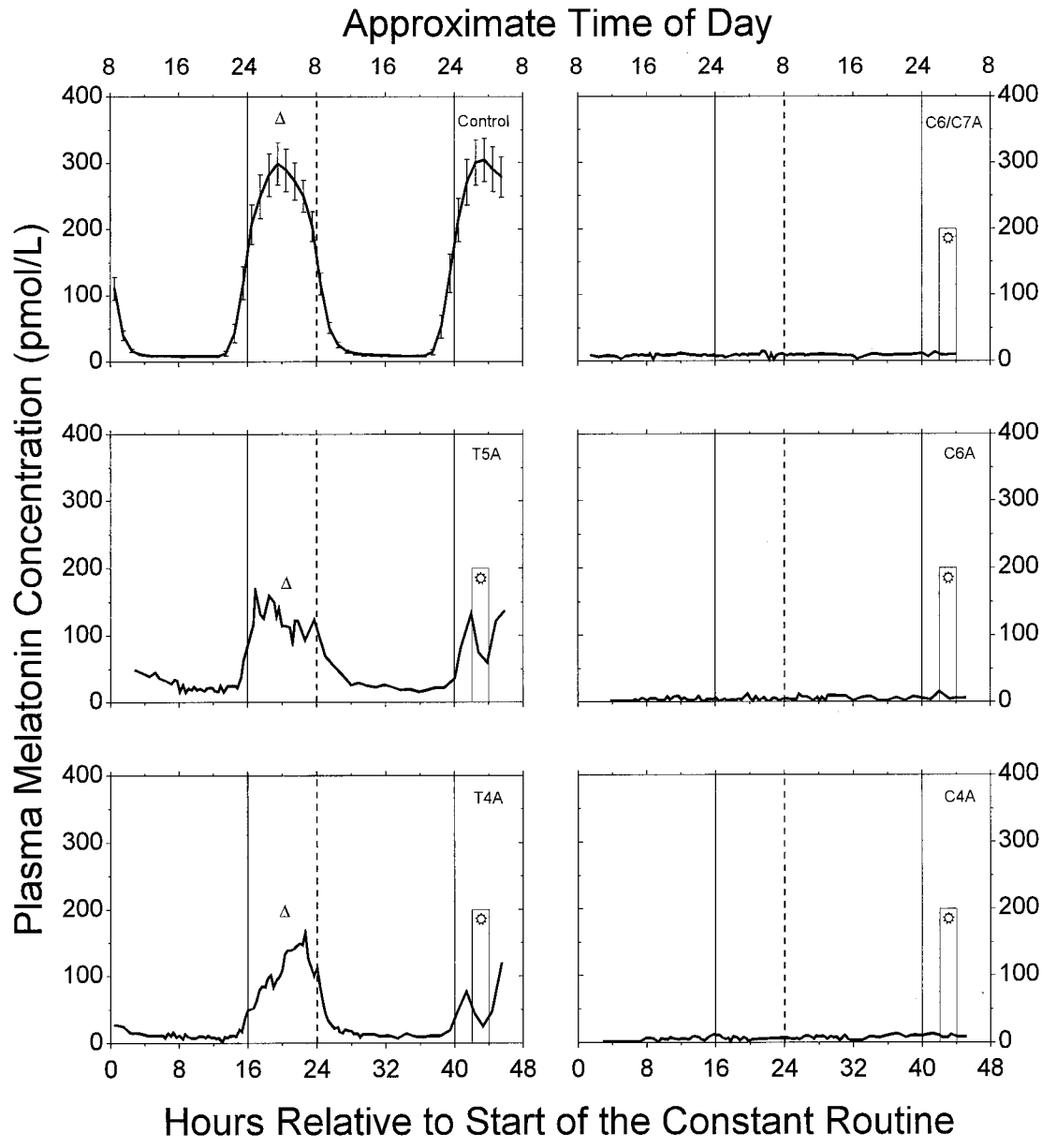

FIG. 2. Melatonin waveforms in a group of able-bodied control subjects (average ± SEM; n = 24; *upper left*) and in T4A paraplegic (*lower left*), T5A paraplegic (*middle left*), C6A tetraplegic (*upper right*), C6/C7A tetraplegic (*upper right*), and C4A tetraplegic (*lower right*) patients during a 46-h constant routine. Control subjects are binned hourly within, then between, subjects. Included in the figure are the time of the melatonin maximum (*open triangle*), the times of habitual bed (*solid line*) and wake time (*dashed line*), and the time of a 2-h episode of brighter light (*open box with* ✲).

sponses to the brighter light exposure in the TSH pattern of any of the patients.

Discussion

There have been two previous studies that have investigated the disruption of the pineal control pathway in SCI (12, 13). Although these earlier studies suggested a lack of melatonin rhythmicity in tetraplegics and preserved rhythmicity in paraplegics, due to their opposing results, it was unclear whether melatonin in tetraplegics was at a constantly elevated or a constantly low level. The former would imply that decentralization of the human pineal gland freed it from the cyclic inhibitory influence of the brain, whereas the latter would imply that the denervated human pineal gland is quiescent (in terms of melatonin secretion). Data from the present study, which employed a more specific and sensitive assay for melatonin than was previously available, indicate that the decentralized pineal gland is quiescent, producing little, if any, melatonin at any time of day. Our data, therefore, support the hypothesis that the human pineal must be stimulated by the sympathetic nervous system to produce melatonin. This conclusion is consistent with data from other mammals (10, 44). Our observations of an absence of circadian rhythmicity in tetraplegia and a preservation in para-

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TABLE 2. Temporal and quantitative hormone data

CODE	1765	1826	1836	18F3	18H6	CONTROL
Level of SCI	T4A	T5A	C6A	C6/C7A	C4A	n/a
Melatonin 24-h avg. (pM)	46.6	62.1	n/a	n/a	n/a	96.1 ± 53.4
ψTWT-Melϕ (h)	3.55	3.50	n/a	n/a	n/a	4.03 ± 0.62
Cortisol 24-h avg. (μg/dL)	7.90	10.4	10.8	8.83	9.75	10.8 ± 2.22
Cortisol circadian amp (μg/dL)	2.80	3.45	3.03	3.35	2.81	3.15 ± 0.65
ψcortϕ-TWT (h)	0.98	1.17	2.42	−0.73*	2.52	2.10 ± 0.78
TSH 24-h avg. (μIU/mL)	1.29	1.89	0.90*	2.71	1.32	2.38 ± 0.70
TSH circadian amp (μIU/mL)	0.68	0.81	0.52	1.39	0.42*	1.26 ± 0.41
ψTBT-TSHϕ (h)	4.12	3.20	3.08	5.27*	3.63	3.26 ± 0.90

SCI and control subjects as explained in text. Control data are presented as average ± SD. SCI subjects with values outside of the 95% control confidence intervals are denoted with an *asterisk*.

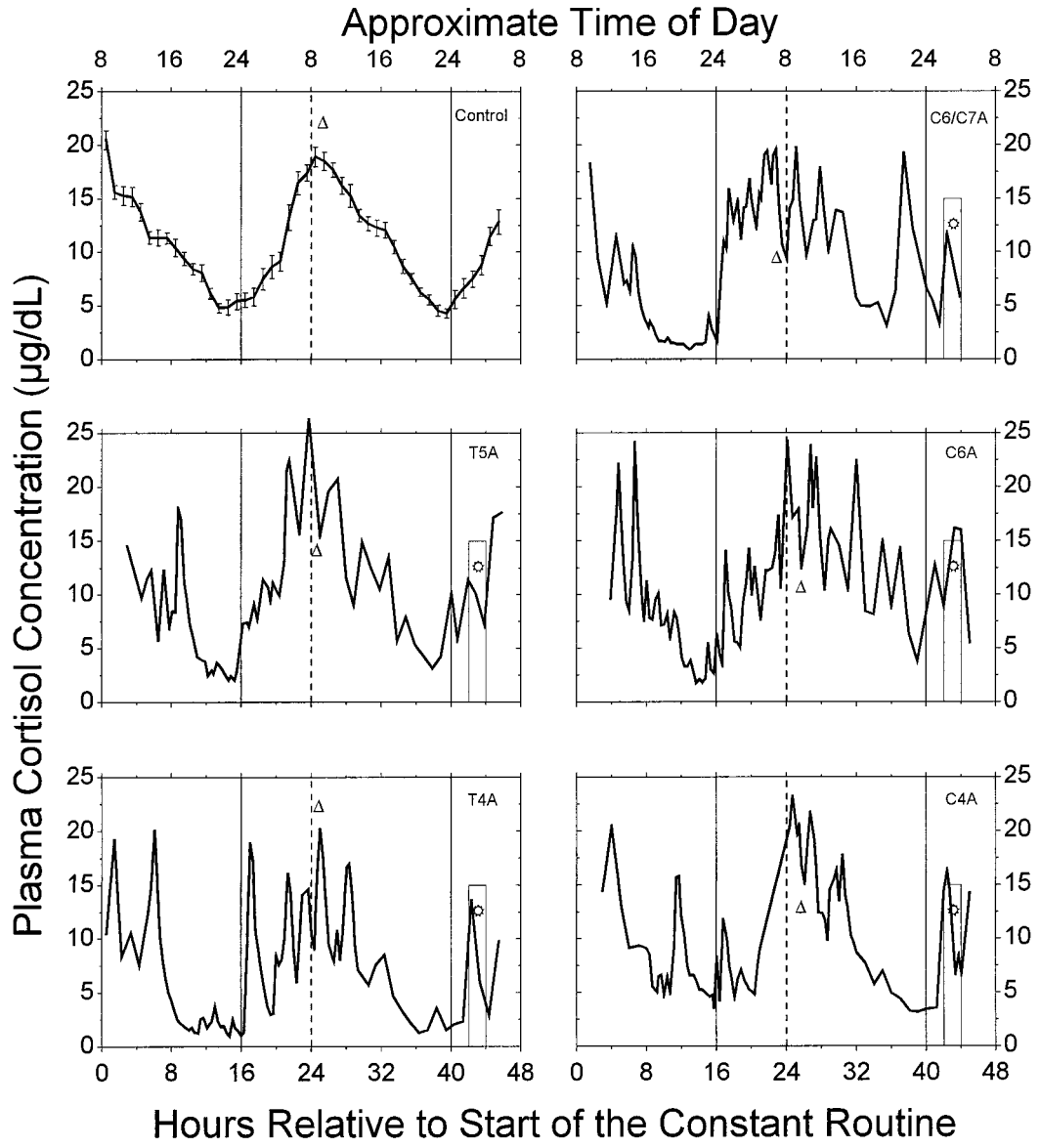

FIG. 3. Cortisol waveforms in a group of control subjects (average ± SEM; n = 24; *upper left*) and in T4A paraplegic (*lower left*), T5A paraplegic (*middle left*), C6A tetraplegic (*middle right*), C6/C7A tetraplegic (*upper right*), and C4A tetraplegic (*lower right*) patients during a 46-h constant routine. Data from control subjects are binned hourly within, then between, subjects—a procedure that smoothes the data and removes the pulsatility that is evident in the plots of each individual subject. The legend is the same as in Fig. 2, except the time of the fitted cortisol maximum (*open triangle*) is plotted. Note that the subject with the spinal cord injury at C6/C7A has a fitted cortisol peak that occurs before the typical waketime, whereas the other subjects have a fitted cortisol peak that occurs just after the typical waketime.

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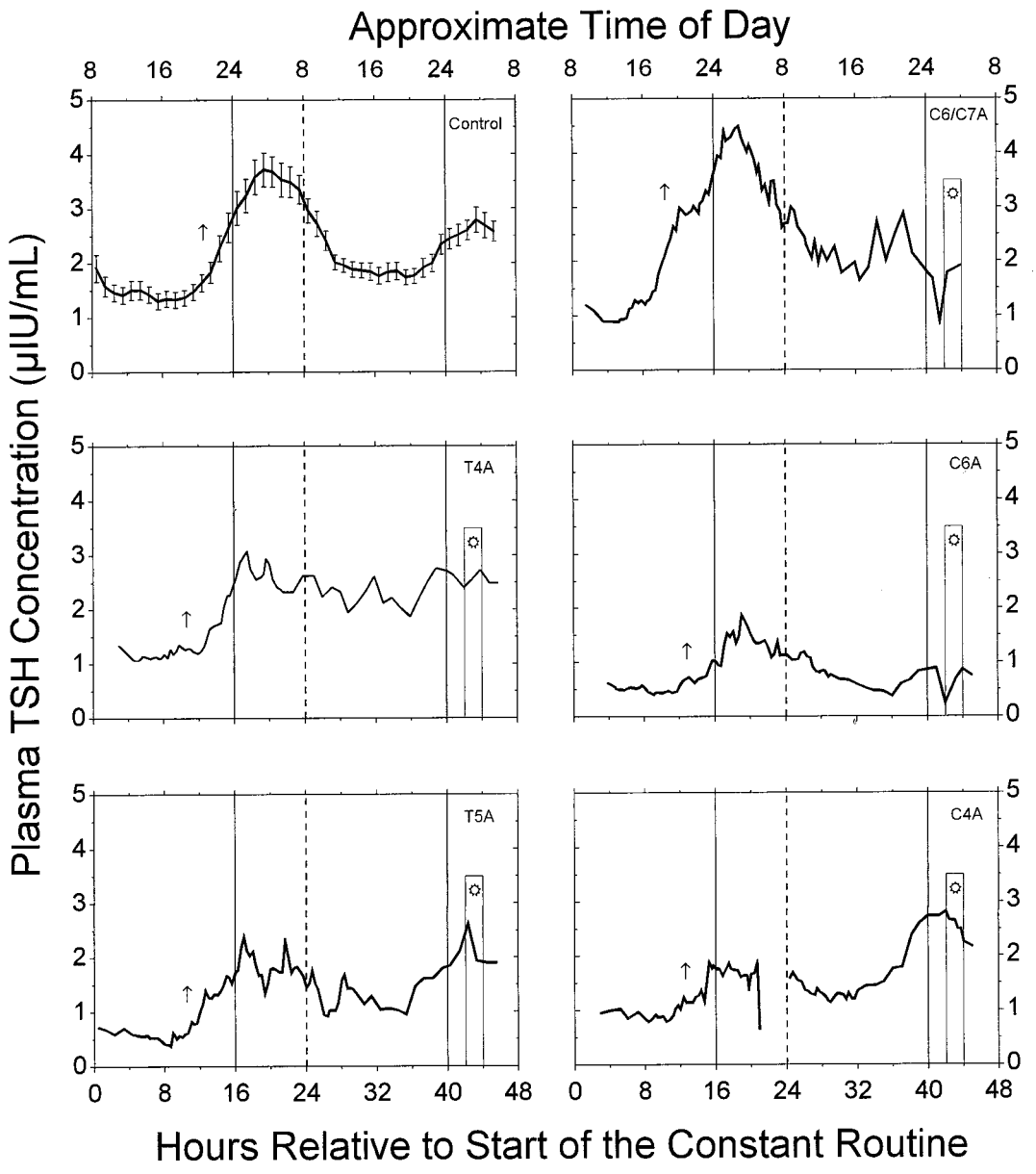

FIG. 4. TSH waveforms in a group of control subjects (average ± SEM; n = 14; *upper left*) and in T4A paraplegic (*middle left*), T5A paraplegic (*lower left*), C6A tetraplegic (*middle right*), C6/C7A tetraplegic (*upper right*), and C4A tetraplegic (*lower right*) patients during a 46-h constant routine. Control subjects are binned hourly within, then between, subjects. The legend is the same as Fig. 2, except the time of TSH onset (↑) is plotted. Note that the subject with spinal cord injury at C6/C7A has a fitted TSH onset that occurs more than 5 h before the typical bedtime, whereas the other subjects have a fitted TSH onset that occurs approximately 3 h before the typical bedtime.

plegics are consistent with the conclusion based on the nonhuman mammalian literature and anatomical studies in humans indicating that the innervation of the pineal arises from thoracic roots 1–4 (9, 10). Our data further suggest that neither the putative pathway that leads directly from the pretectum to the pineal (9, 11) (*i.e.* bypassing the spinal cord) nor parasympathetic innervation (9) is sufficient to generate measurable pineal melatonin secretion in humans.

In contrast to the disruption of the melatonin circuit, the neurohumoral influence of the hypothalamus on cortisol and TSH production remains intact in SCI, although the spinal influences on the adrenal cortex that convey information about acute environmental changes may be disrupted. Previous studies have provided conflicting data on the normalcy of cortisol amplitude in chronic SCI, with some claiming low, others claiming normal, and yet others claiming high circulating concentrations of cortisol or its metabolite 17-hydroxycorticosteroid in such individuals (12, 15–27, 29, 30). However, those studies generally relied upon one or two time points in the determination of amplitude. Under such circumstances, the daily rhythmicity of cortisol concentrations and its inherent pulsatility may have confounded the results. Our study, which employed a frequent blood-sampling protocol (three times per h for 24 h) during a constant routine, demonstrated that both the 24-h average and the circadian amplitude of the cortisol rhythm in our SCI subjects were

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indistinguishable from those in the able-bodied subjects, even among tetraplegics who lacked melatonin.

The literature concerning basal TSH production in chronic SCI is far less extensive than that concerning cortisol. The few studies that have examined TSH have each reported normal plasma concentrations in chronic SCI subjects (14, 18, 19, 27, 28). However, as with much of the cortisol literature, those studies have relied upon a single morning sample in the determination of TSH concentration. TSH also displays a daily rhythmicity, and, as with cortisol, examination of a single time point is insufficient to assess the 24-h profile. In accounting for this variation as well as the suppressive effects of sleep on TSH production, our study has shown that the 24-h average and the circadian amplitude of the TSH rhythm in the chronic SCI subjects were within the low end of the normal range. Whether there actually is a small decline in TSH amplitude in chronic SCI remains a question for further study in a larger sample of subjects.

It has been suggested that melatonin can affect the amplitude of the secretion of TSH and cortisol (45, 46). However, our observation of normal TSH and cortisol amplitudes in the absence of melatonin secretion implies that such an effect is small or absent in humans, assuming that there are no long term compensatory changes. Furthermore, the presence of rhythmic TSH and cortisol in the absence of rhythmic melatonin indicates that pineal melatonin secretion is not necessary to drive these rhythms.

The circadian timing system regulates the oscillation and temporal organization of many biological functions. It has been postulated that the pineal neurohormone melatonin may play a role in the coupling of these downstream rhythms (47), possibly through direct feedback onto the melatonin receptors in the SCN (48, 49) or indirectly by its hypothermic (50) or somnogenic effects (51). We examined the potential role of melatonin in coupling these rhythms by evaluating the phase angles between cortisol, TSH, and sleep in the presence (paraplegics) and absence (tetraplegics) of melatonin. In the two paraplegic subjects, the timing of each of the three hormones was normally aligned with respect to the sleep/wake pattern. In two of the three tetraplegics, the rhythms of cortisol and TSH were normally timed with respect to the sleep/wake pattern. In the third (C6/C7A), the timing of the rhythms of both cortisol and TSH were phase advanced relative to the sleep episode (*i.e.* the onset of TSH and the peak of cortisol occurred earlier than would have been predicted by the subject's sleep/wake times). This could be a random, anomalous result, a possibility difficult to exclude given our small sample size and the observation of such anomalies among able-bodied subjects (52). Alternatively, if data from a larger study were to indicate that it was probable that the rhythms of TSH and cortisol were truly out of phase with the sleep/wake cycle in some tetraplegics compared to those in paraplegics, it would support the hypothesis that melatonin could act as a coupling agent for SCN-controlled variables. This hypothesis may receive further support in the continued examination of the high prevalence of nonapnea-related sleep disturbances in SCI patients (53).

Using the rigorously controlled, constant routine protocol to examine circadian variation in endocrine function, we demonstrated that patients with neurologically complete injury to the lower cervical spinal cord do not produce melatonin. This further indicates that any parasympathetic or direct innervation of the pineal by the pretectum is insufficient to generate significant melatonin secretion in humans. Those subjects with neurologically complete injury to the upper thoracic spinal cord had normal melatonin rhythmicity. Our data also indicate that the long-term loss of melatonin or a substantial amount of peripheral somatic sensory information does not result in substantial changes in the quantitative or temporal characteristics of cortisol or TSH.

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