Involvement of the suprachiasmatic nucleus in diurnal ACTH and corticosterone responsiveness to stress

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Sage, Dominique, Daniel Maurel, and Olivier Bosler. Involvement of the suprachiasmatic nucleus in diurnal ACTH and corticosterone responsiveness to stress. Am J Physiol Endocrinol Metab 280: E260–E269, 2001.—We explored the contribution of the suprachiasmatic nucleus (SCN) in ACTH and corticosterone (CORT) diurnal responsiveness of the rat to restraint stress applied either in the morning (AM) or in the evening (PM). Ablation of the SCN caused the diurnal rhythmicity of the CORT response to disappear but had no effects on AM vs. PM differences in the ACTH response. Stress-response curves in SCN-lesioned rats that had prestress levels of CORT either in the AM range or in the PM range, when compared with those obtained for AM and PM controls, showed that the SCN differentially regulates the stress response depending on the underlying secretory activity of the adrenal cortex. When basal CORT secretion is at its lowest, the SCN inhibits CORT responsiveness to stress by controlling pituitary corticotrophs; but when it is at its highest, it has a permissive action that will bypass the hypophysis and reach the adrenals to adjust the response of the gland to ACTH.

stress; hypothalamic-pituitary-adrenal axis; neuroendocrine responses to stress; circadian rhythm

ACTIVATION OF THE hypothalamic-pituitary-adrenal (HPA) axis in response to stressful stimuli is a major physiological event that contributes to maintaining homeostasis and is therefore of critical importance to the organism’s ability to adapt to environmental conditions. Such activation results in a rapid increase of circulating glucocorticoids that act on virtually every organ in the body to minimize perturbations of the homeostatic state. This neuroendocrine stress response is triggered by increased secretion of adrenocorticotropic hormone (ACTH) by the anterior pituitary in response to a portal blood elevation of corticotropin-releasing hormone and arginine vasopressin, produced by stress-integrative neurons in the parvocellular division of the paraventricular nucleus.

It has long been demonstrated that the HPA axis has another characteristic that is fundamental to homeostatic regulation in mammals: a circadian rhythm in basal activity. In the rat, a daily peak in ACTH and corticosterone (CORT) release occurs near the onset of darkness. It corresponds to the end of inactivity in this nocturnal animal and may serve to prepare the organism for the upcoming period of increased activity. Many findings have demonstrated that the response of the HPA axis to stress is also under rhythmic control, depending on the time of day the stress occurs. Diurnal differences in ACTH and CORT release in response to a variety of stressors have been reported. The responses have been described as higher in the evening (PM) (15, 32), higher in the morning (AM) (22, 31), or equal at these two times of the day (29). Moreover, a dichotomy between nycthemeral variations in stress-induced ACTH and CORT release was further emphasized by Bradbury et al. (5). These authors found that ACTH secretion in response to restraint stress was higher in the AM than in the PM, whereas the CORT response was equal at these times. Using adrenalectomized rats, they also provided convincing evidence that diurnal variations in the ACTH response to stress were independent of basal plasma CORT concentrations and thus could not be accounted for by increased negative feedback signals sent by glucocorticoids to the HPA axis in the PM, when their circadian concentrations are the highest.

Rhythmicity in the secretory activity of the HPA axis is known to be controlled by the suprachiasmatic nucleus (SCN) of the hypothalamus, which is the master component of the central circadian system in mammals (23, 26). Endogenous rhythms are entrained therein by fluctuations in the environment, such as the alternation of light and darkness (12:12-h light-dark cycle). Accordingly, bilateral lesions of the SCN have been shown to eliminate major behavioral and endocrine rhythms, including daily variations in HPA activity (1, 24, 27, 28). According to Szafarczyk et al. (28), however, SCN lesions produce disruption, but not disappearance, of the rhythmic secretion of CORT over the 24-h cycle.

In addition to its role as a central regulator of the rhythmic basal activity of the HPA axis, the SCN is also involved in modulation of the response of this axis to stressful stimuli (6, 17). Because of some discrepnan-
cies among the results, however, its actual role in this context remains unclear. In the present study, we used rats with selective lesions of the SCN to further explore the possible involvement of this nucleus in regulating the diurnal rhythm in the HPA axis response to stress. Especially because it has been demonstrated that the underlying pattern of HPA activity can be a major determinant of reactivity to stress (30), we looked more closely at the question of whether the SCN acts as a differential modulator of the ACTH and/or CORT response to stress in the AM, when concentrations of the hormones are the lowest, and in the PM, when they are at their circadian peak.

MATERIALS AND METHODS

Experimental Animals

Sprague-Dawley rats (Dépré, St-Doulchard, France) were housed individually in temperature-controlled (21°C) and soundproof rooms under a 12:12-h light-dark schedule (lights on at 0700). They were allowed to adapt to this environment at least 2 wk before surgery. Body weights at the time of the experiments ranged between 240 and 260 g. Food and water were available ad libitum. Surgery and perfusions for histochemical controls were carried out under deep equithesine anesthesia. An initial dose (0.4 ml/100 g ip) was followed by an appropriate additional dose when required. All experimental procedures were carried out in strict accordance with European Economic Community guidelines (86/609/EEC) for the care and use of laboratory animals.

Lesion of the SCN

Lesions were made electrically under stereotaxic control. Thirty rats were designated for investigation of postlesional stress responses either in the AM or in the PM. An additional group of 35 rats was subjected to the lesion procedure with the aim of separately examining the effects of SCN ablation on diurnal rhythms (n = 20) as well as on the pulse-like secretory patterns of both hormones within a limited period of time (n = 15). Animals were placed in a David Kopf stereotaxic frame (tooth bar −3.3 mm), and stainless steel lesion electrodes 0.2 mm in diameter (Phymep, France) were lowered bilaterally into the base of the brain at the expected site of the SCN. The following coordinates, adapted from the Paxinos and Watson atlas (25), were used: A, 7.5 mm anterior to the interaural line; H, 0.5 mm dorsal to the interaural line; L, ±0.2 mm lateral to the midline. Electrocoagulation of the SCN was achieved by passing an anodal current of 0.8 mA for 20 s on each side by use of a lesion generator. Tests had been run in advance to make sure that these conditions were appropriate for obtaining total and selective destruction of the SCN. Sham-lesioned rats were prepared by lowering the electrodes onto the appropriate coordinates but not passing any current.

Cannulation

One month after lesion surgery and 4 days before the experiments, all rats were subjected to intracarotid cannula insertion with polyethylene tubing (PE-50), in accordance with Szafarczyk et al. (28), to allow for sequential blood sampling. In the rats destined to be used in a study examining changes in hormone secretion over a short period of time (study 3, see Experiments), a second cannula was inserted into one jugular vein, immediately after which the cannulas were rinsed with heparin-saline solution. Every day for the next 3 days, each rat was handled gently to minimize handling-stress effects on the day of sampling, and the cannulas were flushed with the heparinized saline solution.

Experiments

Three separate studies comparing SCN-lesioned and sham-operated rats were carried out. In each of them, blood samples were collected from the carotid within ±1 min in plastic tubes containing 20 (studies 1 and 2) or 10 μl (study 3) of EDTA at 4%. Samples were kept on ice until centrifuged, and the plasma was stored at −20°C until assay.

Study 1: Patterns of ACTH and CORT release after restraint in AM or PM. Thirty rats subjected to SCN lesion and 11 rats subjected to sham operation were exposed to stress at either 0900 (“zeitgeber” time 2 (ZT 2) in the AM) or 2100 (ZT 14 in the PM). The stress paradigm consisted of placing the rats for 120 min in plastic cylinders for restraint. In each animal, blood samples (0.3 ml) were obtained first, within 1 min after removal from the home cage (time 0), and then after restraint at 15, 30, 45, 60, and 120 min. Each sampling was followed by injection of an equivalent volume of heparin-saline rinsing solution into the cannula to compensate for volume loss.

Study 2: Diurnal profile of basal ACTH and CORT secretion. This study involved 20 lesioned and 10 sham-operated rats moving freely, in which blood samples (0.5 ml each) were taken at 4-h intervals over a 36-h period (10 samples per animal). After each sample, heparin-saline was injected into the cannula.

Study 3: Profile of episodic basal CORT secretion. Fifteen lesioned and eight sham-operated rats moving freely were used. Blood collections (0.15-ml samples) commenced at either 0900 (ZT 2) or at 2100 (ZT 14) and continued every 5 min over a 30-min period. Postsampling injections of the rinsing solution were made through the jugular cannula.

Hormone Measurements

CORT was measured in 20-μl plasma samples after extraction with absolute ethanol using the radioimmunoassay method described by Conte-Devolx et al. (9). The sensitivity of the assay was 7.5 ng/ml and the intra- and interassay coefficients of variation were 6% and 8%, respectively. ACTH was measured in 50-μl plasma samples by means of a radioimmunoassay kit (ACTH-K-PR, CIS Bio-International, Gif-sur-Yvette, France). The antisera used did not cross-react with the following proopiomelanocortin-derived peptides: β- and γ-lipotropin, α- and β-melanocyte-stimulating hormone, and β- and γ-endorphin. The sensitivity of the assay was 10 pg/ml, and the intra- and interassay coefficients of variation were 5% and 6.5%, respectively.

Analysis of Results of Stress Experiments

The stress response in SCN-lesioned vs. sham-lesioned rats at each time of day (AM and PM sessions) or as a function of basal CORT levels was evaluated using three physiological parameters: 1) profile of the stress-response curve over the 120-min exposure, 2) increment of the plasma levels of ACTH and CORT within the first 15 min poststress (A-rise), and 3) area under the stress-response curve (AUC) as a reflection of overall hormone secretion during stress exposure. All results were expressed as means ± SE and were processed in statistical analysis as follows. A one-way ANOVA test with factorial measures was used to evaluate the effects of time of day and SCN lesion on basal
concentrations of ACTH and CORT, as well as on the post-stress \( \Delta \)-rise and 120-min secretion (AUC values) of both hormones. Differences in hormone levels between experimental groups at the various time periods were determined using two-way ANOVA with repeated measures ("group effect": AM vs. PM session in both sham and SCN-lesioned rats; "treatment effect": SCN-lesioned vs. sham-lesioned rats). When a significant effect was detected, a Student-Neuman-Keuls post hoc procedure was applied to assess the specific points in time when the difference occurred. Significance was defined as \( P < 0.05 \).

Analysis of the influence of basal CORT levels on ACTH or CORT responses (AUC or \( \Delta \)-rise) was performed using the Spearman correlation test. Significance was defined as \( P < 0.05 \).

RESULTS

Nycthemeral Variations in ACTH and CORT Responses to Restraint Stress. Effects of the SCN Lesion (Study 1)

Control rats. Histological controls showed that sham-lesioned rats had intact SCNs that were characteristically delineated by dense vasoactive intestinal peptide (VIP) and arginine vasopressin (AVP) neuronal stains. In all of these rats, prestress ACTH and CORT levels as measured just before stress exposure (point 0, basal values) were significantly higher at ZT 14 (PM) than at ZT 2 (AM; ACTH, \( P = 0.0009 \); CORT, \( P = 0.0082 \); Fig. 1). Exposure to restraint resulted in an abrupt increase in the levels of both hormones. Maxima were reached within 15 or 30 min in AM and PM groups alike. ACTH levels declined steadily after that (Fig. 1, A and B), whereas CORT levels stabilized and returned slowly to steady-state values (Fig. 1, C and D).

Patterns of response differed completely depending on the hormone considered (Fig. 1 and Table 1). The ANOVA did not reveal a significant difference between the shape of the ACTH stress-response curves in AM and PM rats, even though the \( \Delta \)-rise at 15 min was significantly higher in the AM than in the PM (Fig. 1, A and B). In terms of the overall 120-min poststress secretion of ACTH (AUC values), AM rats were not statistically different from PM rats.

When applied to CORT, the ANOVA clearly showed a group effect (\( P = 0.02 \)), revealing that the secretion patterns of the adrenocortical hormone in the AM and the PM were different (Fig. 1, C and D). The mean values reached by CORT within the first 15 min post-stress were higher in the PM than in the AM, and concentrations of the hormone remained higher in the
PM until the 120-min point; however, the Δ-rises in CORT levels were equivalent at these two times. In contrast, AUC values were higher in PM than in AM rats (P = 0.013).

SCN-lesioned rats. Of the 30 animals that had undergone a SCN lesion and were exposed to stress either in the AM or in the PM, 17 showed remnant VIP- and/or AVP-immunopositive neurons at the expected place of the SCN, so they were not used for the analysis. The data were thus collected from the 13 rats in which a complete SCN lesion could be observed. In none of these rats did the optic chiasm appear to be sectioned. Seven of the 13 rats selected for the analysis belonged to the AM group, and six belonged to the PM group.

SCN-lesioned rats had basal plasma ACTH levels that were significantly higher in the AM than in the PM (P = 0.023; Fig. 1, A and B), but their basal CORT levels were not different at these two times, being in the same range as the basal evening levels of CORT in control rats (Fig. 1, C and D). In fact, it turned out that the lesion increased basal ACTH and CORT levels in the AM and decreased ACTH levels in the PM, whereas at that time it did not cause any change in CORT secretion.

Restraint stress induced a dramatic increase in both ACTH and CORT (Fig. 1 and Table 1). The general pattern of ACTH release during stress appeared different at both times of day. In the AM, ACTH secretion had a peak at 15 min in every animal and steadily declined thereafter (Fig. 1A), whereas in the PM, rats showed peak secretions at different times between 15 and 60 min, after which concentrations of the hormone returned abruptly to basal values (Fig. 1B). Nevertheless, as found in sham-operated rats, the two-way ANOVA did not yield a significant effect of time of day on the ACTH secretion profile. Accordingly, there was also no significant difference between the AUC values measured in AM and PM rats. Moreover, the Δ-rise in ACTH was not significantly different in the AM and in the PM, even though it tended to be higher in the AM (see Table 1). Comparing SCN-lesioned and control rats, we in fact found no treatment × group interactions, meaning that the SCN lesion had no effect on the stress-induced ACTH response, whether in the AM (Fig. 1A) or in the PM (Fig. 1B).

As found for ACTH, the CORT response curves and the Δ-rise in CORT secretion in SCN-lesioned rats were not statistically different at these two times of day (Fig. 1, C and D). Both AM and PM rats showed sustained secretion of the adrenocortical hormone until the 120-min point, and AUC values were in the same range. Accordingly, when comparing SCN-lesioned with sham-operated rats, the statistics revealed a clear treatment × group effect showing that the lesion had different effects in the AM (Fig. 1C) and in the PM (Fig. 1D; P = 0.007). CORT response to stress after the lesion was indeed significantly greater in the AM at 15, 30, and 60 min (Fig. 1C) and decreased in the PM at 15 min (Fig. 1D).

Regression analysis of the relation between basal CORT levels and either the CORT or the ACTH response to stress resulted in different patterns in sham-operated and SCN-lesioned rats (Fig. 2). In control rats, the correlation was positive for the overall CORT response (AUC values, r = +0.67, P = 0.02) and negative for the increment of ACTH secretion (Δ-rise, r = −0.72, P = 0.02), but the regression lines did not reach significance when the CORT and ACTH responses were considered in terms of Δ-rise and AUC, respectively. After the lesion, the Δ-rises in secretion of ACTH (r = −0.63, P = 0.02) and CORT (r = −0.89, P = 0.001), as well as the overall ACTH response (r = −0.73, P = 0.004), were negatively correlated with basal CORT levels. Regression lines also revealed an apparent negative correlation between the overall CORT response and prestress levels of the adrenocortical hormone (r = −0.52), indicating that the SCN lesion induced inversion of the correlation. However, statistical significance was not reached for this parameter.

Basal CORT levels in pooled SCN-lesioned rats of the AM and PM experimental groups showed a continuous distribution from 20 to 274 ng/ml (Fig. 3). Three rats of the AM group and two rats of the PM group had prestress CORT levels in the range of the basal AM levels that had been measured in sham-operated animals (from 20 to 98 ng/ml), whereas four rats of the AM group and four rats of the PM group had prestress CORT levels in the range of the PM levels (from 143 to 274 ng/ml). This suggested that CORT release in SCN-lesioned rats still fluctuated but independently of time of day. Study 2 was designed to address this question.

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Increments of plasma ACTH and corticosterone (CORT) secretion above the baseline (Δ) within 15 min after the beginning of restraint and areas under the curves (AUC), as calculated from the stress-response curves of each hormone. SCN-lesioned rats (SCN-x) exposed to stress either in the morning (AM) or in the evening (PM) are compared with their respective sham-lesioned controls after reassignment or not to either a morning-like (ML) or evening-like (EL) group. *P < 0.05 vs. PM controls; †P < 0.05 vs. ML SCN-x rats; ‡P < 0.05 vs. EL SCN-x rats.
Effect of SCN Lesion on Nycthemeral Fluctuations of Basal ACTH and CORT (Study 2)

All control rats in this study had phase-related peaks of ACTH and CORT secretion at the beginning of the dark period, in accord with previously described standard patterns (Fig. 4). Of the 20 rats subjected to the lesion protocol, 12 showed, a posteriori, that there was complete destruction of the SCN. The graph representing average hormone levels measured at each sampling time in these rats indicated the disappearance of the nycthemeral fluctuations in ACTH and CORT after the SCN lesion (Fig. 4). However, when analyzed individually, each SCN-lesioned rat exhibited a persistent rhythmic expression of CORT release characterized by the occurrence of several maxima and minima over the 24-h cycle (Fig. 4). CORT concentrations often appeared to be within AM or PM ranges of control rats at any sampling time. There were also some residual variations in plasma ACTH levels in the lesioned rats, but the amplitudes of the fluctuations were much lower than for CORT and were not always correlated with CORT variations.

Effects of SCN Lesion on Stress Response in Relation to Basal CORT Levels

Study 2 showed a lack of synchronization in the time at which hormone peaks occurred after the lesion, which explains why the SCN-lesioned rats in study 1 had basal plasma levels of CORT that were not related to the time of stress exposure, as illustrated in Fig. 3. Results of the two studies, when taken together, then prompted us to reassign a posteriori each SCN-lesioned rat used in stress experiments (study 1) to either a “morning-like levels” group (ML rats) or an “evening-like levels” group (EL rats) according to its individual basal CORT secretion before stress, i.e., irrespective of whether it had been submitted to restraint in the AM or in the PM.
Group reassignment of SCN-lesioned rats. Two criteria were used to determine a threshold concentration of CORT that would allow us to segregate the ML and EL groups in study 1. The first criterion was based on data from study 2. After pooling individual measures in sham rats at the two 22-h sampling points, we established 124 ± 25 ng/ml as a mean concentration characterizing the evening peak of CORT secretion. We therefore defined a cut-off of 99 ng/ml (124 ± 25 ng/ml) between morning-like levels and evening-like levels. This threshold value was further validated by determining the 50th percentile of distribution of basal CORT levels in SCN-lesioned rats as a second criterion. This statistical analysis, which was imposed by data showing a continuous distribution of basal CORT concentrations in these animals (see Fig. 3), was carried out on data from both study 1 (prestress plasma levels in the AM and in the PM, 1 measure/animal) and study 2 (all points over the 36-h sampling period, 10 measures/animal). The 50th percentile of distribution was found to be 98 ng/ml, meaning that 50% of the SCN-lesioned animals had basal plasma levels either below or above this threshold concentration.

At this point, the question arose whether the ML or EL statute of a SCN-lesioned rat in study 1 (below or above 99 ng/ml of basal CORT, respectively) could have merely depended on whether prestress sampling for that particular animal had occurred at a trough or burst in the residual rhythm of basal secretory activity. Study 3 was aimed at evaluating this possibility.

Effect of SCN lesion on pulse-like secretory episodes of CORT (study 3). Of the 15 rats subjected to an SCN ablation in this study, eight exhibited a complete, histologically controlled lesion, and it turned out that six of these had CORT levels in the range of those exhibited by EL rats, whereas two had CORT levels in the range of those exhibited by ML rats. The results indicated noticeable differences between the sham- and SCN-lesioned rats. The AM and PM controls showed the expected pulse-like patterns of CORT release (Fig. 5, A and B). In the SCN-lesioned rats, the amplitude of the episodic fluctuations was conspicuously lower (Fig. 5C), and CORT concentrations throughout the 30-min sampling period remained within the range of AM levels for the two ML rats (mean CORT concentrations of 63 and 65 ng/ml) or within the range of PM levels for the six EL rats (mean CORT concentrations from 146 to 262 ng/ml). It was concluded that the CORT plasma concentrations measured at prestress sampling time in either the AM or the PM in SCN-lesioned rats gave a stable index of the activity of the HPA axis at these times, rendering it unlikely that an ML rat would be wrongly analyzed as an EL rat and vice versa.
Comparing ML rats with AM controls and EL rats with PM controls, the two-way ANOVA revealed a clear treatment group effect (ACTH, \( P < 0.006 \); CORT, \( P < 0.002 \)), showing that the effect of the SCN lesion was dependent on prestress CORT levels. The ML rats did not differ significantly from the sham-lesioned rats restrained in the AM when the \( \Delta \)-rise in ACTH and CORT secretion from baseline levels was considered (Fig. 6, A and B). However, AUC values indicated that there were more sustained overall ACTH and CORT responses in these rats than in their corresponding AM controls (Table 1). Conversely, EL rats compared with PM controls had a smaller \( \Delta \)-rise in CORT secretion after stress, but no change in the \( \Delta \)-rise in ACTH secretion, whereas AUC values relative to either CORT or ACTH secretion were in the same range in both groups of rats (Fig. 6, C and D, and Table 1).

**DISCUSSION**

**Diurnal Rhythmicity in ACTH and CORT Responses to Restraint Stress**

Our control rat data are in line with the results of previous studies showing diurnal variations in the responsiveness of the HPA axis to stress (5, 8, 14, 22, 31). We found that the \( \Delta \)-rise in ACTH secretion after stress was about twice as high at ZT 2 as at ZT 14, confirming the greater response of the hypophysial corticotrophs in the AM. The question of whether this results from higher basal concentrations of circulating corticosteroids in the PM, when both ACTH and CORT are at their circadian peak, than in the AM, was investigated by Bradbury et al. (5). These authors clearly showed continued expression of the diurnal rhythmicity in ACTH secretion induced by restraint stress after adrenalectomy, demonstrating that the diurnal pattern of ACTH responsiveness is not dependent on CORT. It therefore appears to involve neural rather than hormonal mechanisms. Interestingly, there is experimental evidence that responsiveness of the HPA system to stress throughout the 24-h cycle is subordinate to the endogenous rhythm of food intake (4, 16).

The adrenal response patterns in the AM and in the PM did not differ in the same way as those of the pituitary corticotrophs, since absolute concentrations of CORT induced by stress were higher in the PM than in the AM, whereas \( \Delta \)-rises from baseline levels were the same at both times. This result is quite consistent with the data obtained by Kant et al. (22), who used an immobilization stress paradigm comparable to that employed in the present study. The restraint stress thus induced a dichotomous effect on ACTH and CORT responses. This dichotomy is also evident when we consider the AM vs. PM global secretion patterns of both hormones over the 120-min sampling period, as there was a time-of-day effect on overall poststress CORT secretion that was not found for ACTH.
the results of the AUC analysis, it may be concluded that the adrenals respond to stress by a more sustained overall secretion of CORT in the PM than in the AM. Conceivably, the dichotomy between the ACTH and CORT responses may result from greater adrenal sensitivity to ACTH at lights out (10, 12, 13, 20, 21) and/or the lesser efficacy of negative CORT feedback at that time (2, 3).

Similarly, Buijs et al. (6) reported that stress at ZT 10 in the evening, compared with stress at ZT 2 in the morning, resulted in a higher CORT response, even though they measured identical increments in the absolute value of the plasma CORT level at these two times. However, it appeared, in a more recent study by the same group (8), that when stimulation of the HPA axis occurred at ZT 14 or ZT 20 instead of ZT 10 in the PM, the CORT response was higher at ZT 2 in the AM. The fact that the stress paradigm used was mild in the Buijs et al. studies, the so-called novelty stress, could partly explain the conflicting results. Other reports do indicate that the circadian variation in the CORT response may depend on the type of stress (15, 22, 29, 31, 32).

**SCN Involvement in Diurnal Rhythmicity of Stress Response**

We found that SCN ablation had no apparent effect on the diurnal variation in ACTH response to stress but caused the AM vs. PM differences in overall CORT secretion to disappear, demonstrating for the first time that the SCN is critically involved in the diurnal variation of adrenal cortex responsiveness to stressful stimuli. This conclusion could not be reached in previous studies in which novelty stress was used to induce CORT release, given that SCN-lesioned rats were shown to still respond differentially in the morning and in the evening (6). In fact, it appeared in these experiments that, at least in terms of absolute CORT secretion levels, the lesion resulted in an inverse rhythm of adrenal responsiveness, even though CORT concentrations after stress remained higher in SCN-lesioned rats than in intact controls, both in the morning and in the evening. However, as emphasized in the preceding section, experiments by Buijs’s group are not comparable to ours, because the novelty stress used to stimulate CORT secretion can be expected to provide the HPA axis with a much milder stimulation than the...
restraint stress that we used. An additional example showing that experimental results may depend on the stress paradigm used is provided by another report demonstrating impairment of the ACTH surge induced by ether stress after lesion of the SCN (17). The fact that we ourselves did not find any significant effects of the SCN lesion on the ACTH response is consistent with the view that the rhythmic expression of ACTH responsiveness is closely tied to the endogenous food intake rhythm (see above), especially since it has been reported that this rhythm is also maintained in SCN-lesioned rats (1). It is also in keeping with previous results showing that the SCN may influence CORT secretion independently of any action on hypophysial corticotrophs (6, 8, 11, 13, 18–21).

Involvement of SCN in Stress Response as a Function of Basal CORT Levels

As expected from previous data showing that basal HPA activity is an important determinant of the adrenocortical response to acute stress (30), we showed that the overall CORT response in control rats was positively correlated to basal CORT levels. In SCN-lesioned rats, although the significance level was reached only when considering Δ-rise, the stress-induced CORT release still appeared to be correlated with basal CORT levels, but the correlation was negative, suggesting that the role played by the SCN on the response of the adrenal cortex to stress may depend on the prestress hormonal state. Addressing this hypothesis required taking into account the results of study 2, showing that each SCN-lesioned rat had residual fluctuation in plasma CORT that was unrelated to the 12:12-h light-dark cycle and was characterized by the random occurrence of minimal and maximal levels of CORT secretion at any sampling time, as previously emphasized by Szafarczyk et al. (28). It implies that hormonal states in the lesioned rats at prestress sampling time were not predictable and, therefore, that both the AM and PM experimental groups were composed of rats that might have either a low or a high basal CORT level that was not related to the time of stress exposure. Further separation of the lesioned rats on the basis of their individual levels of CORT before stress, instead of their belonging to either the AM or PM experimental group, allowed the comparison of SCN-lesioned rats as ML or EL rats as opposed to sham-operated animals having comparable basal CORT levels, but the correlation was negative, suggesting that the role played by the SCN on the response of the adrenal cortex to stress exposure may depend on the prestress hormonal state. Addressing this hypothesis required taking into account the results of study 2, showing that each SCN-lesioned rat had residual fluctuation in plasma CORT that was unrelated to the 12:12-h light-dark cycle and was characterized by the random occurrence of minimal and maximal levels of CORT secretion at any sampling time, as previously emphasized by Szafarczyk et al. (28). It implies that hormonal states in the lesioned rats at prestress sampling time were not predictable and, therefore, that both the AM and PM experimental groups were composed of rats that might have either a low or a high basal CORT level that was not related to the time of stress exposure. Further separation of the lesioned rats on the basis of their individual levels of CORT before stress, instead of their belonging to either the AM or PM experimental group, allowed the comparison of them with sham-operated animals having comparable circulating CORT levels at prestress sampling time, i.e., ML rats to AM controls and EL rats to PM controls, respectively. The results of study 3, showing that prestress CORT levels reflected stable hormonal states and, therefore, that the classification of the SCN-lesioned rats as ML or EL rats was not a function of the sampling time, allowed the validation of such comparisons.

The results confirmed that the impact of the SCN on the stress response depended upon the prestress level of CORT secretion. Compared with AM controls, ML rats showed a more sustained overall secretion of both CORT and ACTH in response to stress, meaning that the SCN negatively regulates the secretion of both hormones when the basal activity of the HPA axis is at its lowest. In contrast, the overall poststress secretions of CORT and ACTH were not significantly different in EL rats and PM controls, but EL rats responded with a decreased acute release of the adrenal hormone. From this result, it may be concluded that, when the activity of the HPA axis is at its highest, the SCN has a positive regulatory effect on the adrenal response to stress and that this permissive action is independent of any action on pituitary corticotrophs. Conceivably, it could be exerted through adjustment of the sensitivity of the adrenal cortex to ACTH during the acute stress response, a hypothesis which is supported by the fact that patterns of the ACTH stress response were not different in EL rats and PM controls.

In conclusion, this study shows that the SCN plays a crucial role in regulating the diurnal variations of HPA axis responsiveness to stress. It emphasizes that the underlying basal secretory activity of the adrenal cortex is a critical determinant of the stress response and points to the fact that, depending on CORT circulating levels, the SCN has opposite modulatory effects through different mechanisms. It is proposed that, when basal CORT levels are in the morning range, the SCN exerts its action through regulation of the ACTH surge. In contrast, when basal CORT concentrations increase to reach evening levels, SCN messages will bypass the hypophysis and reach the adrenals to adjust the response of the gland to ACTH. Accordingly, a recent study has provided direct anatomic and functional evidence for a polysynaptic SCN-adrenal connection that involves the autonomic division of the paraventricular nucleus of the hypothalamus and the intermediolateral column of the spinal cord as relay structures (7).

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REFERENCES