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Corticosterone-dependent driving influence of the suprachiasmatic nucleus on adrenal sensitivity to ACTH

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Sage, Dominique, Daniel Maurel, and Olivier Bosler. Corticosterone-dependent driving influence of the suprachiasmatic nucleus on adrenal sensitivity to ACTH. *Am J Physiol Endocrinol Metab* 282: E458–E465, 2002. First published October 2, 2001; 10.1152/ajpendo.00287.2001.—We investigated the effects of ablation of the suprachiasmatic nucleus (SCN) on corticosterone (CORT) responses to synthetic ACTH given in either the morning or evening. After dexamethasone treatment, evening ACTH injections in intact rats produced a significantly larger increase in plasma CORT compared with morning ones. In rats with SCN lesions, the ACTH-induced CORT secretion was independent of time of day, providing direct evidence for a driving influence of the SCN on the diurnal rhythm of adrenal sensitivity to ACTH. In the absence of dexamethasone treatment, the SCN-lesioned rats were selected for morning-like (ML) or evening-like (EL) basal levels of CORT. Responses to ACTH were not different in ML rats compared with sham-lesioned morning controls. In contrast, EL rats compared with sham-lesioned evening controls showed an ~60% decrease in increment of CORT levels within the first 15 min postinjection. These results indicate that the SCN upregulates ACTH sensitivity of the adrenal cortex during the ascending phase of the daily CORT secretion and point to a critical role of glucocorticoids in determining SCN action.

adrenocorticotropin hormone; hypothalamic-pituitary-adrenal axis; circadian rhythm; glucocorticoids; adrenal cortex

THE CIRCADIAN RHYTHM of circulating glucocorticoids, characterized by the occurrence of peak levels around the beginning of the daily activity cycle, is normally in phase with the rhythm of adrenocorticotropic hormone (ACTH) secretion. However, diurnal fluctuations in plasma ACTH are of low amplitude and are frequently not significant over a 24-h period (27), so that amplification of this rhythmic signal is necessary to yield a pronounced adrenal rhythm. It has been shown in rats that such an amplification is provided by an increased adrenal sensitivity to ACTH at the peak of the corticosterone (CORT) rhythm, i.e., at lights off in nocturnal rodents (6, 7, 9, 25, 34).

Several studies collectively showed that diurnal changes in adrenocortical sensitivity occurred independently of changes in ACTH. The first demonstration was provided by Dallman et al. (9), who observed, under resting conditions, a persistent adrenal rhythm in dexamethasone-treated rats with no ACTH rhythm and, conversely, persistent diurnal ACTH fluctuations in the absence of adrenal rhythm after treatment with parachlorophenylalanine. Accordingly, changes in plasma glucocorticoids in response to stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis can occur in the absence of concomitant changes in plasma ACTH (2, 5, 16, 50), which provides strong support for the implication of extra-ACTH mechanisms in the driving of rhythmic adrenal responsiveness to ACTH. Diurnal changes in ACTH receptor affinity or configuration on the adrenocortical cell membrane, or in coupling of the receptor with adenylate cyclase, have been proposed as possible mechanisms (26). There are also experimental data to support the view that a mechanism may exist whereby adrenal sensitivity to ACTH is regulated by the functional innervation of the adrenal cortex. Denervation of the adrenal glands did abolish the CORT rhythm (35). Moreover, bilateral section of the splanchnic nerves reduced cortisol secretion in response to exogenous or endogenous ACTH in lambs and calves (11, 13), whereas stimulation of the nerves enhanced cortisol secretion when ACTH was given to hypophysectomized calves and dogs (12, 15).

The present study was designed to explore the involvement of the suprachiasmatic nucleus (SCN) of the hypothalamus, the master component of the circadian timing system generating rhythmic activity of the HPA axis, in driving the diurnal rhythm in adrenal responsiveness to ACTH stimulation. The effects of SCN lesions on the morning (AM) vs. evening (PM) response of the adrenal cortex toward exogenous ACTH was first investigated in rats whose adrenals were deprived of endogenous ACTH after treatment with dexamethasone. To further investigate whether the secretory activity of the adrenal cortex at the time of the ACTH challenge might be critical in determining SCN involvement, as previously found regarding the adrenocortical response to stress (41), experiments were also undertaken in rats not subjected to dexamethasone pretreatment.

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MATERIALS AND METHODS

Experimental animals. Sprague-Dawley rats (Dépré, Saint-Doulchard, France) were housed individually in temperature- (21°C) and humidity-controlled rooms, on a 12:12-h light-dark cycle (lights on at 7 AM). They were allowed to adapt to this environment at least 3 days 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 histological controls were carried out under deep equithesine anesthesia (solution of 4.6% chloral hydrate and 0.96% pentobarbital sodium). 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.

Surgery. Thirty rats were submitted to SCN lesion by electrocoagulation, and 20 rats were submitted to sham operation, as previously described (41). Briefly, stainless-steel electrodes 0.2 mm in diameter with an uninsulated tip of <0.25 mm (Phymep, Paris, France) were lowered bilaterally into the base of the brain at the expected site of the SCN, and an anodal current of 0.8 mA was applied for 20 s on each side. The following coordinates, adapted from the Paxinos and Watson atlas (38), 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 (incisor bar: −3.3 mm). Sham-lesioned rats were prepared by lowering the electrodes onto the appropriate coordinates but not applying any current.

One month after lesion or sham surgery and 4 days before the experiments, all rats were subjected to intracarotid cannulation with polyethylene tubing (PE-50), in accordance with Szafarczyk et al. (42). Every day for the following 3 days, the cannulas were flushed with a heparin-saline solution. Two hours before experiments, an additional piece of PE-50 tubing, ~20 cm in length, was connected to the external end of the cannula to permit intracarotid injections and subsequent blood sampling while the animals were moving freely in their cages.

Experiments. Two separate studies were carried out. *Study 1* was designed to evaluate the effect of the SCN lesions on morning (AM) vs. evening (PM) adrenal CORT responses to ACTH. It involved 14 SCN-lesioned and 11 sham-operated rats. All of them were pretreated 12 h and 2 h before the experiment with dexamethasone to block the basal release of CORT (10 μg/kg body wt ip in 0.9% NaCl containing 0.001% absolute ethanol). Three hundred microliters of synthetic $ACTH_{1-24}$ (Synacthen, Novartis, 3 ng/rat) diluted in 0.9% NaCl (10 ng/ml) were injected through the cannula at "zeitgeber time" 2 (ZT 2) in the AM (SCN lesioned: $n = 7$; sham operated: $n = 6$) or at ZT 14 in the PM (SCN lesioned: $n = 7$; sham operated: $n = 5$). The PM group was injected under constant dim red light of <3 lux.

In *study 2*, the effect of SCN lesions was assessed as a function of basal CORT levels irrespective of the time of day of the injections. We used 16 SCN-lesioned and 9 sham-operated rats that were not pretreated with dexamethasone. The former received Synacthen at ZT 2 ($n = 16$), and sham controls were injected either in the AM (ZT 2, $n = 4$) or in the PM (ZT 14, $n = 5$). With previous results showing in SCN-lesioned rats that several maxima and minima of CORT release occurred with no relation to time of day over the 24-h cycle (41) taken into account, these rats were separated into two groups a posteriori according to their basal CORT levels at the time of injection ($t = 0$).

Blood sampling and assays. Basal levels of ACTH and corticosterone were measured in blood samples (0.3 ml) taken just before ACTH injection ($t = 0$). Further samples were collected at $t = 15$, $t = 30$, $t = 45$, and $t = 60$ min to evaluate plasma ACTH levels restored by the exogenous corticotropic hormone and induced CORT release. Samples were kept on ice until centrifuged, and the plasma was stored at −20°C until the assay.

CORT was measured in 20-μl plasma samples after extraction with absolute ethanol by means of the radioimmunoassay method described by Conte-Devolx et al. (8). 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 with the use of a radioimmunoassay kit (ACTH RIA kit, France Biochem, Meudon, France). The antiserum used had 100% cross-reactivity with human $ACTH_{1-24}$ and rat $ACTH_{1-39}$. It 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. To evaluate the effectiveness and selectivity of the lesion, histological controls were performed using vasoactive intestinal peptide (VIP) and arginine vasopressin (AVP) immunostains as markers for the SCN. We eliminated all rats subjected to SCN ablation, which nevertheless showed remnant AVP or VIP neurons at the expected place of the SCN, as well as those with a damaged optic chiasm. On this basis, 17 SCN-lesioned rats (*study 1*: $n = 3$ in the AM and $n = 5$ in the PM; *study 2*: $n = 9$) and all 20 rats subjected to sham operation (*study 1*: $n = 11$; *study 2*: $n = 9$) in which the presence of intact SCNs was confirmed were included in the analysis.

We evaluated both the kinetics of ACTH concentration in plasma and CORT responsiveness of adrenals after onset of the Synacthen pulse by using three physiological parameters: *1*) profiles of ACTH concentration and CORT response curve over the 60-min postinjection period, *2*) increments of the plasma levels of ACTH and CORT within the first 15 min postinjection (Δ-rise), and *3*) areas under the curves (AUC) as reflections of overall ACTH restoration in plasma or overall CORT secretion within the 60-min postinjection period. All results were expressed as means ± SE.

A one-way analysis of variance (ANOVA) test with factorial measures was used to determine the effects of time of day and SCN lesion (*study 1*) and the influence of basal CORT levels and SCN lesion (*study 2*) on Δ-rises and AUC values. 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 or ML basal CORT vs. EL basal CORT levels; "treatment effect": SCN-lesioned vs. sham-operated rats). When a significant effect was detected, a protected least significant difference Fisher post hoc procedure was applied to assess the specific points in time when the difference occurred. Significance was defined as $P < 0.05$.

RESULTS

Time course of ACTH distribution in plasma after the pulse of exogenous ACTH. Plasma ACTH levels before the ACTH pulse were lower in rats pretreated with dexamethasone (52 ± 7 pg/ml) than in untreated rats (80 ± 10 pg/ml), as expected from an effective blockade of endogenous ACTH secretion by the glucocorticoid agonist. Profiles of the ACTH distribution curves over

the 60-min postinjection period as well as Δ- and AUC values were not statistically different between the SCN-lesioned and sham-operated rats or between each experimental group in either study. Neither did the two-way ANOVA test reveal any group or treatment effect when the rats of *study 1* were compared with those of *study 2* (Table 1). The kinetics of ACTH concentrations within plasma in *study 1* and *study 2* could then be traced after pooling averaged data points obtained at each 15-min interval for all lesioned and sham animals in each study (Fig. 1). Concentrations abruptly increased to 130 ± 12 pg/ml in dexamethasone-treated rats and to 162 ± 18 pg/ml in untreated rats within 15 min of the ACTH injection. They remained stable until 30 min and then declined slowly to steady-state levels.

Diurnal variations in CORT responses to ACTH challenge in dexamethasone-treated rats: effect of the SCN lesion. In control rats, basal CORT levels at the time of the ACTH pulse were very low in both AM (21 ± 5 ng/ml) and PM (24 ± 3 ng/ml), confirming the efficacy of the dexamethasone treatment (Fig. 2*A*). Synacthen injections resulted in significant increases that were much more abrupt in the PM than in the AM. At both times of day, maxima were reached within 15 min, and levels declined after 30 min. The Δ-rises in CORT levels were two times higher in PM rats compared with AM rats ($P = 0.04$), and this was also the case for AUC values ($P = 0.01$), due to the fact that PM plasma concentrations remained much higher from 30 min ($P = 0.04$) to 60 min ($P = 0.001$) postinjection.

Table 1. *Variations of ACTH and corticosterone (CORT) concentrations in plasma increments (Δ) within 15 min after intracarotid injection of Synacthen (3 ng/rat) and areas under the curves (AUC) calculated for the ACTH distribution and the CORT responses within 60 min in rats pretreated or not with dexamethasone*

	ACTH		CORT	
	Δ, pg/ml	AUC, ×10²	Δ, ng/ml	AUC, ×10²
Dexamethasone-treated rats				
AM controls	83 ± 9	60 ± 5	72 ± 23*	41 ± 11*
PM controls	98 ± 22	70 ± 6	152 ± 29	85 ± 13
AM SCN-x	98 ± 5	64 ± 2	88 ± 32	65 ± 11
PM SCN-x	109 ± 17	70 ± 10	101 ± 31	84 ± 9
Untreated rats				
AM controls	76 ± 6	69 ± 4	72 ± 15	60 ± 11*
PM controls	82 ± 27	89 ± 9	126 ± 16	145 ± 22
ML SCN-x	80 ± 32	73 ± 7	83 ± 43*	55 ± 9
EL SCN-x	81 ± 19	88 ± 8	49 ± 24*	159 ± 33

Values are means ± SE, (AUC: arbitrary units). Suprachiasmatic nucleus (SCN)-lesioned (SCN-x) pretreated rats that were submitted to the ACTH pulse either at zeitgeber time (ZT) 2 in the morning (AM) or at ZT 14 in the evening (PM) are compared with their respective controls. The untreated SCN-lesioned rats are referred to as either "morning-like level" (ML) rats compared with AM controls or as "evening-like level" (EL) rats compared with PM controls according to their basal secretion of CORT at the time of the ACTH pulse. *$P < 0.05$ vs. PM controls.

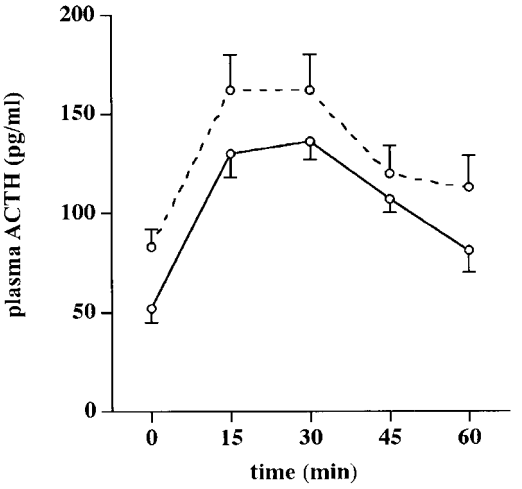

Fig. 1. Time course of ACTH distribution in plasma after intracarotid pulse injections of Synacthen ($t = 0$) in rats pretreated (solid line, $n = 19$) or not (broken line, $n = 18$) with dexamethasone. Data from suprachiasmatic nucleus (SCN)-lesioned and sham-operated rats injected either in the morning (AM) or in the evening (PM) are pooled in each curve.

Accordingly, the ANOVA test clearly showed a significant group effect when both CORT response curves were considered ($P = 0.01$).

In SCN-lesioned rats, basal CORT levels tended to be higher in the PM but remained low at both times of day (69 ± 35 ng/ml in the PM, $n = 5$, vs. 31 ± 23 ng/ml in the AM, $n = 3$), compared with normal values obtained in non-dexamethasone-treated rats (see further, *study 2*) (Fig. 2*B*). After synacthen injections, maximal CORT responses were again obtained within the first 15–30 min. Plasma CORT concentrations declined thereafter to reach values that were in the same range at the 60-min time point (Fig. 2*B*). In contrast to control rats, the ANOVA test did not show any statistical difference between either the Δ-rises in CORT levels ($P = 0.78$) or the AUC values ($P = 0.31$) at both times of day (Table 1). Accordingly, the AM and PM response curves were also not statistically different ($P = 0.25$) (Fig. 2*B*).

Although the SCN lesions tended to increase Δ-rises and AUC values for CORT in the AM (Table 1), they did not appear to significantly modify the kinetics of AM and PM CORT responses to the ACTH; i.e., we found no treatment effect and no treatment × group interaction when we compared SCN-lesioned and control rats.

CORT responses to ACTH challenge in relation to basal levels of circulating CORT in non-dexamethasone-treated rats: effect of the SCN lesion. In control rats, CORT levels as measured just before the ACTH injection were much higher in the PM than in the AM (245 ± 51 ng/ml vs. 61 ± 7 ng/ml), as expected (Fig. 3*A*). The ACTH-induced CORT secretion clearly peaked at 15 min at both times of day. The increase was more abrupt in the PM, and the Δ-rise did tend to be higher at this time (Table 1). However, the AM/PM differences did not reach statistical significance. CORT

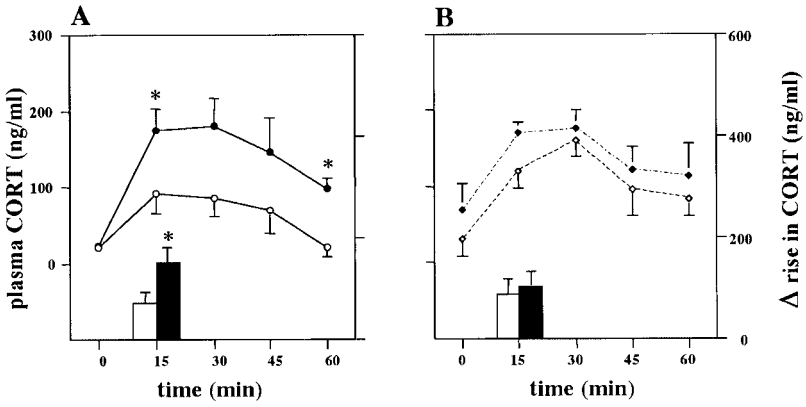

Fig. 2. Plasma corticosterone (CORT) responses to the exogenous ACTH given to control rats (*A*; $n = 11$) or SCN-lesioned rats (*B*; $n = 8$) at $t = 0$ in the AM (○, sham: $n = 6$; lesioned: $n = 3$) or PM (●, sham: $n = 5$; lesioned: $n = 5$) after treatment with dexamethasone. Histograms inserted at the base of the graphs show increments of the CORT secretion (Δ-rise) within the first 15 min after injection in the AM (open bars) or PM (filled bars). $^*P < 0.05$ vs. morning rats.

concentrations also decreased much more rapidly in PM rats than in AM rats and reached levels in the range of basal AM levels at $t = 60$ min. Consequently, AUC values were significantly higher in the PM (Table 1, P = 0.02). The ANOVA test also revealed a clear group effect (P = 0.01), meaning that the patterns of CORT responses were significantly different at both times of day.

As expected from our previous observation that the SCN lesion induced a lack of synchronization regarding the time at which individual CORT peaks occurred over the 24-h cycle (41), basal CORT concentrations ranged from 38 to 373 ng/ml, even though all samples were collected in the AM, when the secretory activity of the adrenal cortex is low in normal controls. Four rats had CORT levels in the range of AM mean concentra-

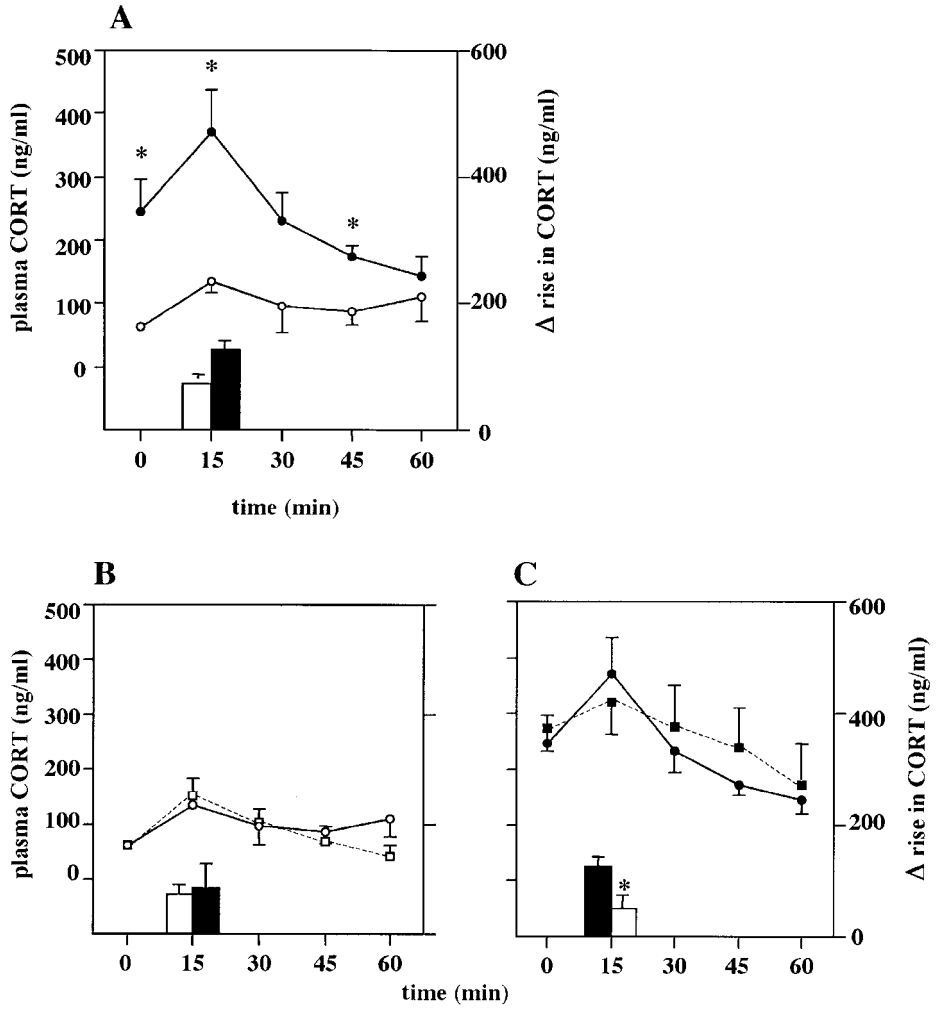

Fig. 3. Plasma CORT responses to exogenous ACTH given at $t = 0$ in non-dexamethasone-treated rats. *A*: effect of time of day in control rats (AM: ○, $n = 4$; PM: ●, $n = 5$). $^*P < 0.05$ vs. AM rats. *B* and *C*: effect of SCN lesion in relation to basal concentrations of CORT in plasma at the time of the ACTH pulse, as evaluated after reassignment of each lesioned rat to either a "morning-like level" group (ML rats, $n = 4$) or an "evening-like level" group (EL rats, $n = 5$), irrespective of whether it had received ACTH in the AM or in the PM (see text). The ML and EL rats (broken lines) are compared with AM controls (*B*) and PM controls (*C*), respectively, shown in *A* (solid lines). Histograms illustrate increments of the CORT responses (Δ-rise) within the first 15 min after injection (open bars: AM controls in *A* and *B* and EL rats in *C*; filled bars: PM controls in *A* and *C* and ML rats in *B*). $^*P < 0.05$ vs. evening controls.

tions measured in normal rats in *study 1* (21 ± 5 ng/ml) and *study 2* (61 ± 7 ng/ml); i.e., they were below the threshold value of 98 ng/ml, which corresponded in the aforementioned study to the 50th percentile of the distribution of SCN-lesioned rats. These rats were referred to as "morning-like level" rats (ML group, mean basal levels: 60 ± 8 ng/ml). The five remaining rats with basal CORT levels above the cut-off concentration (98 ng/ml) were referred to as "evening-like level" rats (EL group, mean basal levels: 273 ± 42 ng/ml).

When a comparison was made between ML rats and AM controls, the ANOVA test did not reveal any statistical difference, when the profiles of the ACTH-response curves, which appeared to be almost superimposable (Fig. 3B), and also the Δ-rises in CORT secretion and the overall responses (AUC values) (Table 1) were taken into account. In contrast, it turned out that the SCN lesion induced, in EL rats compared with PM controls, a significant decrease of ~60% in the increment of CORT secretion (Δ-rise) in response to exogenous ACTH (Table 1). Profiles of the curves (Fig. 3*C*) and AUC parameters (Table 1) were similar in the experimental groups.

DISCUSSION

Involvement of the SCN in the nycthemeral rhythm in adrenal sensitivity to ACTH was investigated by testing the effect of an SCN lesion on AM vs. PM CORT secretion induced by exogenous ACTH in both dexamethasone-treated and untreated rats. In treated rats, the low plasma concentrations of ACTH and CORT, measured before the ACTH pulse, as well as the lack of a daily rhythm in secretion of both hormones, accounted for an effective blocking of HPA axis activity.

In keeping with a previous report (51), ACTH pulses restored plasma ACTH levels that were in the range of nocturnal peaks at the 15- and 30-min time points and slowly decreased thereafter. It was important to assess that they resulted in physiological concentrations of ACTH at both times of day, especially because it has been reported that pharmacological doses of synthetic ACTH can block adrenal sensitivity to the hormone (26). In both lesioned and sham-operated rats, pretreated or not with dexamethasone, plasma CORT levels also abruptly increased in response to the ACTH challenge in either the AM or the PM, indicating that neither the SCN ablation nor the blockade of the HPA axis altered the capacity of adrenals to respond to increases in plasma ACTH levels. We found that the increment of CORT-induced release, as well as overall CORT secretion over the 60-min postinjection period, was about two times higher in the PM than in the AM in all control animals. This result is congruent with previous reports documenting the rhythm of adrenal sensitivity to ACTH in rats (9, 26, 32). The fact that the adrenals retain a greater capacity to respond to the ACTH challenge in the PM after dexamethasone treatment reemphasizes that the diurnal rhythmicity of adrenal secretion is not dependent on, albeit in phase with, the plasma ACTH rhythm (9, 26, 32, 49).

Our data showing that the SCN lesion caused the AM/PM differences in adrenal responsiveness to exogenous ACTH to disappear constitute the first evidence of the critical role played by the SCN in driving the daily rhythm in adrenal sensitivity to the pituitary hormone. This result was not unexpected, as it is known that the adrenal sensitivity rhythm can be entrained by the photoperiod (36); it is consistent with data from a previous study showing that hypothalamic lesions that prevented CORT responses to known stimuli induced responses to ACTH that were essentially identical in the AM and the PM (25). Interestingly, the lesioned rats in that study also showed a reduced metabolic clearance rate of CORT, raising the possibility that the SCN may also have an effect on the clearance of CORT from plasma. This could account for the fact that, in other studies involving selective lesions of the SCN, mean plasma CORT levels in the lesioned rats usually remained in the high range in both the AM and the PM (3, 42), whereas adrenal contents were in the range normally observed for morning values (33).

Comparisons between the SCN-lesioned rats and their respective controls showed that the SCN exerts opposite regulatory roles with regard to adrenal responsiveness to ACTH in the AM and the PM. First, after SCN ablation, the increment of ACTH-induced CORT secretion tended to be increased in the AM, together with the overall CORT response, and decreased in the PM. Moreover, in experiments involving rats not pretreated with dexamethasone, we found a highly significant decreasing effect of the SCN lesion on the adrenal responsiveness of rats with plasma CORT levels in the high range at the time of the ACTH pulse (EL rats) compared with sham-operated rats with comparable concentrations of circulating CORT at the same time. This result not only confirms that the SCN upregulates adrenal responsiveness to ACTH in the PM but also indicates that CORT circulating levels are critical determinants of SCN involvement. This conclusion is in line with the results of our previous study (41) showing that the SCN differentially regulates the stress response depending on the underlying secretory activity of the adrenal cortex. In that study, we provided evidence that the SCN exerts a facilitatory influence on the stress response at the circadian peak of CORT secretion, presumably through adjustment of the response of adrenals to ACTH. This hypothesis is strongly supported by the present results.

On the basis of previous data showing that the magnitude and time course of the adrenal cAMP response to ACTH are increased and prolonged at lights off (9), it is conceivable that the SCN drives changes in adrenal sensitivity through regulation of diurnal changes in the number, affinity, or configuration of ACTH receptors on the adrenocortical cell membrane and/or in intracellular transduction mechanisms (see also Ref. 26). However, a great deal of anatomical evidence has accumulated showing that adrenocortical secretion is regulated not only by systemic factors such as ACTH but also by direct neural influences (18, 37). Neural inputs are thought to account for the apparent disso-

ciation between the rhythm in ACTH and the rhythm in plasma corticosteroids and responsiveness of adrenocortical cells to ACTH (9, 26, 32, 49). One set of fibers supplying the adrenal cortex is derived from adrenal medullary ganglion cells; the other fibers issue from sympathetic and parasympathetic neurons that synthesize norepinephrine (NE), acetylcholine (ACh), and various peptide transmitters (46). These neural inputs are the last link in the polysynaptic SCN-adrenal connection relaying in the paraventricular nucleus of the hypothalamus and the intermediolateral column of the spinal cord, through which the SCN may partly drive the rhythm in adrenal sensitivity to ACTH (4, 43, 48).

The physiological relevance of adrenal innervation in the regulation of corticosteroid secretion has been extensively documented (46). Evidence derives mainly from experiments investigating the effects of stimulation or section of the splanchnic nerves, known to constitute a major source of direct or indirect input to the adrenal cortex, on adrenocortical function (14). These effects were shown to result, in part, from a modulation of adrenal responsiveness to ACTH (10, 15), which was consistent with the fact that no daily rhythm regarding adrenal sensitivity to ACTH can be observed in the absence of adrenal innervation (20). It has been shown in the rat that the splanchnic neural input exerts a tonic inhibitory influence on CORT secretion at the nadir of the circadian rhythm (19). This is consistent with the lower responsiveness to ACTH found in the AM, as well as with the known AVP-mediated inhibition triggered by the SCN as concerns corticosteroid secretion initiated by light-induced activation of hypothalamic neurons (3, 5, 22). Conceivably, in the ascending phase of the daily corticosterone secretion (PM), lower release of AVP by SCN efferents, associated with increase of stimulatory messages (23, 24), could result in progressive suppression of splanchnic tonic inhibition, accounting for restoration of maximal adrenal responsiveness to ACTH and amplification of the plasma corticosterone rhythm.

The data that we obtained after separate analysis of the SCN-lesioned rats having steady-state plasma levels of CORT in either the AM range (ML rats) or the PM range (EL rats) strongly suggest that glucocorticoids are important regulators of SCN signals and/or of the response of adrenals to these signals. That glucocorticoids may influence SCN functioning at the central level, presumably through indirect mechanisms (31), is indicated by experimental data showing that they regulate the diurnal expression of AVP and VIP in the SCN (29). Their modulatory action in the SCN-dependent setting of adrenal sensitivity to ACTH may also be the result of a peripheral action. There is experimental evidence in support of the contention that glucocorticoids interact with ACTH receptors on adrenocortical cells (39). Conceivably, they may also interact with SCN messages through negative feedback regulation of an intraglandular mini-HPA axis (21, 40, 46). In support of the latter possibility, it has been shown that modulation of the adrenocortical sensitivity to ACTH stimulation is a major effect of intra-adrenal corticotropin-releasing hormone (CRH) (45) and that the adrenal content of CRH is significantly reduced by cortisol treatment in rats (1).

Another hypothesis is that glucocorticoids exert a local feedback control of adrenocortical secretion through interactions with other aminergic and peptidergic neural inputs. Although adrenal neurotransmitters undoubtedly have direct effects on adrenocortical cells that presumably provide fine tuning of the responses of adrenals to ACTH, their main target is known to be the vasculature of the gland (47). Through regulation of arteriolar diameter, they may alter blood flow within the inner cortex, thereby acting on steroidogenesis by changing the presentation rate of ACTH (28, 44). There is substantial evidence that such a mechanism plays a critical role in the regulation of adrenal sensitivity to ACTH (14), indicating that the main targets for the action of glucocorticoids, as concerns regulation of the SCN-dependent responses of adrenals to ACTH stimulation, are the nerve supplies regulating blood flow.

A variety of peptide mediators with known effects on adrenal vascular tone are released from these nerves and act in concert with NE and ACh. The most likely to be involved in mediating the increased adrenal blood flow following splanchnic nerve stimulation is VIP, a potent vasodilatator agent (17). Interestingly, VIP is partly colocalized with neuropeptide Y, a peptide with opposite vasoconstrictor effects that is also colocalized with catecholamines in fibers associated with blood vessels (30) and causes a significant decrease in the rate of perfusion medium flow through the gland (17). It is then conceivable that different SCN-dependent effects on cortical vasculature and, hence, on steroid secretion, could be obtained, depending on the respective concentrations in amine and peptide transmitters released from the same or different endings. According to this view, the circadian fluctuations in plasma glucocorticoid levels could adjust adrenal sensitivity to ACTH through differential regulation of the expression and/or release of each mediator. A similar mechanism could partly account for our previous results showing that the underlying basal secretory activity of the adrenal cortex is also a critical determinant of the CORT response to stressful stimuli (41).

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