Suprachiasmatic nucleus lesion increases corticosterone secretion

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Buijs, Ruud M., Andries Kalsbeek, Tjitske P. van der Woude, Joop J. van Heerikhuize, and Susan Shinn. Suprachiasmatic nucleus lesion increases corticosterone secretion. *Am. J. Physiol.* 264 (*Regulatory Integrative Comp. Physiol.* 33): R1186–R1192, 1993.—The diurnal rhythm of corticosteroid secretion is controlled by the suprachiasmatic nucleus (SCN). In rats, plasma corticosteroid levels rise just before the onset of the activity period during the dark phase. Our previous results indicated that vasopressin as a neurotransmitter from the SCN inhibited corticosteroid secretion in the area of the paraventricular/dorsomedial nucleus of the hypothalamus. We hypothesized that during the day the SCN may serve as an inhibitory system for corticosteroid secretion. To investigate this possibility, intact and SCN-lesioned animals were exposed to mild stress in the morning and evening and their plasma corticosteroid levels were monitored. The results indicate that SCN-lesioned animals have higher morning corticosteroid levels and respond both in the morning and evening with higher corticosteroid levels after stress than do intact control animals. We conclude, therefore, that these results indicate an inhibitory role of the SCN on corticosteroid secretion. The apparent discrepancy with the reported stimulatory role of the SCN on adrenocorticotropic hormone secretion is discussed.

circadian rhythm; vasopressin; vasoactive intestinal peptide; diurnal; paraventricular nucleus

SITUATED IN THE PARAVENTRICULAR nucleus of the hypothalamus (PVN) are neuroendocrine "motor" neurons that release corticotropin-releasing factor (CRF) together with vasopressin (VP) in the portal system of the median eminence. Thus adrenocorticotropic hormone (ACTH) is liberated in the general circulation resulting in the release of corticosteroids from the adrenal gland (17, 27). To understand the mechanisms that control the PVN and, e.g., ACTH and corticosteroid release, the neuronal systems that have an input to the PVN have been the focus of a considerable research effort (27). One of these systems is the noradrenergic system from the brain stem that directly innervates the CRF-containing neurons of the PVN. This hind brain ascending system seems largely responsible for the stress-activated corticosteroid secretion (28).

There is considerable evidence that the diurnal circadian variations in corticosteroid secretion stem from the suprachiasmatic nucleus (SCN) (1, 21, 23), although the pathways and mechanisms that the SCN uses to regulate corticosteroid secretion are far from understood.

The SCN has been established as the major pacemaker of the mammalian brain and is able to maintain its own nearly 24-h rhythm under various circumstances, even when isolated in vitro (for review, see Refs. 2 and 22). Numerous lesioning, transplantation, and in vitro studies have provided evidence that the SCN is able to confer its endogenous rhythm onto the central nervous system (CNS). Thus the SCN has been shown responsible for the diurnal rhythm in corticosteroid secretion, which peaks in nocturnal animals just before the dark (the activity) phase of the light-dark cycle (7, 15, 17, 21). Lesioning the SCN results in the disappearance of the rhythm in corticosteroid secretion together with other overt behavioral circadian rhythms (1, 21, 32). However, whether the SCN is the sole responsible structure for the circadian corticosterone secretion is still disputable, because animals kept on a restricted food regime were able to anticipate the moment they received food with a corticosterone surge (20).

The SCN on its own is able to maintain a circadian cycle of approximately 12 h of activity and the same period of inactivity, as has been demonstrated by measuring deoxyglucose, recording electrical activity, and determining VP release, both in vivo and in vitro (for review, see Refs. 2 and 22). The mRNAs of VP and vasoactive intestinal peptide (VIP), two of the primary peptides synthesized in the SCN, are also produced in a circadian cycle that, for VP, has been demonstrated to reflect a 12-h high-secretion period alternating with a 12-h low-secretory period (2, 24). This led us to propose that the SCN, by way of its projections in other areas of the brain and by a periodic release of its transmitters in these target structures, may influence the level of activity in these areas, and hence, circadian rhythmicity. A number of anatomic studies with different approaches have indicated that one of the main target areas of the SCN is located in the hypothalamus and covers the area from just ventral of the PVN to caudal of the PVN, the subparaventricular zone and the dorsomedial nucleus of the hypothalamus (DMH), respectively (3, 13, 25, 33). Recently, we provided evidence that VP, as a neurotransmitter from the SCN, when released in this region of the PVN/DMH, may inhibit corticosterone secretion. Infusion into the PVN/DMH of VP or VP antagonist depressed or elevated, respectively, the levels of plasma corticosterone (14). Since VP seems to be released from the SCN during the light period (24), we proposed that via this neurotransmitter the SCN may have an inhibitory influence on activity and corticosterone secretion during the light period. Other SCN transmitters released at the same or other times during the light-dark cycle may also influence corticosteroid secretion. In view of the VP effect, we, however, hypothesized that the SCN in general may serve more during the light than during the dark period (when the release of VP is much lower) as an inhibitory system for corticosteroid secretion. To investigate this hypothesis, in the present paper a set of experiments is described in which the

SCN was lesioned, and the effect of the lesion on corticosterone secretion was investigated during the early and late light period using novelty stress as an inducer of corticosteroid release.

MATERIALS AND METHODS

Male Wistar rats of 175–200 g housed individually in humidity- and temperature-controlled 12:12-h light-dark cycle rooms had food and water available ad libitum. All of the following animal experiments were conducted under the approval of the Loeb Research Animal Care Committee.

A bilateral lesion of the SCN was carried out under Hypnorm or Innovar Vet anesthesia (Duphar or Janssen; 0.4 ml/kg im). Animals were mounted in a David Kopf stereotact (tooth bar + 5) and sustained an SCN lesion using bilateral lesion electrodes, 0.2 mm diam, with temperature set at 85°C for 1 min (lesion generator, Radiotronics). This temperature was found empirically to result in lesions big enough to eliminate the SCN bilaterally, but small enough to leave the PVN and surrounding tissue intact (Fig. 1 and 2). In the following 3–5 wk, the effectiveness of the lesion was checked continuously by measuring water intake during the light vs. the dark period. If animals drank between 40 and 60% of their daily water consumption during the light period, then they were considered as SCN-lesioned animals and were used for further study. Having reached a weight of 300 g, the arrhythmic animals were implanted with a permanent silicone catheter in the jugular vein, again under Hypnorm or Innovar Vet anesthesia. After this operation animals were handled twice a day for 5 days a week until the end of the experiment. For the control group, intact animals were used that underwent the same surgery without heating the electrodes; these animals also received the jugular vein catheter after reaching 300 g in weight. Typically these animals drank between 0–5% of their daily water consumption during the light period. The experiment started 1 wk after the last operation. Once a day, either in the morning or in the evening, a total of up to four animals, both control and lesioned, were used in the experiments. At the beginning of the experiment (2 h after light on, or 2 h before light off), the animal was removed from its home cage, connected with a PE-20 polyethylene tube to the venous catheter and transferred to a clean experimental cage (25 × 25 × 30 cm). A blood sample (0.1 ml) was taken within 1 min after taking the animal from its home cage and subsequently at 20, 40, 60, 80, 100, and 120 min after transferring the animal to the experimental cage. The same amount of saline solution (0.1 ml) was replaced intravenously each time after blood withdrawal to make up for the loss of volume.

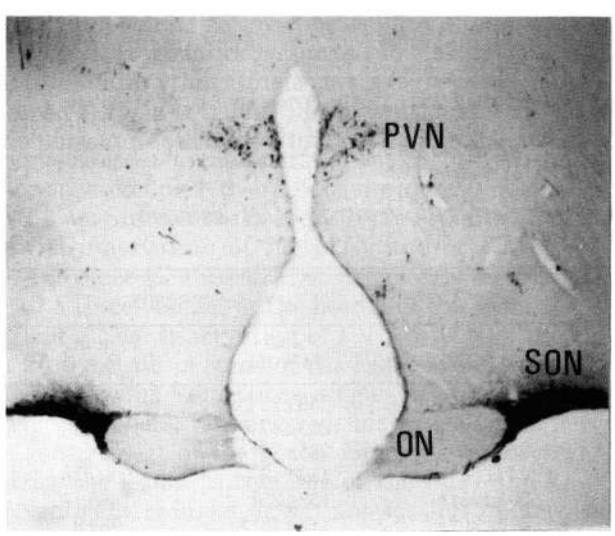

Fig. 1. Size of a complete lesion of suprachiasmatic nucleus (SCN) in a transversal section of hypothalamus stained for vasopressin (VP). Note the relatively small size of the lesion, leaving the supraoptic (SON) and paraventricular nucleus (PVN) intact. ON, optic nerve.

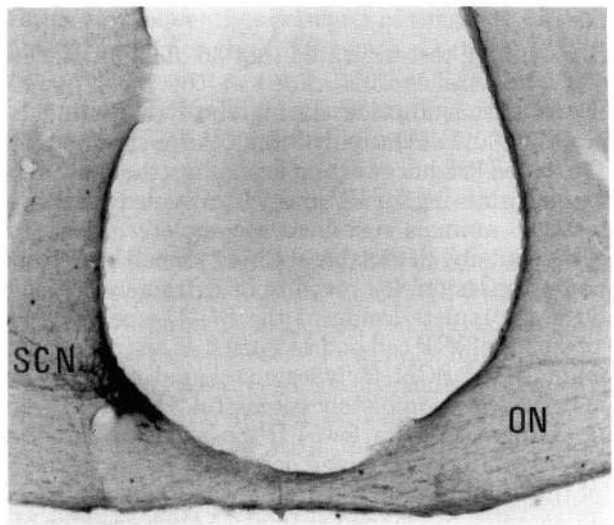

Fig. 2. Lesion that left a tiny part of the SCN intact. Section is stained for vasoactive intestinal peptide (VIP); lesions like this were labeled as partial SCN lesions.

The blood samples were collected in heparinized tubes placed on ice and centrifuged, and the plasma was stored at −20°C until assay. Plasma corticosterone was measured directly without extraction using a radioimmunoassay from ICN Biomedicals (Costa Mesa, CA) with iodinated corticosterone. The interassay coefficient of variation of a 50 ng/ml sample was 12.3%, the intraassay coefficient of variation was 10.2%.

The experiments with each animal were started in a random order, one session in the morning and one in the evening with at least 1 wk between sessions. If the blood catheter permitted, another series of experiments were conducted to investigate the effect of repetition. After the completion of the blood withdrawal experiment the animals were perfused intracardially under deep pentobarbital anesthesia with 4% paraformaldehyde. After 48–72 h postfixation, the hypothalamus was sectioned on a Vibratome, and 50-μm sections were stained for VP or VIP alternatingly. VP and VIP staining was examined in the SCN area and the region of the PVN-DMH. If animals had cell bodies that stained positively for either VP or VIP in the region of the SCN or around the border of the lesion, then they were considered as partial SCN lesions.

Student's t tests on independent measures were used to test the differences between unstressed plasma corticosterone levels of control, SCN-lesioned, and partial SCN-lesioned animals. The response time patterns of plasma corticosterone were evaluated with analysis of variance (ANOVA) followed by the Newman-Keuls post hoc test. Within groups differences between the morning and evening session were analyzed by a two-way ANOVA with both time of day (morning to evening) and sampling time as repeated measures within subject factors (2 levels and 7 levels, respectively). Differences between the three lesion groups during either the morning or evening session were analyzed with a two-way ANOVA (lesion group vs. sample time) followed by a Newman-Keuls post hoc test. To find within treatment groups which time points differed from *time 0* values, a one-way ANOVA was performed, and, if F values were appropriate, this was followed by a Newman-Keuls.

RESULTS

The drinking test indicated that 36 of 53 animals that received bilateral lesions aimed at the SCN increased their water consumption during the light period from 0–5% to 40–60% of the daily intake. After the completion of the blood withdrawal experiments, the immunocytochemical staining for VP and VIP revealed that only 14 of these 36 animals sustained a complete lesion of the SCN without any detectable staining for cell bodies at the border of the lesion. It proved to be extremely difficult to produce a complete lesion of the SCN, especially when the presence of VIP cell bodies is used as an indication for remnants of the SCN. Only lesions that also damaged the optic nerve were completely successful. However, even in some of these cases, a few VIP-positive (or sometimes VP-positive) fibers remained detectable either at the border of the lesion or in the region of the PVN or DMH. Of the 14 completely lesioned animals, 5 were used for blood withdrawal both in the morning and evening, whereas in the other 9 animals the jugular venous catheter became plugged before a second blood withdrawal experiment could be performed.

Both the control and the SCN-lesion group showed a highly significant effect of sampling time ($P < 0.001$), but not the partially lesioned animals ($P = 0.41$). Control animals showed a clear time of the day effect ($P < 0.01$), but not animals of the SCN-lesioned group ($P = 0.055$).

Basal plasma corticosterone levels of control animals during the late light (evening) period were significantly higher than corticosterone levels during the early light (morning) period (Table 1; Fig. 3). In both the morning and the evening periods, the effect of a new environment induced a significant increase in plasma corticosterone after 20 and 40 min, whereafter the increased levels declined steadily (Fig. 3). At $t = 0$, 60, 80, and 100 min, animals during the evening had significantly higher plasma corticosterone levels than the morning animals (Fig. 3). Four intact animals tested in the morning (2 for the first, 2 for the second time) had basal levels similar to the evening values, yet their corticosterone levels after stress were not significantly different from the other morning values. Intact animals that were tested for a second time either in the morning or the evening did not show an altered response to the mild stress paradigm.

Surprisingly, despite their SCN lesions, the lesioned animals responded differently when they were subjected to this mild stress in the morning or in the evening. In the morning the response was more abrupt than in the evening, reaching an earlier peak than in the evening. In the morning after 40 min the levels rapidly declined, until, at 80 min, a new basal level was reached. In the evening, in contrast, the levels did not decrease but remained elevated just below that of the initial stress (Fig. 3). The partially lesioned SCN animals showed a rapid increase in the morning; in the evening no time point differed significantly from *time 0* (Fig. 3).

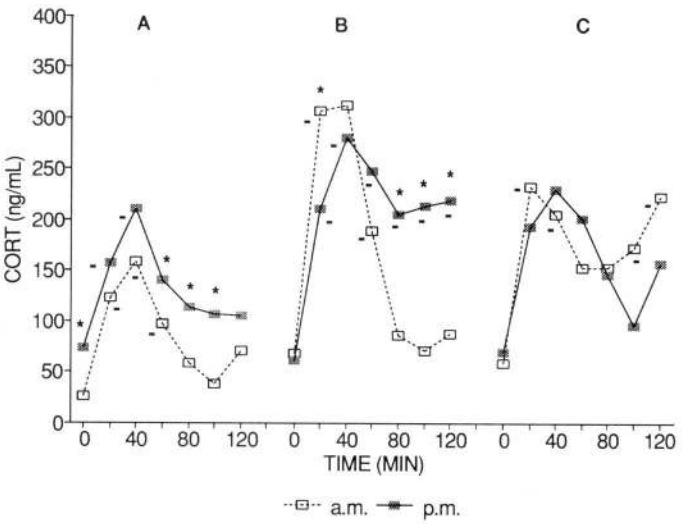

Fig. 3. Basal *time 0* and novelty-induced plasma corticosterone (Cort) levels comparing morning and evening values for intact (*A*), SCN-lesioned (*B*), and partial SCN-lesioned groups (*C*) separately. * $P < 0.05$ compared with morning values. ▬ $P < 0.05$ compared with *time 0* of same group. Animals were moved to a new cage at *time 0*.

On comparing the three treatment groups in both the morning and the evening, we found that there were highly significant effects of lesion and sampling time ($P < 0.001$).

In both the morning and the evening, SCN and partially SCN-lesioned animals had basal corticosterone levels that were at the same level as the evening basal corticosterone levels of the intact animals. But only the basal SCN-lesioned level was significantly different from the intact basal morning level (Table 1; Fig. 4). The novelty stress resulted in a dramatic increase in plasma cor-

Table 1. *Plasma corticosterone concentrations before and after a novelty stress in control, SCN-lesioned, and partial SCN-lesioned animals in morning and evening*

Time, min	Control		SCN-X		Partial SCN-X	
	A.M. ($n = 13$)	P.M. ($n = 13$)	A.M. ($n = 9$)	P.M. ($n = 10$)	A.M. ($n = 15$)	P.M. ($n = 6$)
0	25±10	73±11*	67±12*	61±12*	58±23	69±17*
20	123±15†	157±18†	306±34†‡	210±24†	233±47†‡	143±48
40	158±25†	210±27†	312±43†‡	280±32†§	205±37†	230±67
60	97±14†	140±20	188±34†‡	247±32†§	152±18	209±48
80	57±10	113±16	85±10	205±25†§	152±25†	145±18
100	38±7	107±11	71±14	213±20†§	172±28†‡	95±9
120	71±18	104±12	87±23	218±24†§	223±32†‡	157±22

Values are means ± SE; *n*, no. of experiments. These data were used to prepare Figs. 3 and 4. SCN-X, suprachiasmatic nucleus lesion. * $P < 0.05$, basal corticosterone concentration higher than A.M. control value by *t* test. † $P < 0.05$, compared with *time 0* value in same column. ‡ $P < 0.05$, compared with corresponding A.M. level in control group. § $P < 0.05$, compared with corresponding P.M. level in control group.

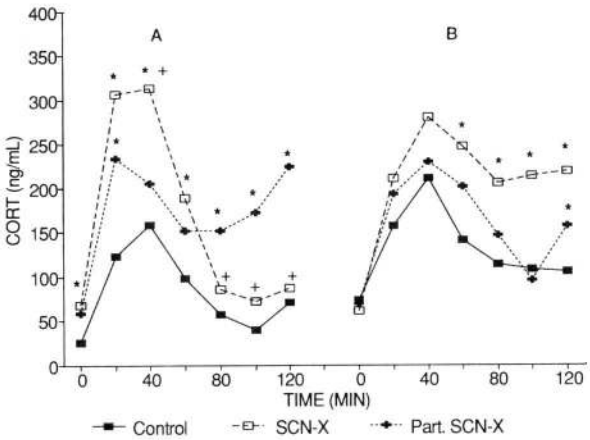

Fig. 4. Basal level of plasma corticosterone in intact and lesioned animals, at *time 0*, and effect of moving to a new cage. *A*: morning. *B*: evening. * $P < 0.05$ compared with control values; + $P < 0.05$ SCN-lesioned vs. partial SCN-lesioned in morning and in evening.

ticosterone levels two- to threefold higher in the SCN-lesioned rats both during morning and evening than the comparable morning or evening levels in intact control animals (Fig. 4).

The partially SCN-lesioned animals responded as a group with measurements somewhere between the complete SCN-lesioned and the control group. Within the partially lesioned group, five animals had basal levels in the morning comparable to control animals; however, after stress, these animals had much higher levels at $t = 100$ and $t = 120$. The other animals had levels more comparable to SCN-lesioned animals.

DISCUSSION

The present study illustrates the difficulty of completely eliminating the SCN with lesions still small enough to leave the largest part of the surrounding tissue and the PVN intact. Only 14 of 53 animals sustained a complete SCN lesion. Initially, the drinking test indicated a larger number of successful SCN lesions. However, on histological examination, often small parts of the SCN remained visible, but only after staining for VIP or VP. A regular Nissl control stain did not reveal these remnants of SCN neurons. This result confirms previous observations that small parts of the SCN are insufficient to impose complex overt behavioral rhythms onto the CNS (31). Since these neurons often still provided a substantial innervation of the PVN and/or DMH, we decided to place all animals that had clearly distinguishable VP or VIP cell bodies at the border of the lesions, with or without a clear innervation of PVN or DMH, in a different group. Still, in some of the lesions that were considered successful, sometimes single VP fibers, but more often VIP fibers, remained visible in the optic nerve at the ventral part of the lesion. Since it was necessary to restrict the lesion to the area of the SCN and keep the PVN and DMH intact, it was not possible to increase the size of the lesion. The results of the partially lesioned animals (that lost their drinking rhythm) indicate that in some animals the part of the SCN remaining was sufficient to maintain basal levels in corticosterone in the morning. Consequently, remnants of the SCN still might be able to influence the CNS to some extent. This confirms as does a previous study (23) that a drinking test, or activity monitoring only, is not sufficient to establish whether the SCN is lesioned completely. The present results strongly indicate that a histological examination that includes a specific stain for SCN neurons is also required.

The present data showing higher basal plasma corticosterone levels in the evening compared with the morning in intact animals fully corroborate those data in many previous reports (see, for example, Refs. 7, 9, 15, 17, 21). In addition, the present results indicate that a light stress in the evening results in slightly higher corticosterone levels compared with the morning at all time points, albeit these are only significant at $t = 0$, 60, 80, and 100 min. Also, this result confirms earlier observations using different stress paradigms (see Refs. 5, 9, and 16). One may argue that the increase in the morning is relatively higher than that in the evening, i.e., a factor of 6 compared with a factor of 3 in the evening. However, our results indicate that a higher basal level does not automatically lead to a higher stress level. Animals in the morning that had a substantially higher basal level did not reach higher final stress levels compared with their other morning companions, and certainly not by the same factor. The present results indicate that the stress, both in the morning and in the evening, results in identical increments of absolute values in plasma corticosterone levels. Consequently, enhanced basal levels of corticosterone in the evening may be explained by a lesser inhibition of corticosterone secretion by the SCN or by an enhanced stimulatory input from the SCN, whereby the SCN sets the system at a different level.

To investigate whether the SCN has an inhibitory influence in the morning or a stimulating influence on corticosterone secretion in the evening, this nucleus was lesioned. In the available literature, studies already exist in which the effect of SCN lesions on basal levels of corticosterone is investigated at several points in the light-dark cycle (1, 32). Also, these experiments, as in the present results, failed to find a daily change in basal corticosteroid secretion in SCN-lesioned animals, resulting in the general conclusion that the SCN controls the circadian rhythm of corticoid secretion. The characteristics of this control, however, remained unclear. In the case of the SCN having a stimulatory role in the evening, one would expect to find in SCN-lesioned animals low basal levels, both in the evening and in the morning; whereas, if the SCN has an inhibitory role in the morning, then one would expect to find the opposite. The present results illustrate that the plasma corticosterone level in the morning in SCN-lesioned animals is higher than in the control rats sampled at the same period. This already suggests that elimination of the SCN results in a removal of an inhibitory influence on corticosteroid secretion. In addition, in the evening, basal levels of SCN-lesioned and SCN-intact animals are comparable, suggesting that the inhibitory influence of the SCN on corticosteroid secretion is absent, or at least

lower, in the evening. These present results are in agreement with previous observations (1, 32) demonstrating variable levels of corticosterone in SCN-lesioned animals, but close examination of these published data indicates that all SCN-lesioned rats fail to exhibit the low morning values found in intact animals. The hypothesis that the SCN has an inhibitory influence on corticosteroid secretion is further supported by the results of the mild stress experiment. Here plasma corticosterone levels in SCN-lesioned animals rose much more rapidly and higher than intact controls in the morning. The fact that the corticosterone levels in SCN-lesioned animals were also significantly higher than in intact animals during the evening suggests that in the intact animals the SCN still exerts an inhibitory input during evening, although less strongly than in the morning.

The data on the partially lesioned animals indicate that in this group corticosterone levels were elevated but less so than in the completely lesioned group. The corticosterone levels in this group are also less homogeneous. Some animals ($n = 7$) had levels at $t = 0$, 20, 40, and 60 min quite comparable with intact control animals, but much more elevated at $t = 80$, 100, and 120 min. The other 12 were more comparable with complete SCN-lesioned animals. The variability of the site and the size of the lesion in this group did not allow us to draw conclusions as to which area of the SCN needed to be lesioned to be effective. The animals that showed most VIP or VP immunoreactivity in the SCN unilaterally ($n = 2$) or caudally ($n = 3$) were the same animals that had also basal corticosterone levels comparable to intact controls. This may suggest in these animals that the amount of SCN tissue remaining was not sufficient to completely synchronize the activity of the animal but was sufficient to maintain a low corticosterone level in the morning.

The absence of a decline in corticosterone in SCN-lesioned animals in the evening compared with SCN-lesioned animals in the morning suggests that in these animals either a stimulatory input is present in the evening or an inhibitory one is present in the morning. This latter observation could be explained as an indication that, apart from the SCN, other timekeeping mechanisms may also be present, as suggested by experiments with food restriction regimens (20). Other data that may support this indicate that brain stem noradrenergic mechanisms may be the activating factor in this process (12). However, another more likely possibility is that, since all experiments were conducted in the animal quarters where both SCN-lesioned and the intact animals used in this experiment were housed, the SCN-lesioned animals remain more disturbed in the evening because of the higher arousal of their intact roommates during this time of the day. No obvious behavioral differences between SCN-lesioned animals in the morning or in the evening could be noted, however. The absence of an inhibitory influence from the SCN may then result in the sustained elevated levels. Further experiments aimed at this problem may clarify this possibility.

Another issue that requires clarification is the apparent dichotomy that exists between the release of ACTH and corticosterone in the morning and evening (7, 15, 17). Even though basal plasma ACTH levels are somewhat higher during evening than morning, stress-released ACTH is much more elevated in the morning than in the evening. Recently, Bradbury et al. (5) showed that this diurnal difference in ACTH release is largely independent of corticosterone. In addition, it was reported that a bilateral lesion of the SCN eliminated the evening rise in ACTH, suggesting that the SCN stimulates evening ACTH secretion (7). Apparently, a regulatory system exists at the level of the hypothalamus that controls the release of ACTH and corticosterone separately and which can be influenced by the SCN. Unfortunately, the sample size that we used to limit side effects of blood withdrawal did not allow us to measure ACTH as well. Consequently, we have no insight as to what happens to ACTH values in SCN-lesioned animals subjected to stress.

All anatomic data thus far have failed to provide evidence for a direct interaction of SCN projections with CRF-containing cell bodies that may explain the diurnal rhythm in either ACTH or corticosterone (3, 25, 27, 33). However, other indirect pathways should exist that may explain the impact of the SCN on ACTH and corticosteroid secretion. In a recent anatomic tracing study we labeled the projections of the SCN and identified stress-activated neurons by means of c-*fos* immunocytochemistry. The results indicated that SCN neurons contacted *fos*-labeled cells not in the CRF-containing medial parvicellular part of the PVN but in the periventricular and

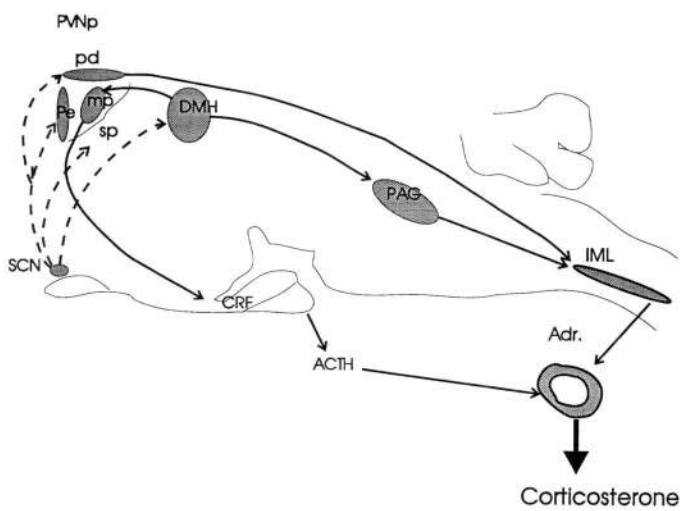

Fig. 5. Proposed pathways used by SCN to induce a diurnal rhythm in corticosterone secretion. Direct connections with dorsomedial nucleus of hypothalamus (DMH) and periventricular nucleus (Pe) most likely serve to transmit SCN information via interneurons onto corticotropin-releasing factor (CRF) neurons located in medial parvicellular (mp) part of parvicellular PVN (PVNp). These neurons release CRF into the portal vascular system of the median eminence, which releases ACTH. Via bloodstream this hormone affects adrenal cortex to induce release of corticosterone. The other pathway that the SCN may use to influence corticosterone secretion is by way of a direct interaction with parvicellular neurons in the dorsal cap of the PVN (pd) that project directly to the intermediolateral column (IML) in the spinal cord. Another pathway that the SCN may use is as follows: by contacting DMH neurons that project to the periaquaductal grey (PAG) and then onward to the IML. From IML a direct, and possibly indirect, pathway exists to the adrenal (Adr.) medulla and cortex. This scheme is based on several different anatomical and physiological studies. Projections of the subparaventricular zone (sp) of PVN are not, as yet, known. For further details see references and text.

rostral PVN and in the DMH (6). The DMH has been shown to project heavily onto the medial parvicellular part of the PVN (29), whereas these CRF-containing neurons of the PVN may be also connected with interneurons in the periventricular part and rostal part of the PVN (30). Therefore, we concluded that by innervating these regions the SCN is able to influence CRF neurons and the stress response indirectly. Since the SCN may directly innervate neurons in the dorsal cap of the PVN (3, 25, 27) and in the DMH that project to the spinal cord and brain stem, the SCN may use this pathway to regulate corticosteroid secretion independently from that of CRF/ACTH.

Apart from the hypothalamo-hypophysial-adrenal axis that has been shown to be essential for the control of the secretion of corticosteroids, substantial evidence is available that indicates the possibility of a direct central CNS control of corticosteroid secretion. Anatomic data indicate the presence of a direct peptidergic, i.e., VIP or catecholaminergic, innervation of the adrenal cortex (18, 19). This innervation may be derived from sympathetic preganglionic nuclei situated in the intermediolateral column of the spinal cord or from one of the prevertebral ganglia (18, 26). The physiological evidence indicating a direct CNS control over the adrenal cortex relates to effects of splanchnic nerve stimulation and the role of VIP in corticosteroid secretion (4, 8, 10). This might be one of the CNS pathways other than the hypothalamo-hypophysial-adrenal axis responsible for the diurnal control of corticosterone secretion. Further evidence for the existence of such a pathway is the diurnal change in the sensitivity of the adrenal cortex to ACTH (15). This PVN-sympathetic spinal cord-adrenal pathway (Fig. 5), however, is still not completely elucidated and requires substantiation by careful anatomic analysis of the innervation of the different subareas of the PVN and DMH together with the identification of the projections of these nuclei.

The present observations that the SCN may have an inhibitory role on corticosteroid secretion tie nicely into our previous observation that VP from the SCN inhibits corticosterone release when infused into the area of the DMH/PVN (14). There is ample evidence that VP is released from SCN neurons during the daytime (24). Thus the daily occurring peak of corticosterone may be explained by a lesser inhibition of the SCN via a lower release of VP. Our experiments, which showed that infusion of VP antagonists in the DMH/PVN area elevated corticosterone levels, support this view. Consequently, the present results indicate that in nocturnal animals the general role of the SCN may be to suppress activity during the light period to avoid unnecessary arousal. Further physiological and morphological studies will be essential to prove this hypothesis.

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