Fetal grafts containing suprachiasmatic nuclei restore the diurnal rhythm of CRH and POMC mRNA in aging rats

Aihua Cai, Kathryn Scarbrough, David A. Hinkle, and Phyllis M. Wise. Fetal grafts containing suprachiasmatic nuclei restore the diurnal rhythm of CRH and POMC mRNA in aging rats. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1764–R1770, 1997.—We assessed whether fetal tissue containing the suprachiasmatic nuclei (SCN) can restore age-related changes in the diurnal rhythm of hypothalamic corticotropin-releasing hormone (CRH) and anterior pituitary proopiomelanocortin (POMC) mRNA. Young, middle-aged, and middle-aged SCN-transplanted rats were killed at seven times of day. In young rats, CRH mRNA exhibited a diurnal rhythm in the dorsomedial paraventricular nucleus but not in other subdivisions of the nuclei. No rhythm was detected in aging rats. SCN transplants restored a rhythm in CRH mRNA, but the timing was not precisely the same as in young animals. POMC mRNA exhibited a daily rhythm in young rats. Aging abolished the rhythm and decreased the average mRNA level; fetal transplants restored the rhythm, but the amplitude remained attenuated. These data are the first demonstration that fetal tissue can restore the diurnal rhythm of a neuroendocrine axis that is driven by the SCN. We conclude that the neuroendocrine substrate from the aging host remains capable of responding to diurnal cues to express diurnal rhythmicity in CRH/POMC mRNA when fetal SCN transplants confer the appropriate signals.

MATERIALS AND METHODS

Animals and procedures. In this study, we used young and middle-aged female Sprague-Dawley rats that were ovariectomized and treated with a specific dose of estradiol for a controlled period of time because evidence demonstrates that the diurnal rhythm of CRH mRNA levels in the anterior pituitary of old rats, whereas others detected no change (19, 42). These studies only measured POMC mRNA levels at a single time of day. Changes in the rhythmicity of CRH mRNA levels in middle-aged rats do not appear to be translated into dampened rhythmicity of corticosterone secretion (7, 48), although the secretory rhythm is dampened by the time female rats are old (48).

Aging of the SCN itself may explain changes in the HPA axis. We reported a phase advance in the diurnal rhythm of local cerebral glucose utilization (51) in the SCN of middle-aged rats, which suggests that changes occur in the ability of the neural substrate to maintain rhythmicity with age. Light and electron microscopic analysis revealed morphologic changes in the aged SCN (53). Although the total number of cells in the SCN does not change (30), several specific neuronal types may decrease or no longer exhibit rhythmicity with age (10, 32, 39).

Transplantation of fetal tissue containing the SCN into old hosts can restore the age-related behavioral changes in running rhythm (20, 45); responsiveness of circadian activity to a phase shifting stimulus, such as triazolam in hamsters (44); and light-induced Fos expression in the SCN in middle-aged rats (6). However, previous studies have been largely unsuccessful in restoring diurnal rhythms in endocrine outputs that are driven by the SCN (3, 29, 38). Thus the purpose of this study was to 1) determine whether transplantation of fetal tissue containing the SCN into middle-aged rats can restore the diurnal pattern of CRH gene expression in the PVN, 2) evaluate whether age-related changes in the CRH mRNA rhythm are reflected in changes in the pattern of POMC mRNA expression in the anterior pituitary, and 3) determine whether transplantation of fetal tissue containing the SCN into middle-aged hosts can restore any age-related changes in POMC expression to that of young rats.

THE SUPRACHIASMATIC NUCLEI (SCN) are sometimes called the biological clock because they are the critical endogenous neural pacemaker that drives most circadian rhythms in mammals. Several lines of evidence demonstrate that the SCN drive the corticotropin-releasing hormone (CRH) rhythm, which, in turn, governs the adrenocorticotropic hormone (ACTH)/glucocorticoid rhythm (2, 9, 27, 40). Electrophysiological and anatomical studies demonstrate that there are direct neural inputs from the SCN to the paraventricular nucleus (PVN) (5, 18, 46). In addition, recent anterograde tracing methods have identified pathways from the SCN to the paraventricular thalamus, the lateral geniculate, and the dorsomedial hypothalamus (4). Together, these targets are likely to mediate SCN influence on the circadian rhythm of the CRH/ACTH/corticosterone secretion.

Aging alters the diurnal rhythm of several aspects of the hypothalamic-pituitary-adrenal cortex (HPA) axis (36). We have previously reported that the diurnal rhythm of CRH mRNA levels in the PVN is abolished in middle-aged rats (7). It is unclear whether age-related changes in CRH mRNA rhythm are reflected in changes in the pattern of proopiomelanocortin (POMC) gene expression. Cizza et al. (11) reported a decrease in POMC mRNA levels in the anterior pituitary of old rats, whereas others detected no change (19, 42). These data are the first demonstration that fetal tissue containing the SCN can restore age-related changes in the diurnal rhythm of CRH and POMC mRNA in aging rats.
cerebral glucose utilization in the SCN is blunted and phase advanced (51), transplantation of SCN tissue restores light-induced Fos (6) and Jun (8) expression, and disruption of a key neuropeptide of the SCN mimics aging (17).

Young (2–3 mo) and middle-aged (11–12 mo) Sprague-Dawley female rats were housed in a 14:10-h light-dark cycle (lights on at 0400) for at least 2 wk. Rats were maintained under a constant temperature conditions (21°C) and were fed Purina rat chow ad libitum. Daily vaginal lavage was performed to monitor estrous cyclicity. Only regularly cycling young rats and irregularly cycling and constant estrous middle-aged rats were used for the following experiments. Young rats were sham operated. No grafts were transplanted in young animals because previous studies (31) have shown that the transplanted SCN determine the phase of the host when it is transplanted into a young host whose own SCN have been lesioned. Middle-aged rats were either sham operated or received control cerebral cortical tissue transplants. We have previously shown that the pattern of light-induced expression of immediate early genes is identical in both of these control groups of animals (8). Another group of middle-aged rats received fetal hypothalamic transplants containing the SCN according to the methods of Lehman et al. (29). In brief, recipient animals were anesthetized with Ketaset (ketamine, 8.7 mg/kg body wt) and PromAce (acepromazine, 0.52 mg/kg body wt) as directed by the manufacturer’s instructions and placed in a stereotaxic instrument. Fetuses (embryonic day 18) were removed immediately after the pregnant female was decapitated and were kept on ice. Fetal tissue was transplanted into middle-aged hosts within 60 min of killing of the pregnant rat. The fetal SCN was dissected under sterile conditions on ice from the ventral surface of the hypothalamus, with coronal cuts just rostral and caudal to the optic chiasm and with lateral cuts equidistant from the midline. Approximately 3–4 µl of hypothalamic tissue containing the SCN or cerebral cortex from two fetuses were collected into a cannula (Drummond Scientific, Broomall, PA) and implanted into the third ventricle of hosts [coordinates = 1.2 mm anterior to bregma and −7.5 mm (young) or −8.2 mm (middle aged) ventral to the surface of the dura mater]. After 4 wk (day 0), all rats were ovariolectomized bilaterally under methoxyflurane anesthesia. One week later (day 7) rats were implanted subcutaneously with Silastic capsules containing 17β-estradiol (180 µg/ml sesame oil; ID = 20 × 0.625 mm for young rats and 30 × 0.625 mm for middle-aged rats) to equalize estradiol levels among all age and treatment groups. The presence of an equivalent estrogen milieu may be important because estrogen can modulate the expression of circadian behavioral rhythms (1) and the diurnal rhythm of corticosterone secretion (48). Animals were rapidly decapitated at 2300 on day 9 (n = 6–9 per experimental group per time point). At each time, no more than three rats were killed by two people within 2 min. Rats in each treatment group were killed and tissues were collected over a 2-yr period; animals from each experimental group were killed and tissues were collected over the entire 2-yr interval. Trunk blood was collected for corticosterone measurement. Brains were rapidly removed, frozen on dry ice, and stored at −70°C until they were sectioned. Pituitaries were removed and the anterior pituitary was dissected away from the posterior and intermediate lobes, quickly frozen in Freon (Aldrich Chemical, Milwaukee, WI) on dry ice, and stored at −70°C until POMC RNA levels were measured. After all animals were collected, brains were sectioned (12 µm) for CRH mRNA in situ hybridization assay. Total RNA from the anterior pituitary was extracted to quantitate POMC gene expression using a solution hybridization/ribonuclease (RNase) protection assay.

In situ hybridization histochemistry. Methods used to quantitate CRH mRNA levels were the same as those that we have used previously (7) with minor modifications. In brief, we used a 380-base pair (bp) Pvu II to Sph I fragment of CRH cDNA, a generous gift from Drs. A. G. Watts and K. Mayo. This cDNA was ligated into pGEM4 (Promega, Madison, WI) and corresponds to the carboxy terminus of the prepro-CRH molecule, the entire CRH-41 peptide coding region, and a portion of the 3′-untranslated region of rat CRH mRNA (21, 41). The CRH riboprobe was transcribed using 50-µM total α-thio-UTP (15 µM 35S-labeled, 35 µM unlabeled UTP), which yielded an antisense cRNA with a specific activity of 7 × 106 disintegrations-min⁻¹-µg⁻¹. A control sense riboprobe was transcribed in a similar manner and was of the same specific activity.

In situ hybridization histochemistry was performed according to the method of Wise et al. (52) with minor modifications. On the day of hybridization, sections were rapidly dried, fixed in RNase-free phosphate-buffered 4% paraformaldehyde, then sequentially washed in phosphate buffer, diethylpyrocarbonate-treated water, acetic anhydride, triethanolamine buffer, and 0.3 M sodium citrate. We have previously established that under these conditions a concentration of 0.2 µg/ml CRH cRNA achieved saturation (7). Therefore, this concentration (47 µl) was applied to each slide. Slides were covered with glass coverslips and incubated overnight (16 h) at 55°C in a humidified incubator. After hybridization, slides were immersed in 4× SSC to remove coverslips. Slides were then rinsed in 4× SSC, treated with RNase A, and stringently washed. After dehybridization, slides were dipped in Kodak NTB-2 emulsion (Eastman Kodak, Rochester, NY) and exposed for 14 days.

A preliminary study was performed to locate the CRH mRNA signal from the rostral to caudal portion of the PVN (data not shown). Three slides of the medial PVN containing the greatest number of CRH mRNA-positive cells were used for in situ hybridization histochemistry. For the middle-aged animals that received implants, only those that had viable grafts that were located in the third ventricle were used (6). Functional viability of the graft was assessed by the presence of VIP- and neurophysin-immunoreactive cells in the graft (6). These criteria have been used by previous investigators (29).

The levels of CRH mRNA were measured bilaterally in the PVN using a Bioquant OS2 (R&M Biometrics, Nashville, TN). We measured CRH mRNA levels in the PVN in four size-fixed windows: the PVN window included ~95% CRH-containing neurons of the medial PVN; the dorsomedial, periventricular, and lateral PVN windows included only those specific subdivisions of the PVN. CRH mRNA levels were measured as “video count area,” which represents the total area covered by silver grains within a window. A gray level threshold was set such that the histological stained nucleus was below the threshold and was not measured, whereas the majority of the silver grains were above the threshold and enhanced by the computer. We observed no obvious difference between the size, shape, or rostral-caudal extent of the PVN in young compared with middle-aged animals using thionin staining. Therefore, we used the same-sized windows and the same relative placement of the windows for young and middle-aged rats.

RNase protection/solution hybridization assay. RNA was isolated as previously described with minor modifications (35). [32P]UTP-labeled POMC cRNA was transcribed from a 470-bp segment of the rat POMC gene containing the last 20 bp of intron A, all 180 bp of exon 2, and the first 270 bp of
intron B in pBS(+) vector (Stratagene, La Jolla, CA). Cyclophilin (1B15) was used as an internal control. Both POMC and cyclophilin cDNAs were gifts from Dr. J. L. Roberts. Five micrograms of cytoplasmic RNA from each pituitary were hybridized with excess [32P]UTP-labeled POMC and cyclophilin antisense cDNA (0.5 ng/sample) at 45°C overnight. After hybridization, RNase A digestion, and washes, samples were loaded on 6% native polyacrylamide gels and fractionated. Gels were dried and exposed on a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Radioimmunoassay. The concentration of serum corticosterone from all of the rats was measured in a single assay using a kit from ICN Biomedicals (Costa Mesa, CA). The limit of detection was 2.5 µg/dl corticosterone.

Data analysis and statistics. Data were analyzed using two-way analysis of variance (ANOVA) to test the effects of group (young, middle-aged, middle-aged + transplant), time of day, and the group × time interaction. To assess whether transplant completely restored rhythmicity to that observed in young rats, two-way ANOVA was performed to compare the effect of group (young and middle-aged + transplant). When a significant interaction was detected, further analyses were performed to determine effects of group and time of day within each experimental group. To determine the presence or absence of a diurnal rhythm within each group, data were analyzed using one-way ANOVA for effect of time. If there was a significant effect of time, Duncan’s multiple-range analysis was used to determine which times were different from each other.

RESULTS

CRH gene expression in different regions of the PVN. The distribution of CRH mRNA signal from the rostral to caudal PVN was analyzed by in situ hybridization. Approximately six to eight slides (2 brain sections on each slide) contained the highest levels of CRH mRNA in the medial PVN, where we (7) and other investigators (28, 47) have reported a diurnal rhythm in CRH mRNA levels. Therefore, we analyzed the level of gene expression using three slides (every other slide) in this region of the PVN. Figure 1 shows a schematic diagram of different subpopulations of the PVN that we analyzed.

Two-way ANOVA (group [young, middle-age, middle-age + transplant] × time of day [7 times], n = 6–9 rats per group per time point) revealed no significant effect of group and a significant effect of time of day (P < 0.05) in the whole PVN (Fig. 2) and the dorsomedial PVN (Fig. 3). The lack of any significant effect of group indicates that the overall levels of CRH mRNA in different regions of PVN remained unaltered during aging. A group × time of day interaction was detected in the dorsomedial PVN (P < 0.05). One-way ANOVA revealed that, in young rats, there was a diurnal pattern of expression of CRH mRNA in the whole PVN (Fig. 2, P < 0.05) and dorsomedial PVN (Fig. 3, P < 0.001): CRH mRNA levels were low at 2300, increased significantly at 0600, decreased gradually during the day, and were statistically lower than peak levels at 1800 and 2300. In contrast, in middle-aged rats, no rhythm was detected in the whole (P = 0.46, Fig. 2) or dorsomedial (P = 0.73, Fig. 3) PVN. Therefore, one of the sources of the interaction in the dorsomedial PVN (group × time) was the lack of effect of time in

![Fig. 1. Schematic diagram of the medial paraventricular nucleus (PVN). The whole PVN window, shown in dotted line, enclosed an area (1.2 mm²) enclosing ~95% of the CRH mRNA-labeled cells. The other windows, shown in solid lines, depict the subdivisions of the PVN, dorsomedial (dm), lateral (lat), and periventricular (peri) PVN, representing ~0.5, 0.48, and 0.53 mm², respectively. 3V, 3rd ventricle.](http://ajpregu.physiology.org/)

![Fig. 2. Corticotropin-releasing hormone (CRH) mRNA levels in the whole PVN in young, middle-aged, and middle-aged suprachiasmatic nucleus (SCN)-transplanted rats. In young rats, CRH mRNA levels exhibited a diurnal rhythm (P < 0.05); no rhythms were detected in middle-aged rats. Transplantation of fetal SCN restored the diurnal rhythm in middle-aged rats (P < 0.05).](http://ajpregu.physiology.org/)

![Fig. 3. CRH mRNA levels in the dorsomedial PVN in young, middle-aged, and middle-aged SCN-transplanted rats. In young rats, CRH mRNA levels exhibited a diurnal rhythm (P < 0.001). No rhythms were detected in middle-aged rats (P = 0.73). Transplantation of fetal SCN restored a diurnal rhythm in middle-aged rats (P < 0.001). Two-way analysis of variance (ANOVA) revealed a significant age-by-time interaction (P < 0.05), but no significant effect of age.](http://ajpregu.physiology.org/)
middle-aged rats. No rhythms were detectable in the periventricular (Table 1) and lateral (Table 2) PVN of young or middle-aged animals.

Transplantation of fetal SCN grafts into middle-aged animals restored the diurnal rhythm in CRH mRNA levels in the whole PVN (P < 0.05) and dorsomedial PVN (P < 0.0001). Two-way ANOVA, comparing CRH mRNA levels in the dorsomedial PVN in the young compared with middle-aged plus transplant rats, revealed a significant interaction. Thus, although both groups exhibited an effect of time, the temporal pattern of the rhythm in CRH mRNA levels in middle-aged rats receiving a transplant was different from that of the young. CRH mRNA levels in dorsomedial PVN rose significantly at 0600, were maintained at high levels for at least 8 h until 1600 before they declined at 1800 to levels significantly lower than the peak. CRH mRNA levels at 1800 were not significantly different from the levels at 0600, but statistically lower than that at 1400. CRH mRNA levels returned to the basal level at 2300. Fetal tissue transplantation did not influence the pattern of CRH mRNA in periventricular (Table 1) and lateral (Table 2) PVN.

POMC gene expression in the anterior pituitary. Two-way ANOVA of POMC mRNA levels in the anterior pituitary gland revealed a significant effect of group (P < 0.0001) and time (P < 0.001). Young rats exhibited a diurnal rhythm (P < 0.04, Fig. 4): levels were high from 2300 to 1000 and decreased between 1400 and 1800. In middle-aged rats, POMC mRNA levels did not exhibit a diurnal rhythm (P = 0.60), and the mean POMC mRNA levels were significantly lower than those of young rats (P < 0.001). Two-way ANOVA comparing young and middle-aged plus transplant rats and between middle-aged and middle-aged plus transplant rats showed that transplantation of fetal SCN tissue into middle-aged rats restored the diurnal pattern in POMC mRNA (P < 0.02); however, the average levels of POMC mRNA remained as low as those in the middle-aged controls.

Serum corticosterone levels. Serum corticosterone concentrations expressed robust diurnal rhythms (P < 0.001) in all experimental groups (Fig. 5). No obvious changes in the pattern or level of serum corticosterone occurred in middle-aged rats. Corticosterone levels reached a nadir at 0600–1000, increased at 1300 (P < 0.05), peaked at 1500–1800 (P < 0.05), and decreased at 2300 (P < 0.05).

DISCUSSION

The present study provides the first demonstration that transplantation of fetal tissue can restore the diurnal pattern of expression of neuroendocrine events that are driven by the biological clock. Our results constitute strong evidence that 1) aging affects the diurnal rhythm of CRH and POMC gene expression, 2) transplantation of fetal tissue that contains the SCN restores a diurnal pattern of CRH mRNA levels in middle-aged female rats, and 3) the transplant-induced restoration of a CRH mRNA rhythm correlates with a partial restoration of the pituitary POMC mRNA rhythm.

CRH neurons are localized in different regions of the PVN. These subdivisions may have distinct functions; therefore, we analyzed separately three regions and the entire PVN in this study. Our data demonstrate that the rhythm in CRH mRNA observed in the whole PVN can be attributed to a diurnal rhythm of CRH mRNA that exists selectively in the dorsomedial PVN. We observed no significant rhythm in the lateral and the periventricular PVN. The pattern that we observed is similar to that reported by Watts and Swanson (47), but not the same as that reported by Kwak et al. (28). In the former work, CRH mRNA was measured by in situ hybridization methods similar to those used in the

Table 1. CRH mRNA levels in periventricular PVN in young, middle-aged, and middle-aged SCN-transplanted rats

<table>
<thead>
<tr>
<th>Video Count Area of CRH mRNA, µm²</th>
<th>day 8, 2300</th>
<th>day 9, 0600</th>
<th>day 9, 1000</th>
<th>day 9, 1400</th>
<th>day 9, 1600</th>
<th>day 9, 1800</th>
<th>day 9, 2300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>1,243 ± 429</td>
<td>1,669 ± 203</td>
<td>1,187 ± 242</td>
<td>1,709 ± 244</td>
<td>1,174 ± 159</td>
<td>1,179 ± 103</td>
<td></td>
</tr>
<tr>
<td>Middle-aged</td>
<td>1,650 ± 302</td>
<td>2,004 ± 595</td>
<td>1,936 ± 313</td>
<td>1,907 ± 176</td>
<td>1,373 ± 183</td>
<td>1,406 ± 261</td>
<td>1,605 ± 282</td>
</tr>
<tr>
<td>SCN transplant</td>
<td>1,542 ± 189</td>
<td>1,603 ± 126</td>
<td>1,686 ± 108</td>
<td>1,747 ± 189</td>
<td>1,616 ± 146</td>
<td>2,284 ± 309</td>
<td>1,452 ± 320</td>
</tr>
</tbody>
</table>

Values are means ± SE. CRH, corticotropin-releasing hormone; SCN, suprachiasmatic nucleus; PVN, paraventricular nucleus. Two-way analysis of variance (ANOVA) revealed no effects of time of day (P = 0.43), age (P = 0.12), or age-by-time interaction (P = 0.16) in any groups.

Table 2. CRH mRNA levels in lateral PVN in young, middle-aged, and middle-aged SCN-transplanted rats

<table>
<thead>
<tr>
<th>Video Count Area of CRH mRNA, µm²</th>
<th>day 8, 2300</th>
<th>day 9, 0600</th>
<th>day 9, 1000</th>
<th>day 9, 1400</th>
<th>day 9, 1600</th>
<th>day 9, 1800</th>
<th>day 9, 2300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>2,126 ± 292</td>
<td>1,802 ± 206</td>
<td>2,030 ± 124</td>
<td>1,956 ± 401</td>
<td>3,236 ± 546</td>
<td>2,127 ± 294</td>
<td>1,597 ± 233</td>
</tr>
<tr>
<td>Middle-aged</td>
<td>1,615 ± 134</td>
<td>2,073 ± 232</td>
<td>2,025 ± 287</td>
<td>2,890 ± 525</td>
<td>2,190 ± 323</td>
<td>1,695 ± 169</td>
<td>1,686 ± 220</td>
</tr>
<tr>
<td>SCN transplant</td>
<td>2,353 ± 438</td>
<td>2,411 ± 320</td>
<td>2,411 ± 32</td>
<td>2,363 ± 217</td>
<td>2,218 ± 582</td>
<td>1,645 ± 302</td>
<td>2,210 ± 417</td>
</tr>
</tbody>
</table>

Values are means ± SE. Two-way ANOVA revealed no effects of time of day (P = 0.10), age (P = 0.65), or age-by-time interaction (P = 0.41) in any groups.
present study, whereas, in the latter report, CRH mRNA was measured in larger hypothalamic tissue chunks by RNAase protection methods.

When animals reached middle age, we could no longer detect any significant rhythm in CRH gene expression. Although the rhythmic expression of CRH mRNA is lost, no age-related changes occur in overall average level of CRH mRNA in any region measured. Similarly, the rhythm of POMC mRNA in the anterior pituitary was no longer detectable in middle-aged rats. Although our experimental design did not allow us to obtain information of whether diurnal rhythm in CRH peptide content in the hypothalamus and the secretion into portal blood changes with age, the simultaneous loss of both rhythms of CRH and POMC mRNAs suggests that the rhythmic secretion of CRH peptide into hypophysial portal blood may no longer exist in middle-aged animals. The loss of CRH rhythm combined with other age-related changes in the anterior pituitary, such as the decrease of CRH receptor expression, may contribute to the decrease of the average levels of POMC mRNA in the anterior pituitary.

Transplantation of fetal hypothalamic tissue containing the SCN has been reported to restore many circadian rhythms abolished by SCN lesions in young animals, such as running activity and drinking behavior (13, 14, 29, 33, 34). However, SCN transplants have not been able to restore all SCN-driven functions in previously SCN-lesioned animals, such as endocrine rhythms and gonadal regression (29, 38). Few studies have been performed in aging models in which the endogenous, possibly deteriorating, host SCN remains intact (6, 20, 44, 45). None of these studies have examined whether SCN-driven neuroendocrine outputs can be completely or partially restored. In the present study, we provide the first evidence that fetal SCN grafts can partially restore a neuroendocrine output: we detected a rhythm, albeit temporally altered, in CRH mRNA in middle-aged rats that received SCN transplants. Our ability to restore CRH/ACTH function contradicts the lack of ability of transplants to restore reproductive function in SCN-lesioned male hamsters (3). This may indicate that 1) the SCN has more direct circuitry to HPA axis compared with hypothalamic-pituitary-gonadal axis or 2) the aging host SCN provides important factors that interact with factors provided by the graft to restore endocrine function. Previous data from our laboratory (6) suggest that fetal SCN grafts provide unique factors that rejuvenate the host SCN and enable it to function in a manner characteristic of the young. In previously SCN-lesioned animals, any ability of the transplant to drive and/or synergize with the host SCN would not be possible.

The pattern of CRH mRNA rhythm restored in the SCN-transplanted, middle-aged animals was not exactly the same as that of the young. The CRH mRNA level remained high for a longer interval compared with young rats, indicating that SCN transplants cannot restore the precise timing of outputs that are characteristic of young animals. It is possible that multiple other regulatory factors, such as the inputs from the hippocampus, other nuclei of hypothalamus, and pituitary, may also contribute to the changes in the rhythm of CRH mRNA. Studies have shown that aging influences glucocorticoid receptors in the brain (16, 43), and SCN transplants are likely to correct only some of these age-related changes.

Transplantation of fetal SCN tissue into middle-aged rats restored a diurnal rhythm of POMC mRNA in the anterior pituitary. However, SCN transplantation did not completely restore the exact pattern of expression; the average levels of POMC mRNA remained low. The precise timing of CRH rhythm may be very critical in generating the young pattern of POMC gene expression. The expression of POMC gene is regulated by CRH, as well as by glucocorticoids, and CRH and glucocorticoid regulation of POMC gene transcription are interdependent (15, 37). Using an in vitro nuclear run-on assay, Eberwine et al. (15) reported that dexamethasone, given 30 min before CRH treatment, can
completely block CRH-stimulated POMC gene transcription, and, conversely, CRH can neutralize the glucocorticoid-inhibited effects on POMC gene expression. Thus the timing of steroid or CRH administration determines whether CRH stimulates or the glucocorticoid inhibits POMC gene expression. This may explain why SCN transplants only partially restored the expression of the POMC gene in the anterior pituitary. Up to date, no studies have been done in regard to the age-related rhythmic changes of CRH and glucocorticoid receptors in the anterior pituitary. These data suggest, once again, that multiple regulatory elements may change with age and that SCN transplants can restore some, but not all, of them.

At the present time, the factors that allow the maintenance of normal glucocorticoid function in the face of alterations in hypothalamic CRH/anterior pituitary POMC mRNA patterns of expression are unclear. Some evidence indicates that the CRH/POMC components of the HPA axis may not be the only pathway that regulates the diurnal pattern of corticosterone. Anatomic studies (4, 22, 23) demonstrate that the dorsomedial hypothalamus, which receives the direct inputs from the vasopressin neurons of the SCN, regulates corticosterone rhythm. Preliminary data (25) from our laboratory indicate that the rhythm of vasopressin mRNA of the SCN remains unchanged during aging, indicating that this diurnal input to corticosterone rhythm remains intact in middle-aged rats. Furthermore, the dorsomedial hypothalamus also regulates rhythmic food intake, and the diurnal pattern of corticosterone is tightly regulated by feeding. Finally, the SCN is also able to directly influence the sensitivity of the adrenal to ACTH (24). Therefore, the diurnal rhythm of corticosterone may remain unchanged in middle-aged rats in the face of the changes in CRH/POMC gene expression due to stability in the SCN-driven rhythm of the AVP, feeding rhythms, and variation of sensitivity to corticosterone, all of which influence the corticosterone rhythm.

Perspectives

In summary, we have demonstrated that some age-related changes in the CRH/POMC axis can be restored by fetal transplants that contain the SCN. This strongly suggests that the aging hypothalamic/pituitary axis remains capable of responding to cues from the biological clock and that fundamental changes in the SCN or its ability to communicate with neuroendocrine outputs underpins the changes in the CRH/POMC axis. In the future, it will be necessary to investigate the age-related changes in the rhythms of CRH receptor and glucocorticoid receptors. It will also be important to investigate the cellular and molecular mechanisms by which the neural pacemaker maintains function in aging animals and the crucial factors from fetal tissues that communicate with hosts and the possible connections of donor-host.

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Address for reprint requests: P. M. Wise, Dept. of Physiology, Univ. of Kentucky College of Medicine, 800 Rose St., Lexington, KY 40536.

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