invited review

Neuroendocrine modulation of the "menopause": insights into the aging brain

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Wise, Phyllis M. Neuroendocrine modulation of the "menopause": insights into the aging brain. *Am. J. Physiol.* 277 (*Endocrinol. Metab.* 40): E965–E970, 1999.—The menopause marks the permanent end of fertility in women. It was once thought that this dramatic physiological change could be explained simply by the exhaustion of the reservoir of ovarian follicles. New data from studies performed in women and animal models make us reassess this assumption. An increasing body of evidence suggests that there are multiple pacemakers that contribute to the transition to irregular cycles, decreasing fertility, and the timing of the menopause. We will present evidence that lends credence to the possibility that a dampening and desynchronization of the precisely orchestrated neural signals lead to miscommunication between the brain and the pituitary-ovarian axis, and that this constellation of hypothalamic-pituitary-ovarian events leads to the deterioration of regular cyclicity and heralds menopausal transition.

estrogen; luteinizing hormone; gonadotropin-releasing hormone; menstrual cycle; circadian rhythm; neuroprotection

THE END OF THE REPRODUCTIVE LIFE SPAN has far-reaching consequences for women, because the ovarian follicle is not only the source of oocytes but also the primary source of estrogens that are important for maintaining normal functions as disparate as bone and mineral metabolism (27), memory and cognition (50, 51), cardiovascular function (47, 53, 61), and the frequency of age-related neurodegenerative diseases such as Alzheimer's disease (14, 34). The rapid increase in the average life span of humans, in the face of a fairly unchanging age of the menopause, has led to an increase in the number of women who will spend a larger proportion of their lives in a chronic hypoestrogenic state (56). Thus it becomes increasingly important to understand more thoroughly the mechanisms that govern the menopausal transition. Furthermore, a deeper understanding of the mechanisms regulating female reproductive aging will be important to gerontologists interested in brain aging, because, if the central nervous system is a key pacemaker of reproductive senescence, then we may gain a clearer view of the fundamental process of brain aging. In addition, because the female reproductive system undergoes such dramatic changes relatively early during aging, we hope that this system will allow us to address important questions regarding the biology of aging, in the absence of confounding pathological changes that often confound gerontological studies.

For many years, it was accepted that the menopause resulted simply from an exhaustion of the postmitotic endowment of ovarian follicles that is set down during embryonic development (60); the hypothalamic/pituitary changes that accompany the menopause were thought to be merely a consequence of declining ovarian function. More recently, several lines of evidence have led to the suggestion that the brain plays an important role in the sequence of events leading to reproductive senescence. It appears that the temporal patterns of neural signals are altered during middle age in both women and animal models, before the cessation of reproductive cycles, and may contribute to the loss of follicles leading to the menopause.

THE RODENT AS A MODEL FOR HUMAN REPRODUCTIVE AGING

These conclusions are based predominantly on studies performed in rodent models. Therefore, it is appropriate to ask whether data that are derived from these species will provide insights into the human menopause. In fact, there has been a lively discussion as to whether rodents are adequate models for human reproductive aging. In some sense, because rodents do not undergo a real menstrual cycle, by definition they do not undergo a true "menopause." Arguments that rodents are not good models are based on two observations. First, in postmenopausal women, plasma gonadotropin levels are high because of the lack of estrogen (68). In contrast, luteinizing hormone (LH) concentrations remain relatively normal in aged, acyclic, repeatedly pseudopregnant rats (29) despite substantial decreases in estradiol. This suggests that decreased hypothalamic influences are paramount to the postreproductive state in rats and may not be as critical in the human female. Second, it has been claimed that the dynamics of loss of the ovarian follicular reserve is fundamentally different in women compared with rodents. Richardson et al. (40) showed that the loss of primordial follicles is log-linear during the initial stages of life and accelerates dramatically around the time women are 37 yr old, leading to the total absence of follicles when women are between 50 and 55 yr old, when they are postmenopausal. Interestingly, no equivalent study has been performed in rodent models; therefore, we do not really know the dynamics of follicular

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loss in rodents. However, follicles have been reported in old acyclic rats, suggesting that exhaustion of ovarian follicles is not the limiting factor in rodents.

Despite these differences in older postmenopausal women and older acyclic rats, there are striking parallels between the human female and the female rat during the middle-aged pre- and perimenopausal periods. Therefore, we believe that rodents are likely to serve as excellent models in which to examine the factors that initiate the process of reproductive aging during middle age. For example, changes in estradiol, LH, and follicle-stimulating hormone (FSH) during the transition period are very similar. Several investigators (20, 26, 30, 45) have shown that estradiol concentrations do not decrease during the pre- and perimenopausal periods but in fact remain normal or are elevated. These new findings are strikingly similar to what Lu (29) observed in middle-aged rats as they enter the transition to irregular cyclicity. Likewise, in both humans and rats, an elevation in FSH concentrations has been considered one of the earliest hallmarks of the pre- and perimenopausal periods in women and the transition to irregular estrous cycles in rats (8, 18, 19, 49). In humans, the change is prominent during the periovulatory phase of the menstrual cycle. In a similar manner, middle-aged rats exhibit elevated FSH levels during estrous afternoon. These changes may result from decreased inhibin levels and/or alterations in the pattern of gonadotropin-releasing hormone (GnRH) secretion (8, 20). Another change that is similar in middle-aged women and female rats is the pattern of pulsatile LH release. The most recent report of Matt et al. (32) shows that, in regularly cycling middle-aged women, the duration of LH pulses increases and the frequency of pulses decreases. These data mirror the changes that we observed in middle-aged regularly cycling rats (46). To our knowledge, the work of Matt et al. (32) is the only study performed in regularly cycling premenopausal women. None of the other studies in humans has controlled for changing cycle length during the perimenopausal period when investigators monitored LH pulses, and this may underlie the discrepancy among studies in humans. In addition, the ability of estradiol to induce LH surges is attenuated in both perimenopausal women and middle-aged rats. Van Look et al. (59) showed that estradiol was able to induce LH surges of attenuated amplitude in only a small portion of the women studied. This parallels precisely the changes that we observed in middle-aged rats (63). Together, these studies argue strongly that pre- and perimenopausal women and middle-aged regularly and irregularly cycling rats share significant commonalities, particularly with regard to changes in exposure to estradiol, elevated FSH concentrations, patterns of LH secretion, and responsiveness to estradiol. Therefore, we firmly believe that the rat is an appropriate model for the study of reproductive aging. We anticipate that the results of studies performed in rodents will shed light on fundamental concepts underlying female reproductive aging that are likely to be applicable to the menopausal transition in women.

Some of the earliest evidence suggesting that the hypothalamus plays a role in reproductive aging came from two classical experimental approaches. First, transplantation of ovaries of old animals to the kidney capsule of young ovariectomized female hosts demonstrated that a permanently aged ovary could not explain the lack of cyclicity and ovulation. These ovaries revealed that follicular development and ovulation occurred under the influence of neuroendocrine signals of the young host (1, 36). Second, pharmacological methods showed that administration of drugs that restored the level of activity of monoaminergic neurotransmitters could restore cyclicity, albeit temporarily. Furthermore, progesterone treatment, which was thought to act centrally, or electrochemical stimulation of the preoptic area of old rats (3–6, 9–11, 13, 17, 38) resulted in restoration of estrous cyclicity. These results implicate changing hypothalamic function as a crucial element in reproductive decline. More recent studies that have focused more on the middle-age transition period suggest that hypothalamic changes may contribute to the onset of irregular cycles that ultimately lead to acyclicity (for review see Ref. 65).

ALTERATIONS IN THE TIMING AND INTENSITY OF ACTIVATION OF GnRH NEURONS MAY CONTRIBUTE TO CHANGES IN THE PATTERN OF LH SECRETION

Analysis of the secretory patterns of GnRH neurons has been difficult for several reasons. There are only ~1,000 GnRH-containing neurons, and they are scattered throughout the septo-preoptico-infundibular pathway of rodents and the preoptic and medial basal hypothalamus of humans (52). Because GnRH receptors are expressed in diverse regions of the brain, it is thought that these neurons subserve multiple functions, not all of which are directly related to gonadotropin secretion. It has been difficult to determine whether anatomically or morphologically discrete subpopulations of GnRH neurons are specifically dedicated to regulating LH and FSH, although recent data (37, 39) suggest that they may exist. For all of these reasons, it has been extremely difficult to monitor GnRH release patterns over time in individual animals under controlled experimental conditions. Other methods, including quantitation of mRNA levels to assess gene expression and use of dual-label immunocytochemistry to identify activated GnRH neurons, have allowed us to view the activity of GnRH neurons in aging animals.

Expression of immediate early gene products, such as Fos or Jun, by individual neurons can be used as markers of change in cellular activity. We have used this method to test whether alterations in the timing and amplitude of the proestrous LH surge result from alterations in the GnRH neuronal activity by assessing the expression of Fos within the nuclei of GnRH neurons. We and others have found that, in young animals, Fos is expressed in GnRH neurons coincident with both proestrous and steroid-induced LH surges (23–25). In contrast, in middle-aged, regularly cycling rats, the intensity of Fos staining in GnRH neurons is lower, the percentage of Fos-expressing GnRH neurons

is dramatically lower around the time of peak LH release, and this no longer correlates with serum LH levels (28). This implies that there is an age-related blunting or desynchronization of the neurochemical signals that are required to activate GnRH neurons involved in generating the proestrous LH surge. Rubin and Bridges (42) reported alterations in GnRH release from the mediobasal hypothalamus of steroid-primed middle-aged rats, as monitored by push-pull cannula methods. These functional changes become apparent before any detectable change in the number of immunoreactive GnRH neurons assessed in our study (28), in morphology or distribution of GnRH neurons of aging male rats (66) or any age-related differences in the distribution of GnRH-immunoreactive forms expressed in GnRH neurons (16). Thus the neurochemical signals that stimulate GnRH secretion and/or the ability of GnRH neurons to respond to stimuli change during the middle-age period, and these changes appear to precede changes in the ability to maintain regular estrous cyclicity.

Equivalent functional studies are virtually impossible in humans. To our knowledge, only two studies measuring GnRH have been performed in human females. Both monitored GnRH in postmenopausal women; no studies have attempted to follow GnRH neuronal changes before or during the perimenopausal transition. Parker and Porter (35) reported that radioimmunoassayable GnRH concentrations in the mediobasal hypothalamus were lower in postmenopausal women than in young women. More recently, Rance and Uswandi (39) found that GnRH mRNA levels in the tuberoinfundibular region, but not in the preoptic area, were elevated in postmenopausal women. Thus it is possible that transcription of the GnRH gene increases and that release of the peptide is elevated to an even greater extent such that steady-state mRNA levels are elevated, but that the stored pool of GnRH in the mediobasal hypothalamus is lower than in young women. Obviously much more needs to be done before one can clearly interpret these data or draw conclusions as to the factors that lead to such changes.

CHANGES IN THE TEMPORAL PATTERN AND SYNCHRONY OF NEUROTRANSMITTER EXPRESSION MAY ACCOUNT FOR ALTERATIONS IN THE PATTERN OF LH SECRETION

Changes in the pattern of GnRH expression and secretion may result from changes in one or more of the repertoire of neurotransmitters and neuropeptides that modulate neuronal activity. Over the last several years we have examined monoamine activity, neurotransmitter receptor densities, and the gene expression of some of the neuropeptidergic modulators of GnRH. The common theme that emerges from these studies is that the daily rhythmicity in the activity of many neurotransmitters, the density of their receptors, and/or the level of gene expression is greatly dampened or altered with age in hypothalamic regions involved in regulating the pattern of GnRH neuronal activity. We consistently observed a change by the time animals were middle-aged, as they were entering the transition to irregular cycles; often the change was progressive and more exaggerated in older rats that had completed the transition to acyclicity. These changes in rhythms are subtle and sometimes did not translate into any change in the average level of the end point that we quantified. Indeed, the gerontological literature is replete with seemingly contradictory data, some investigators finding changes and others unable to replicate these findings or reporting the opposite effects of age. Our data would suggest that investigators who measure these end points at any one time of day in aging animals may not have the complete picture of the potential changes that occur with age. Our data would suggest that disruption of the synchrony and coordination of multiple neural signals that govern the precise timing of GnRH release may ultimately lead to a decreased ability of rats to maintain regular estrous cycles. The studies of Everett et al. (12) clearly established the stringent temporal requirements for the maintenance of consistently timed cyclic LH surges that repeat every 4 days. They found that if the neurochemical signals that are prerequisite for the LH surge did not occur within a 2-h window, designated as the "critical period," then the surge was delayed by an entire day. The LH surge occurred on the following day at the proper time, and the cycle was lengthened by 1 day. Thus, it is clear that small changes in the temporal integrity of neurochemical events become greatly magnified in terms of the ability to maintain regular estrous cycles. The presence of a critical period is completely different from the effects of timing on other neuroendocrine rhythms, which can be phase-shifted by several hours without any major compounding impact on the peripheral endocrine rhythms that they drive. For example, desynchronization of neurochemical messages does not cause the corticotropin-releasing hormone-adrenocorticotropic hormone-glucocorticoid rhythm to skip an entire day. There is some evidence that the human menstrual cycle also has a circadian basis (7, 31, 41, 48). However, rigorous testing of the relative importance of circadian rhythms vis-à-vis the maintenance of regular menstrual cyclicity has never been tested.

CHANGES IN THE ABILITY OF THE CIRCADIAN BIOLOGICAL CLOCK TO KEEP TIME MAY UNDERLIE THE DESYNCHRONIZATION OF MULTIPLE NEUROCHEMICAL AND NEUROENDOCRINE RHYTHMS

Our findings that reproductive decline is accompanied by changes in such a broad spectrum of neural rhythms led us to hypothesize that a fundamental deterioration in the integrity of the "biological clock" or its intercellular coupling to outputs may cause increasing temporal disorganization in the broad array of neurotransmitter rhythms that are critical for stable, precise, and regular reproductive cycles. The suprachiasmatic nuclei (SCN) are the master circadian neural pacemakers, or biological clock, in mammals (33, 55). These bilateral nuclei, which consist of ~10,000 neurons located at the ventral border of the brain, dorsal to the optic chiasm, exhibit endogenous circadian rhyth-

micity: they continue to exhibit endogenous 24-h rhythmicity in electrophysiological activity and neuropeptide secretion patterns, even when maintained in vitro (54). Efferent connections to various regions of the brain communicate time-of-day information and drive the timing of multiple outputs, resulting in circadian rhythmicity in most physiological functions. If destroyed, the circadian rhythms of most functions (e.g., drinking, rest/activity, endocrine, temperature, metabolic) disappear. To test whether the overall neural rhythm of the SCN is altered with age, we assessed local cerebral glucose utilization (LCGU) in the SCN of young and middle-aged rats (64). Glucose utilization is an indirect measure of the level of neural activity. Because the brain utilizes predominantly glucose as its metabolic substrate, glucose utilization increases when neural activity increases, and vice versa. We found that the diurnal rhythm in LCGU in the SCN was altered by the time rats reached middle age: the response to the light-dark cycle was dampened, and LCGU increased in advance of lights on and decreased earlier in anticipation of lights off in middle-aged compared with young rats. It is possible that the altered timing of activity of cells within this circadian pacemaker region of the brain may lead to alterations in the timing of neurotransmitter signals required to trigger an LH surge or to maintain LH pulses of normal duration, amplitude, and frequency. A delay or increased variability of diurnal hormone release may, in turn, lead to estrous cycles of irregular and unpredictable length, and, ultimately, to acyclicity.

Support for the concept that changes in the biological clock may occur during middle age comes from three lines of evidence. First, transplantation of fetal tissue containing the SCN into middle-aged rats restored the light-induced pattern of Fos expression in the host (2). We utilized this index of the integrity of the SCN because Fos immunoreactivity clearly increases after lights on in young rats, and the intensity of the light stimulus is directly related to its ability to entrain running behavior and its ability to induce Fos in the SCN (21, 44, 67). Fos expression in young rats was virtually undetectable during the dark, and light onset induced a dramatic increase. In contrast, middle-aged rats exhibited premature Fos expression during the dark and a markedly blunted response to light. When we transplanted fetal SCN into the third ventricle of middle-aged animals, the pattern of Fos immunoreactivity was restored to a pattern that was temporally and anatomically similar to that of young animals. These data clearly show that the aging host SCN retains functional capacities, remains plastic and responsive to environmental cues, and is not permanently damaged by death of cells involved in responding to retinal input. They infer that important factors that regulate the temporal pattern of expression of the SCN are absent by the time rats reach middle age, and fetal tissue transplants that contain the SCN can provide these factors or induce their expression in the host.

Second, we tested whether we could produce aging-like effects on the LH surge by mimicking the changes that normally occur in one of the neuropeptides within the SCN that is thought to regulate the timing of GnRH release. Recent evidence suggests that vasoactive intestinal peptide (VIP) is one of the most abundantly expressed neuropeptides in the SCN (43) and communicates time-of-day information from the SCN to GnRH neurons (57, 58). Furthermore, the rhythmic expression of VIP mRNA disappears by the time females reach middle age (22). Therefore, we used antisense oligonucleotides, which targeted VIP-containing neurons in the SCN, to test the hypothesis that this treatment selectively suppresses VIP in this region of the brain and that this would lead to an aging-like effect on cyclic LH secretion (15). We infused antisense oligos into the peri-SCN region through stereotaxically placed bilateral cannulas and monitored the effects on the estradiol-induced LH surge 2 days later. Peak LH levels during the surge were delayed and attenuated in antisense-treated, compared with random oligo-treated, control rats in a manner that is strikingly similar to that observed previously in middle-aged rats (62). The effects of antisense VIP were anatomically and neurochemically discrete, as they did not influence VIP concentrations in other regions of the brain or vasopressin concentrations in the SCN. These data clearly show that suppression of a single neuropeptide in the SCN can mimic the effects of age on the estradiol-induced surges of LH. They support the concept that VIP neurons in the SCN play a central role in the regulation of the LH surge and suggest that age-related perturbations in the integrity of this axis may account for alterations in the pattern of LH secretion observed during middle age.

SUMMARY

During the past few years, increasing evidence suggests that no single player in the brain-pituitary-ovarian axis can explain the dramatic change that occurs during the perimenopausal transition. Evidence suggests that changes in both neural and ovarian pacemakers lead to a decline in regular reproductive cycles and increasing infertility and, ultimately, to the postreproductive state. It appears that the precision with which neurochemical signals are communicated to GnRH neurons deteriorates, leading to alterations in the timing of the preovulatory LH surge and changes in the frequency of LH pulses. Ovarian steroid and peptide secretion may also be altered due to the diminishing pool of ovarian follicles. Together, the interplay of these factors brings about the gradual age-related decline in reproductive function.

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