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20. Aging of the Endocrine Tissue

AGING OF THE HYPOTHALAMO-PITUITARY-OVARIAN AXIS: HORMONAL INFLUENCES AND CELLULAR MECHANISMS

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Summary—Longitudinal studies employing heterochronic ovarian grafts and long-term ovariectomy indicate that there is no single pacemaker of reproductive aging. Neuroendocrine dysfunction, the declining follicular reserve, and ovarian secretions all contribute to reproductive decline, and their relative importance to the different stages of reproductive aging varies markedly. Moreover, although ovarian secretions during adulthood potentiate certain aspects of the reproductive aging process, their behavior does not fit a simple model of cumulative steroidal damage incurred over the lifespan. Current data are more consistent with temporally distinct windows of steroidal vulnerability for the events affected: cycle lengthening is affected by ovarian secretions during the period of cyclicity, and post-cyclic neuroendocrine failure is potentiated by ovarian secretions during the peri- and post-cyclic period of the lifespan. Recent examination of estradiol receptor dynamics reveals multiple, albeit selective, changes during aging that may contribute to the age-related impairments of tissue sensitivity to estrogen. These changes vary qualitatively and quantitatively among target tissues. Thus, aging of the hypothalamo-pituitary-ovarian axis at the cellular level mirrors, in its multifactorial nature, aging at the organismic level.

INTRODUCTION

Among the most striking and detrimental aspects of mammalian aging is the declining ability of the organism to adequately respond to the internal and external demands placed upon it. The mobilization of energy reserves during stress [1], of immunological defenses during infection [2, 3] and the preparation for and maintenance of pregnancy [4] all show marked impairments with advancing age. Because most of these responses involve one or more endocrine systems, the endocrinology of aging has become an important area of clinical and basic gerontology. Although the hormonal correlates of altered endocrine responsiveness during aging have been widely studied, we remain largely ignorant of molecular mechanisms and etiological factors.

In this report, we summarize some of our recent findings on the hormonal and cellular mechanisms associated with reproductive aging in the female. Our approach has been to identify major physiological changes regulated by the hypothalamo-pituitary-ovarian (HPO) axis, and then to search for the hormonal and molecular mechanisms that are responsible for these changes. The major findings of this work fall into two areas. The first concerns the relative contributions of three elements of the HPO system—the hypothalamic–pituitary unit, the aging ovary, and chronic ovarian secretions—to the reproductive aging process. The second involves the potential role of altered estradiol receptor dynamics in the diminished sensitivity of aging target tissues to the effects of estradiol.

The studies described have used the C57BL/6J mouse as a model of reproductive aging. Although the general aspects of the reproductive aging process are common to most mammalian species, the detailed patterns and, undoubtedly, the mechanisms vary considerable [4]. Thus, the present findings may not apply *in toto* to other strains or species, a caveat that calls for extending these studies to other genotypes.

MAJOR TRANSITIONS IN THE AGING OF ESTROUS CYCLICITY

The major events preceding and following cessation of cyclicity in the C57BL/6J mouse are shared by many genotypes [4]. After a peripubertal period of irregular, predominantly long cycles, cycles shorten to a modal length of 4 days. Peak cyclicity lasts for 4–6 months. The onset of HPO aging is signalled by a transition from predominantly 4-day to predominantly 5-day cycles around 9 months [5]. This shift is followed by a period of progressively lengthening cycles which terminate in acyclicity between 12 and 14 months of age. Cessation of cyclicity is followed by one of two states: persistent vaginal cornification (PVC) or persistent diestrus (PD). PVC usually lasts for 2–4 months [6], and is characterized by anovulatory, polyfollicular ovaries and an elevated estradiol: progesterone ratio relative to that of the cycling state [7]. The probability of entering PVC diminishes with advancing age of onset of acyclicity [6]. The final stage in all mice is persistent

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diestrus, a hypogonadal hypergonadotropic state [8] which continues until death.

NEUROENDOCRINE VERSUS OVARIAN CONTRIBUTIONS TO CYCLE LENGHTENING AND CESSATION

Which components of the HPO axis are primarily responsible for the various events of reproductive aging described above? The preovulatory LH surge declines in magnitude prior to the cessation of cyclicity in both rats (9) and mice (10), as do follicular reserves [11–15]. These and other decrements in both neuroendocrine and ovarian function [4, 16] indicate that either locus could be responsible. Thus, their relative rates of decline and minimal functional thresholds will largely determine whether neuroendocrine or ovarian aging plays the major role in limiting a particular event of reproductive aging.

Krohn [17] was among the first to employ heterochronic transplantation of tissues to determine the relative contributions of a tissue and its environment to its altered function. Applying this approach to the aging reproductive system, he found that replacing ovaries of middle-aged CBA mice with ovaries from young donors restored the hosts' cyclicity [18]. This was the first study to show conclusively that ovarian aging was the principle factor limiting the cycling lifespan of a species. Age changes extrinsic to the ovary (i.e. neuroendocrine) were clearly secondary.

We have employed Krohn's paradigm to factor out the relative importance of ovarian and extra-ovarian (presumably neuroendocrine) contributions to the first three stages of the HPO aging outlined earlier: (1) the 4- to 5-day transition, (2) the 5- to >5-day transition, and (3) cessation of cyclicity. In this study, young (Y, 2 month) ovaries were grafted into middle-aged (M, 13-month) mice shortly before they would normally have ceased cycling. Only middle-aged hosts that were still cycling were used, to avoid confounding the results with the potentially disruptive effect of the acyclic state on the ability to cycle. Cycles were monitored until all mice had ceased cycling.

In middle-aged hosts bearing ovarian grafts from young donors (Y→M) the cycling lifespan was prolonged by about 60% relative to the normal age of cycle cessation (cf. M; Fig. 1). These data unequivocally demonstrate that the aging ovary plays the major role in limiting the cycling lifespan. The functional capacity of the neuroendocrine component to support cyclicity extends well beyond the normal age of cycle cessation, and is at most a secondary influence on this event. That the aging ovary is the major pacemaker of this cycle cessation was confirmed by the observation that ovaries from

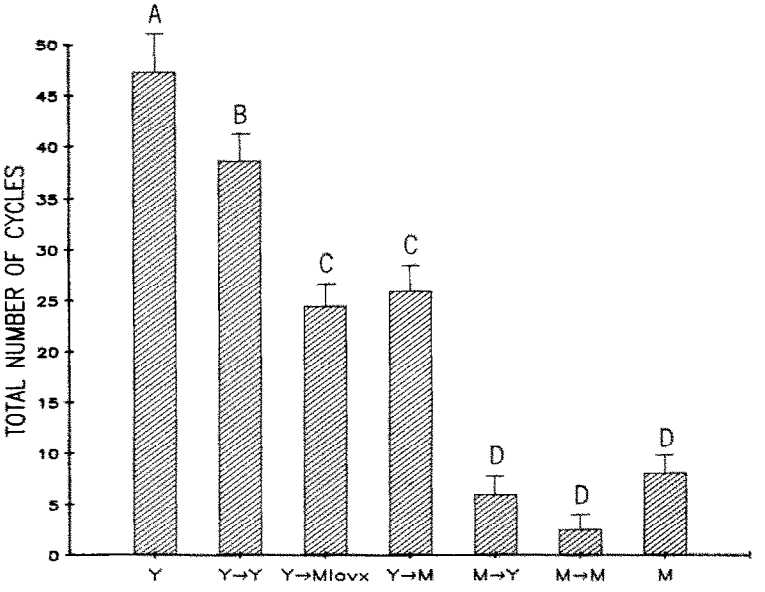

Fig. 1. Effect of grafts of hetero- and homochronic ovaries under the renal capsule on total number of estrous cycles ($X \pm$ SEM) experienced by hosts. Total cycles are calculated from the time of grafting and are compared to the number of cycles experienced by intact, age-matched mice. Young (Y) (2-month-old) or middle-aged (M) (12-month-old) ovaries were grafted into young and middle-aged mice that were acutely ovariectomized. Young ovaries were also grafted into middle-aged mice that had been ovariectomized (Y→Mlovx) at 3 months of age. This experiment was done to assess the effect of exposure to ovarian secretions before the cessation of cyclicity on the response to the grafted young ovaries. Intact control groups are designated Y and M. Recipients of grafted ovaries are designated with two letters, the first indicating the age of the donor and the second indicating the age of the host (e.g. Y→M designates a Y donor and an M host). Total cycles experienced by groups with different alphabetical letters are significantly different ($P < 0.05$, Duncan's Multiple Range Test). (Redrawn from Ref. [29].)

middle-aged donors grafted into young hosts (M→Y) reduced their cycling lifespan to that of the middle-aged donors (Fig. 1).

In addition to prolonging the cycling lifespan, ovarian grafts from young donors reduced the incidence of very long (i.e. >5-day) cycles in middle-aged mice to that of young mice (cf. M vs Y→M, Fig. 2). This finding, coupled with the observation that middle-aged grafts increased the incidence of very long cycles in young hosts to that of middle-aged mice, indicates that ovarian aging is also the primary cause of the transition from 5-day to longer cycles. Moreover, the completeness of the age reversals achieved by these reciprocal grafts suggests that neuroendocrine aging plays virtually no role in this transition.

Although ovarian grafts from young mice extended cyclicity and delayed one cycle length transition, they were completely unable to increase the incidence of 4-day cycles in middle-aged mice (cf. M vs Y→M, Fig. 2). Thus, neuroendocrine dysfunction appears to play an important role in the early transition from 4- to 5-day cycles. Yet, a potentiating role of the aging ovary is also indicated by the observation that ovaries from middle-aged donors grafted into young mice halved the incidence of 4-day cycles (cf. Y vs M→Y, Fig. 2).

Thus, the relative contributions of the aging ovary and neuroendocrine dysfunction to cycle prolongation and to cycle cessation vary markedly. In addition to revealing the multifocal etiology of reproductive decline, these results have facilitated our efforts to ascertain the underlying causes by revealing, for each event of aging, the most likely sites of aberrant cellular function and hormonal activity.

HORMONAL INFLUENCES AND CELLULAR MECHANISMS OF HYPOTHALAMO-PITUITARY-OVARIAN AGING

What clues do we have concerning the mechanisms of HPO aging? Evidence about the mechanisms that are acutely responsible have come largely from what is known about the biochemical and neuroendocrine systems that are involved in regulation of normal reproductive function [4, 16, 19, 20]. Evidence about the factors that precipitate these changes—about the etiology of reproductive aging—is much more limited, but mainly is derived from early work which revealed that exposure to ovarian steroids, both during a critical neonatal period [21] and during adulthood [22, 23] led to the premature loss of estrous cyclicity.

The role of ovarian secretions: event specificity and windows of vulnerability

Ascheim [22] first showed that ovariectomy of rats in early adulthood preserved their ability to cycle upon receipt of ovarian grafts in old age. Animals that had thus been exposed to ovarian secretions throughout adulthood failed to cycle when given young ovaries. Moreover, chronic administration of low doses of estradiol accelerated the onset of acyclicity in rats [23]. These results led to the hypothesis that cumulative exposure to the ovarian secretions of successive estrous cycles gradually damaged neuroendocrine centers regulating ovulation and thereby resulted in acyclicity. In the rat, this hypothesis has not been properly tested, although some indirect evidence is consistent with it [24–27].

In the C57BL/6J mouse, however, neuroendo-

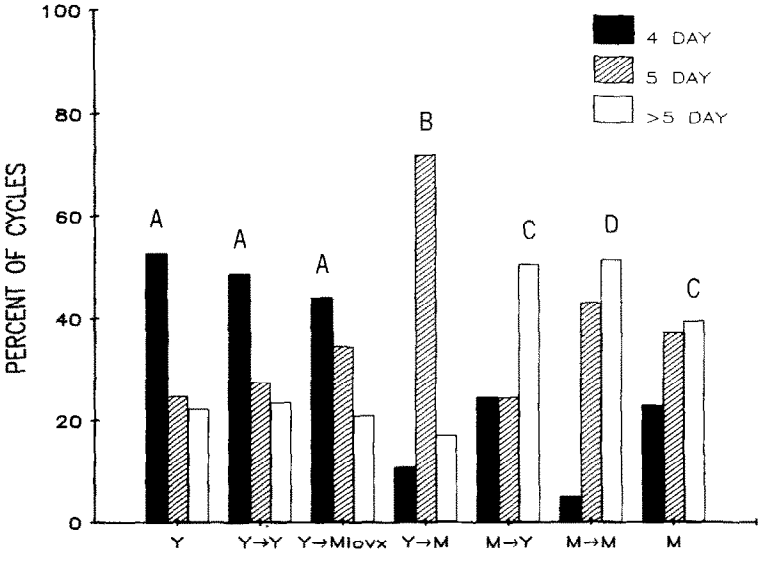

Fig. 2. Effect of grafts of hetero- and homochronic ovaries on the frequency distribution of cycle lengths during the first 2 months following grafting. See Fig. 1 for description of treatment groups. Groups with different alphabetical letters have significantly different frequency distributions ($P < 0.05$, G-test). (Redrawn from Ref. [29].)

crine damage from cumulative exposure to ovarian secretions during the cycling lifespan clearly does not play a major role in the cessation of cyclicity; heterochronic grafting has shown that ovarian failure is the major cause of acyclicity (Fig. 1). Moreover, withdrawal from ovarian secretions during the cycling lifespan confers no advantage to the cycling lifespan of middle-aged animals given young ovaries (cf. Y→M vs Y→Mlovx, Fig. 1). The question arises whether there is any deleterious role of ovarian secretions in mice? We have obtained evidence that there are at least two roles: one is played before the cessation of cyclicity, and the other appears to be played predominantly thereafter.

Although long-term ovariectomy has no perceptible influence on the cycling lifespan of middle-aged mice, it protects against the initial neuroendocrine-dependent transition from 4- to 5-day cycles. If mice have been ovariectomized since early adulthood and given grafts of young ovaries, their modal cycle length returns to 4 days and to a level that is indistinguishable from that of young controls (cf. Y→M vs Y→Mlovx, Fig. 2).

In addition to its protective effect on the 4- to 5-day transition, long-term ovariectomy protects against the loss of neuroendocrine function which arises after the cessation of cyclicity [28]. Young ovaries can markedly extend the cycling lifespan of middle-aged mice but become progressively less able to do so as mice grow older, unless they have been long-term ovariectomized (cf. [28, 29]). The evidence that this deleterious action of ovarian secretions occurs predominantly post-cyclically and thus is temporally distinct from the effect of ovarian secretions on the 4- to 5-day transition is as follows. First, when withdrawal from ovarian secretions is restricted to the period of regular cyclicity, no prolongation of the functional lifespan of neuroendocrine mechanisms regulating cyclicity is achieved. Only if the period of steroidal withdrawal includes the post-cyclic period is prolongation achieved. Second, even a 6-week withdrawal from ovarian secretions during the post-cyclic period potentiates cyclicity [30], although a similar period of withdrawal at an earlier age confers no benefit to an already functionally impaired cycling system.

There are several possible reasons why ovarian secretions during the post-cyclic period may be more deleterious. The hormonal milieu associated with PVC, the most common postcyclic state, may be more pathogenic than that associated with cyclicity. In young rats exposed to a PVC state, the arcuate nucleus is more vulnerable to glial hyperactivity than in regularly cycling rats [31]. Moreover, in young mice the PVC state may be slightly more deleterious to the ability to cycle than the hormonal milieu associated with cyclicity [32]. However, not all mice enter PVC after ceasing to cycle, yet all mice show ovarian-steroidal dependent acyclicity [28]. This suggests that hormonal milieus other than those associated with PVC are also deleterious. We recently simulated the PVC state in young mice by radically depleting their ovarian follicular reserves. The resultant PVC was only transiently, and slightly, more deleterious to the cycling potential of mice than their normal hormonal state associated with regular cyclicity [32]. Although this PVC may not be hormonally homologous with that occurring spontaneously, the result of this study is consistent with the possibility that the vulnerability to ovarian secretions, regardless of their nature, may increase with age. Whether and how increased steroidal vulnerability occurs remains to be determined.

Table 1 summarizes our knowledge of the relative contributions of ovarian and neuroendocrine aging, and of chronic ovarian secretions to the major events of reproductive aging that we have examined. Although our knowledge is poorly resolved at this point, the present data indicate a greater complexity to the etiology of reproductive aging at the tissue and organ level than heretofore demonstrated. Recent studies reveal a similar complexity at the cellular and molecular level.

Altered estradiol receptor dynamics during aging: multiple defects and tissue differences

Estradiol (E2) plays three key roles in female reproductive aging. It is believed to be the major component of those ovarian secretions which

Table 1. Role of ovarian aging, neuroendocrine dysfunction and chronic exposure to ovarian secretions to major events of hypothalamo-pituitary-ovarian aging in the C57BL/6J mouse

	Etiological component			
		Neuroendocrine		
Event	Ovarian	Ovarian-secretion-dependent	Ovarian-secretion-independent	Ref.
4–5-day cycle	Yes	Yes	?	29
5–>5-day cycle	Yes	No	No	29
Acyclicity	Yes	No	No	28, 29
Post-cyclic loss of cycling potential	No	Yes	Yes	28, 29

potentiate certain neuroendocrinological aspects of the aging process [4, 19]. Second, the circulating concentrations of E2 generally, though not always, decrease with age, thereby contributing to suboptimal function of target tissues [4, 20]. Finally, target tissue sensitivity to E2, at least in terms of certain responses, diminishes [4, 19]. Understandably, a number of laboratories have begun to examine the intracellular estradiol receptor (ER) to determine whether there are any alterations in its properties that might provide a molecular basis for altered tissue responsiveness to estrogen.

Most studies have only measured basal levels of ER or ER content at a single time after challenging the organism with exogenous E2. The results of those studies indicate losses in both cytosolic [33, 34] and nuclear ER [35, 36]. However, none of these studies has evaluated the dynamics of nuclear ER binding. Thus, it is difficult to determine whether the reduced nuclear binding that has been observed is a consequence of an absolute defect in translocation or only the result of a delay in achievement of maximal nuclear binding. The question of duration of nuclear binding has also received little attention. These questions are important given the evidence that the duration as well as the magnitude of cell nuclear steroid binding is a determinant of steroidal effectiveness [37, 38]. One set of studies has assessed the intracellular dynamics of ER during aging [39, 40]. Although peak ERn was reduced, sampling frequency during the period of peak nuclear binding was insufficient to distinguish among the possible explanations outlined above for this apparent reduction of ERn. Moreover, pooling of hypothalamic and pituitary tissue hindered the interpretation of the data [40].

We therefore undertook a detailed examination of the effect of aging on the intracellular dynamics of ER following injection of a bolus of E2 in hypothalamus, pituitary and uterus. This approach was taken in an attempt to factor out the relative importance of absolute loss of receptor, retarded rate of nuclear translocation and absolute loss of translocatability on the reduced nuclear binding observed by others.

Figure 3 shows the results of nuclear binding in the hypothalamus. Overall, ERn was significantly reduced in aged hypothalamus ($P < 0.001$ ANOVA), as well as in pituitary and uterus (data not shown). However, the lower profile of elevated ERn in old mice was not due to an inability of ER to translocate, but to a more rapid loss of ER from the cell nuclei ($P < 0.05$, age × time interaction). Peak nuclear binding in old hypothalami did not differ from that in young, nor did it differ in the uterus or pituitary, though all tissues showed a lower exposure to elevated ERn following E2 injection (Table 2). Although the decline of ERn following attainment of peak levels was more rapid in the aged hypothalamus, as well as the pituitary, the aging uterus remained unchanged in this regard (Table 2). However, both the uterus and the pituitary showed a retarded rate of translocation; peak binding was not achieved until 2 h following E2 injection in old tissues, whereas it occurred by 1 h in young tissues. Thus, the relative contributions of these various

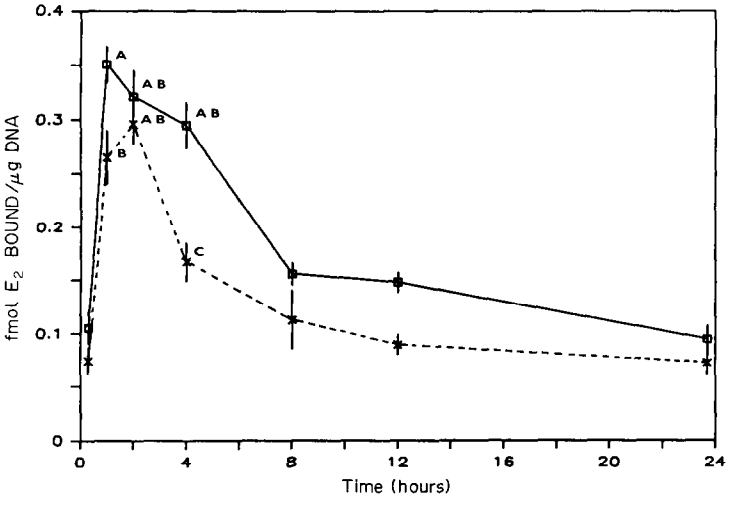

Fig. 3. The effect of aging on the profiles of hypothalamic nuclear estradiol receptors (ERn) following injection of E2. Intact young (□—□) (3–6-month-old) and old (×—×) (22–24-month-old) mice were injected subcutaneously with E2 in corn oil at a dose sufficient to achieve maximal ERn (0.05 μg/10 g body wt) and sacrificed 1, 2, 4, 8, 12, and 24 h later (time 0 = vehicle). ERn was measured by a sodium thiocyanate-potentiated exchange assay at 4°C, validated for the mouse. ERn was assayed by incubation with 3H-E2 +/− 100-fold excess radioinert E2. Bound and free steroid were separated by LH-20 chromatography. Points with alphabetical letters are significantly elevated above baseline (time 0) (Student Newman–Keuls; $P < 0.05$). Points with different letters are significantly different from each other (SNK; $P < 0.05$). Each point represents the mean ± SE of 4–14 determinations at a saturating concentration of 3H-E2, with 2–3 animals per determination.

Table 2. Effect of aging on cell nuclear estradiol-receptor dynamics following a bolus injection of E2 in three target tissues of C57BL/6J mice*

Tissue	Elevated nuclear binding† (old compared to young)			
	Peak	Rise	Fall	Integrated area
Hypo	ND‡	ND	Faster	−25%
Pituitary	ND	Slower	Faster	−25%
Uterus	ND	Slower	ND	−34%

*Tissues were assayed for nuclear estrogen-receptor binding as described in Fig. 3.

†Elevated nuclear binding consisted of all binding points significantly above baseline. Areas under these points above baseline were cut out and weighed in young and old mice to obtain a measure of overall cell nuclear exposure to estradiol receptors.

‡Not different.

lesions to declining cell nuclear exposure to ERn vary across target tissues.

CONCLUSIONS

These studies indicate that the etiology of reproductive aging involves complex, but discernible, sets of interactions between functionally declining components and intrinsic potentiating factors. Moreover, they have revealed that this complexity is present at both the tissue and molecular levels. It is probable that the aging of other physiological systems are similarly complex. Thus, a multifactorial, multidisciplinary approach is called for to elucidate the causes of declining physiological function with advancing age.

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