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THE ROLE OF THE HYPOTHALAMUS IN AGING OF THE FEMALE REPRODUCTIVE SYSTEM

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Summary—We have investigated the role of neuroendocrine and neurochemical changes in the age-related deterioration of cyclic female reproductive function. During middle age the timing and amplitude of the proestrous and estradiol-induced LH surge is altered. We have found that the diurnal pattern of norepinephrine turnover is altered in critical hypothalamic areas known to regulate the release of LHRH. These changes may contribute to alterations in the timing and the amplitude of LH release, which may, in turn, affect the ability of rats to maintain regular estrous cycles.

INTRODUCTION

The reproductive system has been used as a model system in gerontological research because of several reasons. First, there are several theories on the relative importance of extrinsic vs intrinsic factors in the aging process [1]. The reproductive system is one which can be manipulated to assess the role of hormonal milieu (extrinsic) vs the genome (intrinsic) in the timing of age-related changes. Second, the reproductive system of females exhibits overt deterioration relatively early during the lifespan of the animal in several species (for review see Ref. [2]). Therefore, we can anticipate that alterations that we observe in reproductive function are likely to be related to changes in this particular axis. The observed changes in the hypothalamus, pituitary gland and gonad during middle age are probably not secondary to generalized deterioration of the circulatory or nervous system. Third, in the female successful reproduction depends upon an exquisitely interactive and complex set of rhythmic events. Hence, this system provides the researcher with the opportunity to differentiate primary alterations in one component of the axis from secondary repercussions in other components of the axis.

In most mammalian species, females reproduce during a limited period of time during their lives. Reproductive capacity is maintained from the time of sexual maturity through the first third of their lifespan. Thereafter, the frequency of normal births decreases and reproductive cycles cease well before the average lifespan is reached. The "perimenopausal" period is characterized by cycles of variable length. In humans, Korenman[3] reported that the length of each menstrual cycle during the perimenopausal period may be variable due to changes in the length of the follicular phase of the cycle. In laboratory animals, estrous cycles of variable length have been documented in middle-aged rodents from the time they are 8–18 months of age [4, 5]. In addition, during the middle-age period, there is decreased fertility and an increasing frequency of fetal mortality and abnormal young at birth [6–10]. The goal of several laboratories has been to better understand the causes of the increasingly irregular cycles and ultimate total acyclicity and infertility. We have focused our attention upon the role of changing hypothalamic function in the transition to estrous acyclicity.

Several studies suggest that hypothalamic function is altered by the time that reproductive cycles no longer occur. First, estrous cyclicity can be reinstated when old, acyclic constant estrous rats are treated with a variety of centrally acting pharmacological agents such as 1-dopa [10–14], lergotrile mesylate [15], iproniazid [13], ether [12] or electrochemical stimulation of the hypothalamus [16]. Second, aging rats are less responsive to the positive [17] and negative [18] feedback effects of estradiol. Since estrogen's effects on gonadotropin secretion are thought to be mediated predominantly by action at the hypothalamic level [19, 20], the age-related changes in responsiveness to steroid may be due to changes at the hypothalamic level. Third, in old rats, monoamine activity is altered in several brain areas, including key hypothalamic areas known to be involved in gonadotropin secretion [21, 22]. Finally, that changes at the level of the hypothalamus contribute to the onset of acyclicity is suggested by the studies of Aschheim [23] and Peng and Huang [24], who found that ovaries of old animals transplanted into young hosts were able to maintain ovulatory cycles. More recently, Felicio *et al.*[25] reported that young ovarian grafts placed in aging mice were able to restore ovulatory cycles; however, the duration of this restorative effect was limited by progressive neuroendocrine dysfunction. These elegant data clearly demonstrate that both neuroendocrine and ovarian factors contribute to the age-related deterioration of the female reproductive system.

We have focused our attention on the possible

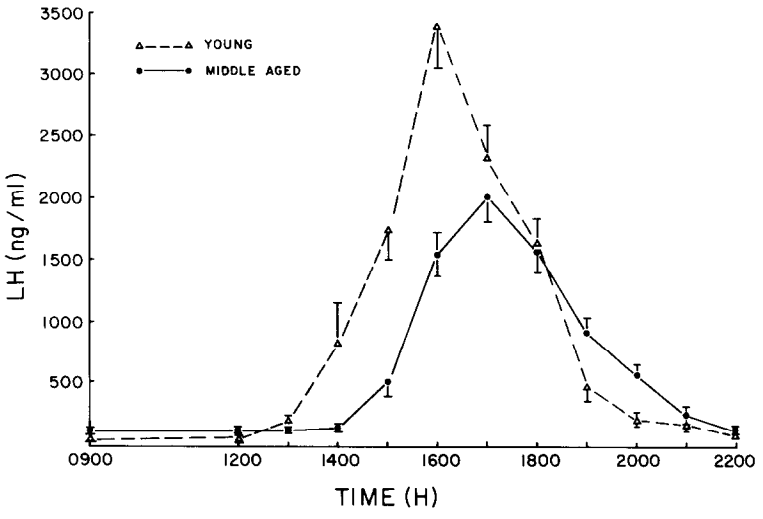

Fig. 1A. Plasma LH (RP-1) concentrations in young and middle-aged rats on proestrus. Rats were bled from right atrial cannulae a maximum of 9 times during the day; all rats were bled at hourly intervals between 1400 and 1800 h. Values represent mean ±SE. (From Ref. [26] with permission.)

hypothalamic changes that occur in middle-aged rats that may contribute to the onset of irregular estrous cycles. We [26] and others [11, 27] have reported that changes in the timing and the amplitude of preovulatory LH surges occur with age. We used young (3–4-month-old) and middle-aged (7–9-month-old) Sprague–Dawley rats. They were cannulated via the external jugular vein to the level of the right atrium early on proestrous morning and then sequentially bled during the entire day. LH, FSH and prolactin levels were measured by radioimmunoassay. The data show that both LH and FSH rose later during the afternoon and peak concentrations of both of these hormones were significantly lower in middle-aged rats compared to their young counterparts (Fig. 1). In contrast, the timing and the amplitude of the preovulatory prolactin surge was not affected by age.

Several hypothalamic factors could be altered on proestrus in middle-aged rats and contribute to the observed alteration in pattern of preovulatory gonadotropin release. We first considered the possibility that plasma estradiol concentrations and/or hypothalamic or pituitary concentrations of estradiol receptors were altered and would therefore not allow estradiol positive feedback to occur normally. Groups of young and middle-aged proestrous rats were decapitated at 0900, 1200, 1500 or 1800 h and serum estradiol was measured by radioimmunoassay [28]. Estradiol concentrations were virtually the same in both age-groups and therefore inadequate estradiol cannot explain the

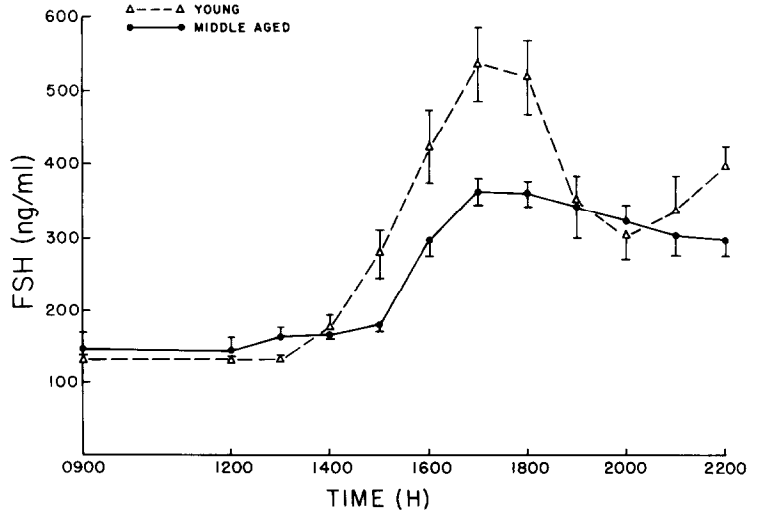

Fig. 1B. Plasma FSH concentrations in young and middle-aged rats on proestrus. The same rats were used as shown in Fig. 1A. (From Ref. [26] with permission.)

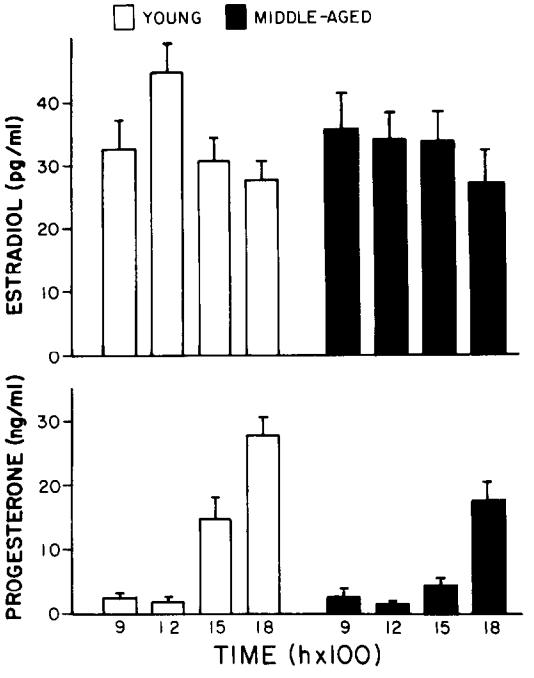

Fig. 2. Serum estradiol (above) and progesterone (below) concentrations on proestrus in young (clear bars) and middle-aged (dark bars) rats. Columns represent mean ± SE. (From Ref. [28] with permission.)

delay and attenuation of the preovulatory LH (Fig. 2) surge observed in middle-aged rats. More recent evidence by Nass and colleagues [27] and Sopelak and Butcher [29] demonstrates that estradiol concentrations are elevated for a longer period of time in middle-aged proestrous rats than in young rats. Together these data suggest that adequate levels of estradiol exist in circulation to allow normal positive feedback to occur.

We next assessed estradiol receptor concentrations in hypothalamic areas and the pituitary gland of middle-aged rats, since estradiol positive feedback depends upon normal estradiol receptor levels in these neuroendocrine target areas [30]. The dissociation constant and maximal estradiol nuclear receptor concentrations were determined in the preoptic area, medial basal hypothalamus and pituitary gland of young (3–4-month-old), middle-aged (8–11-month-old) and old (16–18-month-old) female rats [31] using an exchange assay [32]. The results demonstrate that by middle age significant changes occurred in the maximal number of estradiol nuclear receptors in the preoptic area only. No change was observed in the medial basal hypothalamus, the amygdala or the pituitary gland (Table 1). In older rats (16–18-month-old) a significant decline in the maximal number of estradiol nuclear receptors expanded anatomically to include the medial basal hypothalamus and the pituitary as well. That we observed the first significant decrease in maximal estradiol nuclear receptor number in the preoptic area is intriguing since this area of the brain is thought to be particularly important in the induction of preovulatory LH surges [19, 33] and the maintenance of biological rhythms including reproductive cycles [34]. Thus, changes in function in this area of the brain may affect the ability of aging females to maintain regular cyclic functions. Decreased estradiol uptake in the hypothalamus and pituitary [35] and decreased binding to cytoplasmic [36, 37] and nuclear [38] estradiol receptors in various areas of the brain and pituitary of non-cycling rats has been reported previously. In addition, age-related changes in other estradiol target tissues have been documented in rats [39] and mice [40]. In a follow-up study we [41] determined whether (1) the changes we observed in the preoptic area of middle-aged rats were evident in selected specific areas of this brain region and (2) changes in estradiol receptor concentrations were detectable in other regions of the brain when a microdissection technique, which permitted finer separation of discrete brain areas, was utilized. Young (3–4-month-old) and middle-aged (10–11-month-old) cycling rats were ovariectomized for 1 week prior to sacrifice to allow maximal translocation of receptor into the cytoplasm. Eleven brain areas were microdissected and the pituitary gland was removed and analyzed for receptor content using the assay of Rainbow *et al.*[42]. We observed significant changes in the

Table 1. Effect of age on dissociation constant and maximal number estradiol nuclear receptors in various brain areas and the pituitary gland

		Dissociation constant (10^{-10} M)	B_{max} (fmol/mg DNA)
Preoptic area	Young	1.01	450
	Middle-aged	0.81	358
Medial basal hypothalamus	Young	1.42	363
	Middle-aged	0.89	282
Amygdala	Young	0.81	260
	Middle-aged	0.98	266
Pituitary	Young	2.92	1175
	Middle-aged	4.15	1124

suprachiasmatic–preoptic area and the medial preoptic nucleus, with a similar trend in other areas (Fig. 3) which comprise the "preoptic area" dissected in our previous study [31]. The power of the more discrete dissection was most evident when analyzing the data from the several different brain areas which comprised the "medial basal hypothalamus" used previously. In the above study, a significant decrease in cytosol estrogen receptors was detected within the ventromedial nucleus (Fig. 3), one component of the "medial basal hypothalamus". No such change was detected with the gross dissection methodology [31], since the periventricular–anterior hypothalamic area and dorsomedial nucleus exhibit no change and therefore obscured changes within the smaller ventromedial nucleus. These data support the hypothesis that changes at the receptor level may contribute to the changes in the ability of estradiol to induce LH surges observed in middle-aged rats.

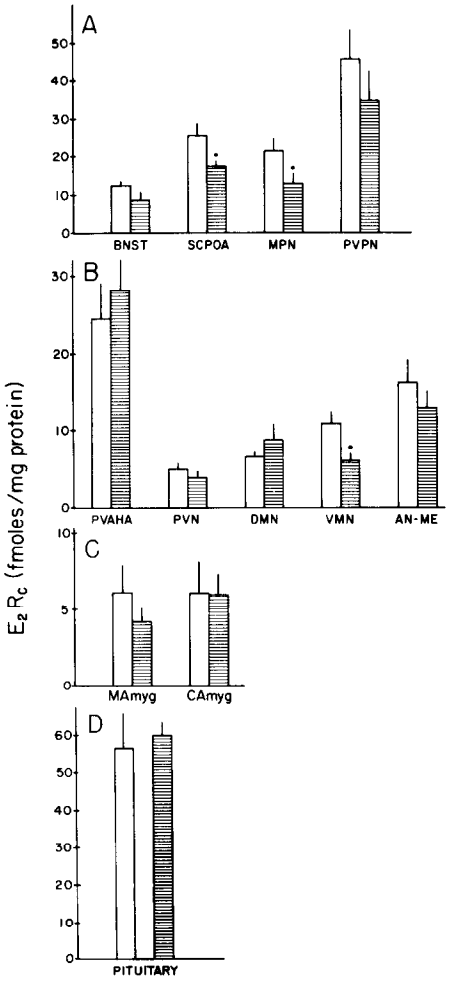

Fig. 3A–D. Cytoplasmic estradiol receptor concentrations in young (▯) and middle-aged (▯) ovariectomized rats. Microdissected brain areas in panel A were previously included in the grossly dissected "preoptic area". Microdissected brain areas in panel B were previously included in the grossly dissected "medial basal hypothalamus". The medial (MAmyg) and cortical (CAmyg) data in panel C were previously dissected as one piece of tissue. Bars represent mean ± SE. (From Ref. [42] with permission.)

Considerable evidence suggests that catecholamines, in particular norepinephrine, play an important role in regulating the release of LHRH (for reviews see Refs [33, 43, 44]). Thus, intraventricularly administered norepinephrine can trigger an ovulatory-like surge of LH [45]. Progesterone-induced LH surges can be blocked by diethyldithiocarbamate, an inhibitor of norepinerphrine synthesis [46]. Finally, norepinephrine turnover rates are elevated in specific hypothalamic nuclei during proestrous [47] and steroid-induced [48] LH surges. Conversely, norepinephrine turnover rates are low when preovulatory LH surges are blocked [49, 50]. This body of evidence strongly suggests that norepinephrine stimulates the release of preovulatory LHRH, directly or indirectly, and that this, in turn, stimulates the preovulatory surge of LH. Therefore we performed a series of studies to determine whether (1) the pattern of norepinephrine activity is altered during preovulatory and/or estradiol-induced LH surges and (2) the alterations are limited to specific hypothalamic areas. Proestrous young (3–5-month-old) and middle-aged (8–10-month-old) rats were killed at 0900, 1200 or 1500 h; since the LH surge had been shown to be delayed in middle-aged rats, an additional group of middle-aged rats were killed at 1800 h. Other groups of young and middle-aged rats were treated with α-methyl-paratyrosine at the above times, to block further synthesis of catecholamines, and were killed 60 or 120 min later. The brains were removed, frozen, sliced and microdissected according to the method of Palkovits[51]. We analyzed catecholamine activity in the medial preoptic nucleus, the suprachiasmatic nucleus, the median eminence and the arcuate nucleus [52]. The medial preoptic nucleus and the suprachiasmatic nucleus are two anterior hypothalamic areas that are thought to regulate the rhythmic biological functions; whereas the median eminence and the arcuate nucleus are areas of the medial basal hypothalamus generally thought to be involved with basal hormone release [33]. We found that in young proestrous rats, norepinephrine turnover rates increased during the day and peaked at 1500 h in all brain areas. The diurnal rhythm of activity was such that activity was high when the LH surge occurred and low during the morning when LH concentrations were basal (Fig. 4). In contrast, in middle-aged rats norepinephrine turnover rates increased during the afternoon only in the medial basal hypothalamic nuclei (median eminence and arcuate nucleus) but exhibited no significant increase during the afternoon in the anterior hypothalamic areas (medial preoptic nucleus and suprachiasmatic nucleus). These results suggest that changes in the diurnal pattern of norepinephrine turnover rates are apparent during

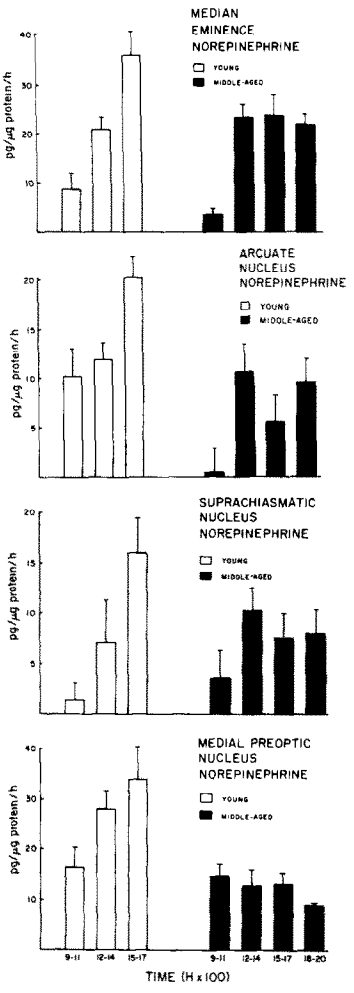

Fig. 4. Norepinephrine turnover rates in the median eminence, arcuate nucleus, suprachiasmatic nucleus and medial preoptic nucleus of young (clear bars) and middle-aged (dark bars) rats on proestrus. (From Ref. [52] with permission.)

middle age when the preovulatory LH surge is delayed and attenuated. Furthermore, the pattern is altered predominantly in the suprachiasmatic nucleus and the medial preoptic nucleus, two anterior hypothalamic areas known to regulate cyclic physiological events and in which we have observed changes in estradiol receptor concentrations. Finally, norepinephrine turnover rates in middle-aged rats are not simply lower than in young rats. Instead, there is a relatively steady turnover rate during the entire day with no significant diurnal rhythm. It is possible that increased norepinephrine turnover rates in the medial basal hypothalamic nuclei are necessary for LH surges to occur and increased turnover in the anterior hypothalamic nuclei serve to modulate the timing and the amplitude of the surge. Thus, the absence of a diurnal rhythm of norepinephrine activity in the anterior hypothalamic areas of middle-aged proestrous rats may result in subtle changes in the profile of the proestrous LH surge that may contribute to the onset of irregular estrous cyclicity.

We have reported that plasma estradiol concentrations were not altered in middle-aged compared to young rats on proestrus [28]. Therefore, we tested whether altered responsiveness to estradiol could explain the attenuated preovulatory LH surges that we previously observed in middle-aged rats on proestrus [26]. Young (3–4-months-old) and middle-aged (9–12-months-old) rats were ovariectomized. One week later, they received Silastic capsules containing estradiol-17β. Groups of young and middle-aged rats were sequentially bled through the indwelling right atrial cannula throughout the day 1, 2, 3 or 4 days after the implantation of the estradiol capsule. We found that a maximal positive feedback response to estradiol was observed 2, 3 and 4 in young rats. In contrast, middle-aged rats required the presence of estradiol for at least 3 days before a maximal positive feedback response was achieved. Even at these times, the timing of the LH rise was delayed by 1 h and peak concentrations were lower in middle-aged rats [17]. Since the diurnal pattern of catecholamine turnover was altered in middle-aged rats during the proestrous LH surge, we wondered whether an equivalent defect might explain the changes in the ability of estradiol to LH release in middle-aged rats. Young and middle-aged rats were treated with estradiol capsules as described above and catecholamine turnover rates were examined. On days 2 and 4, young and middle-aged rats were killed at 1000 or 1500 h or treated with α-methyl-paratyrosine at those times and killed 45 or 90 min later. Brains were removed, frozen, sliced and microdissected. The medial preoptic nucleus, suprachiasmatic nucleus and median eminence were analyzed for catecholamine content. In young rats, norepinephrine turnover rates increased during the afternoon compared to the morning in all brain areas examined on both days (Fig. 5). In contrast, in middle-aged rats, no increase in norepinephrine turnover rates was observed during the afternoon of day 2. By day 4, the delayed and attenuated LH surge was accompanied by increased turnover rates in the median eminence only, no change occurred in the suprachiasmatic nucleus or medial preoptic nucleus. The alteration in the catecholamine profile is remarkably similar to that which we observed in middle-aged proestrous rats. This suggests to us that age-related neurochemical changes influence both the proestrous and steroid-induced surge during middle age. We were able to detect these changes in the anterior hypothalamic areas only, suggesting that aging may affect this area of the hypothalamus initially.

In summary, our data demonstrate that neuroendocrine and neurochemical alterations are detectable early during the aging process in female rats. Changes in the diurnal pattern of norepinephrine turnover may contribute to alterations in the timing

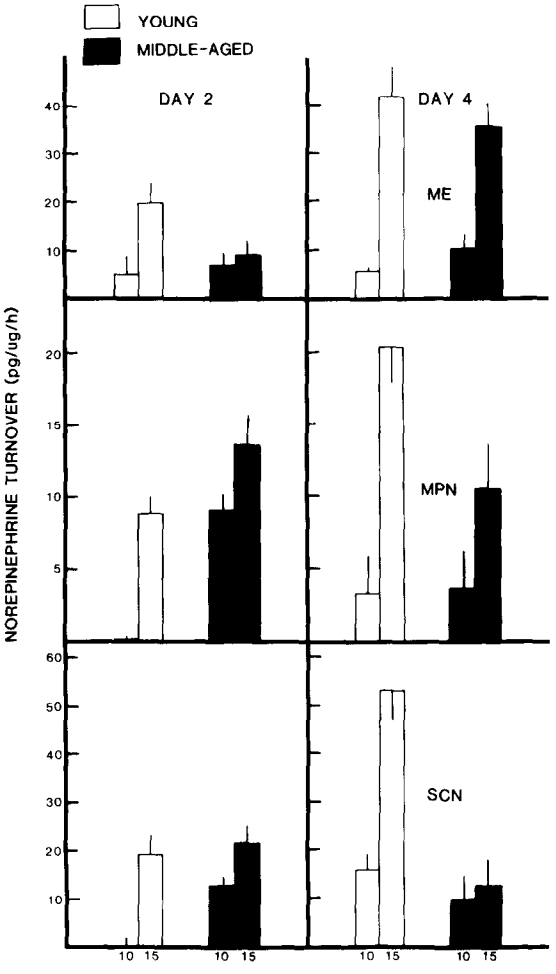

Fig. 5. Norepinephrine turnover rates in the median eminence, medial preoptic nucleus and suprachiasmatic nucleus of young (clear bars) and middle-aged (dark bars) rats on day 2 and day 4 after the administration of an estradiol-containing capsule. (From Ref. [17] with permission.)

and the amplitude of LH release. We are presently testing the hypothesis that multiple diurnal neurochemical and neuroendocrine events, which are critical to cyclic LH release and which are regulated by the suprachiasmatic nucleus and the medial preoptic nucleus, deteriorate during middle age.

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