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# ESTROGEN-INDUCED HYPOTHALAMIC BETA-ENDORPHIN NEURON LOSS: A POSSIBLE MODEL OF HYPOTHALAMIC AGING

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Abstract — Over the course of normal aging, all female mammals with regular cycles display an irreversible arrest of cyclicity at mid-life. Males, in contrast, exhibit gametogenesis until death. Although it is widely accepted that exposure to estradiol throughout life contributes to reproductive aging, a unified hypothesis of the role of estradiol in reproductive senescence has yet to emerge. Recent evidence derived from a rodent model of chronic estradiol-mediated accelerated reproductive senescence now suggests such a hypothesis. It has been shown that chronic estradiol exposure results in the destruction of greater than 60% of all beta-endorphin neurons in the arcuate nucleus while leaving other neuronal populations spared. This loss of opioid neurons is prevented by treatment with antioxidants indicating that it results from estradiol-induced formation of free radicals. Furthermore, we have shown that this beta-endorphin cell loss is followed by a compensatory upregulation of mu opioid receptors in the vicinity of LHRH cell bodies. The increment in mu opioid receptors presumably renders the opioid target cells supersensitive to either residual betaendorphin or other endogenous mu ligands, such as met-enkephalin, thus resulting in chronic opioid suppression of the pattern of LHRH release, and subsequently that of LH. Indeed, prevention of the neurotoxic effects of estradiol by antioxidant treatment also prevents the cascade of neuroendocrine aberrations resulting in anovulatory acyclicity. The loss of beta-endorphin neurons along with the paradoxical opioid supersensitivity which ensues, provides a unifying framework in which to interpret the diverse features that characterize the reproductively senescent female.

Key Words: opiod receptors, antioxidant, free radicals

#### INTRODUCTION

In FEMALE rodents, as in other mammals, the reproductive stages representing oocyte maturation, ovulation, and preparation of the uterus for implantation of the embryo succeed each other in a highly regulated and cyclic fashion. Collectively, these stages

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comprise the estrous or menstrual cycle. The control of the estrous and menstrual cycles is exerted by hypothalamic LHRH neurons and by the negative and positive feedback influences of ovarian steroids on the hypothalamic-pituitary axis. Although pituitary gonadotropes are themselves sensitive to estradiol (Simantov *et al.*, 1977), LHRH neurons have been shown to be devoid of estradiol receptors (Shivers *et al.*, 1983). The actions of estradiol on LHRH are thus mediated by a variety of estradiol-sensitive neural inputs which impinge on LHRH neurons amongst which the hypothalamic opioid system figures prominently.

Opioids, more particularly those acting on the mu opioid receptor type such as beta-endorphin, have been shown, in both rodents and primates, to potently inhibit the release of LHRH and LH (Drouva et al., 1981; reviewed by Kalra and Kalra, 1983; VanVugt et al., 1981; VanVugt et al., 1984). During the normal estrous/menstrual cycle, decreased opioid tone, manifested by decreased mu opioid receptors and diminished beta-endorphin release in the region of LHRH neurons has been associated with the induction of the LH surge (Kalra and Leadem, 1984; Allen et al., 1988; Jacobsen and Kalra, 1989). In addition, the reduced secretion of LH observed during the luteal or estrous phase has been shown to be dependant on opioid action (Ferin et al., 1984), thus illustrating the normal involvement of opioids in the regulation of the estrous/menstrual cycle.

During the course of normal aging, female mammals exhibit a transition from regular cycles to variable cycles of greater length followed by the complete cessation of cyclicity (Finch, 1990). In rats and mice with 4 day estrous cycles, the acyclic state begins at 12–16 months of age but varies among species and individuals (Thung, 1956; Finch, 1978). In women, the mean age of menopause is 51 years of age (Treloar *et al.*, 1981). It has been noted by several investigators that the period of fertility across many different female species is a remarkably consistent fraction of life span (Harman *et al.*, 1985). Thus, in rodent and human females, more than one-third of the individual's life span is postreproductive. This is in marked contrast to males which have been shown to exhibit gametogenesis throughout life (Finch, 1990). In an extensive phylogenetic analysis of female reproductive aging, Finch has concluded that the mid-life arrest of cyclicity is a universal feature of female mammalian senescence which may have been selected for during evolution. This, along with the demonstrated role of estradiol in promoting acyclicity, suggests that the mechanism(s) underlying the arrest of cyclicity at midlife may be widely generalizable across species.

# CRITICAL ROLE OF ESTRADIOL IN THE GENERATION OF THE MID-LIFE ARREST OF CYCLICITY

It is now well established that a variety of morphological and endocrine manifestations of female reproductive senescence are delayed by ovariectomy and are induced in young animals following chronic treatment with estradiol. These include lengthening of estrous cycles (Farookhi et al., 1985; Felicio et al., 1986), cessation of estrous cycles (Ascheim, 1964; 1976; Brawer et al., 1983; Felicio et al., 1983; Mobbs et al., 1984; Kohama et al., 1989b), impairments in induced LH surges (Blake et al., 1983; Mobbs et al., 1984), decreases in the postovariectomy rise in LH (Blake et al., 1983; Gee et al., 1983; Mobbs et al., 1984; Schulster et al., 1984; Simard et al., 1987), increases in pituitary dopamine (Telford et al., 1986), increases in pituitary glucose-6-phosphate

dehydrogenase, increases in lactotrope adenomas and prolactinemia (Giok, 1961; Brawer, 1975; Nelson et al., 1981; Casanueva et al., 1982; Wiklund and Gorski, 1982; Brawer et al., 1983; Takahashi and Kawashima, 1983; Mobbs et al., 1984) as well as the generation of a hypothalamic lesion of the arcuate nucleus (Ascheim, 1964, 1976; Brawer et al., 1975; Brawer et al., 1978; Schipper et al., 1981; Brawer et al., 1983).

The transition period of abnormal cycling observed before acyclicity in aged rodents has been shown to be accompanied by elevations in serum estradiol concentrations and followed by diminutions in progesterone concentrations (Gray and Wexler, 1980; Miller and Riegle, 1980: Wise, 1982). Mean serum estradiol levels were found to be significantly elevated during the diestrous phase of the estrous cycle in a subpopulation of normally cycling middle-aged rats that became irregular a few months later (Nass et al... 1984). In addition, the preovulatory rise in estradiol was found to occur prematurely in middle-aged animals with lengthened ovulatory cycles (Page, 1982). Although mean plasma estradiol concentrations in old acyclic rats have been found to be only slightly elevated compared with those normally observed at estrus or diestrus (Huang et al., 1978; Lu, 1983), estradiol exposure during this period is tonic and uninterrupted, due to the lack of intermittent progesterone surges (Wise et al., 1991). This period of chronic. unopposed estradiol exposure appears to be particularly critical in initiating permanent damage to the hypothalamic-pituitary-ovarian axis, which results in acyclicity (Felicio et al., 1983; Felicio et al., 1986; Kohama et al., 1989a, 1989b; Mobbs et al., 1984; Naftolin et al., 1990; Nelson et al., 1982; Nelson and Felicio, 1985).

Felicio and collaborators (1986), have reported that most of the damage to centers regulating reproductive cyclicity occurs between 12–16 months in mice, that is to say, during the 4-month period preceding acyclicity. These and other studies (Schipper et al., 1981) have nonetheless also demonstrated that degenerative changes in reproductive control centers may also occur from 0–12 months following exposure to normal physiological estradiol levels. It may be concluded from the above-mentioned studies that the effects of estradiol in initiating the arrest of cyclicity are not directly cumulative throughout life but rather depend upon a critical pattern of estradiol exposure that is both chronic and unopposed. This pattern of estradiol exposure is what is observed in the period of irregular cycling just prior to acyclicity and what is mimicked in a variety of conditions of experimental estradiol exposure.

#### SITE OF IRREVERSIBLE ESTRADIOL-INDUCED DAMAGE

A major question arising from the study of female reproductive senescence concerns the primary site of estradiol-induced irreversible age-related change. In rodents, it is now fairly clear that permanent alterations in hypothalamic function contribute to the arrest of cyclicity and the endocrine changes observed in the postreproductive female. In classic transplantation experiments, the ovaries of a young animal became incapable of supporting normal ovulatory cycles when transplanted in an old acyclic rat whereas the ovaries from old acyclic animals reverted to healthy ovarian morphology and supported normal ovulatory cycles when transplanted to young animals (Peng and Huang, 1972; Ascheim, 1976; but see Krohn, 1966). Analagous transplantation experiments in mice have largely confirmed these findings, although the duration of restoration of cyclicity varied with age (Felicio *et al.*, 1986). In these experiments, age-associated

hypothalamic damage was prevented by early ovariectomy such that old animals ovariectomized when young were capable of supporting normal estrous cycles, including ovulation, when transplanted with the ovaries of old animals (Ascheim, 1965; Felicio *et al.*, 1986). These experiments provided strong support for the notion that the hypothalamus was the primary site of estradiol-induced damage.

In support of evidence indicating that permanent damage to hypothalamic circuitry plays a major role in reproductive aging in females, several studies have demonstrated neuronal cell loss in the hypothalami of aged rats (Ascheim, 1976; Hsu, 1978; Mervis, 1981; McNeil, 1983) and mice (Machado-Salas, 1977). Furthermore, increased glial reactivity associated with neurodegeneration has specifically been observed in the arcuate nucleus of aged rats and mice (Brawer et al., 1975; Brawer et al., 1978; Schipper et al., 1981). A hypothalamic lesion restricted to the arcuate nucleus has been experimentally induced in a multiplicity of rodent models of chronic estradiol exposure, including implantation with chronic release capsules (Schipper et al., 1981; Brawer et al., 1983; Finch et al., 1984; Mobbs et al., 1984), feeding with estradiol (Kohama et al., 1989b), exposure to constant light (Campbell and Schwartz, 1980) and injection with estradiol-valerate (Brawer et al., 1983; Brawer et al., 1986; Garcia-Segura et al., 1986).

This lesion, characterized most extensively in the estradiol-valerate treated rat, is comprised of multiple isolated foci of degenenerating axons and dendrites, collapsed myelin figures and increased size of unique peroxidase positive astrocytes (Brawer et al., 1978, Brawer et al., 1980; Brawer et al., 1986). A similar hypothalamic lesion has also been described in sheep having grazed on phytoestrogen containing clover leaves (Adams, 1976), thereby indicating that a variety of species are susceptible to the neurotoxic effects of estradiol. Chronic estradiol-mediated hypothalamic neuronal degeneration was found, in rats and in mice, to be progressive with time (Schipper et al., 1981; Mobbs et al., 1984). Neither androgens (Brawer et al., 1983) nor progestins (Schipper, 1990b) were found to generate the arcuate pathology. Male rats implanted with estradiol also developed the arcuate pathology (Schipper et al., 1981), suggesting that the estradiol neurotoxicity occurs independent of genotype.

## ESTRADIOL-INDUCED HYPOTHALAMIC AND PITUITARY DEFICITS

The permanent damage to hypothalamic neurons induced following chronic estradiol exposure is accompanied by a series of hypothalamic-pituitary-ovarian deficits that participate in the generation and maintenance of acyclicity (Brawer et al., 1986). These endocrine features, observed in estradiol-treated animals, closely parallel what is observed in normal aged females (Mobbs et al., 1984; Finch et al., 1984). For example, in EV-injected and old acyclic animals, postovariectomy elevations in serum LH are significantly reduced with respect to controls (Mobbs et al., 1984; Simard et al., 1987; Weiland and Wise, 1990; Takahashi et al., 1980; Steger et al., 1980; Shaar et al., 1975; Huang, 1976; Estes et al., 1980; Estes et al., 1982) and LH release in response to LHRH stimulation is reduced in groups as compared to young cycling controls (Hemmings et al., 1983; Howland, 1976; Watkins et al., 1975; Cooper et al., 1984). These reductions in sensitivity to LHRH may be accounted for by the decrease in LHRH receptor numbers observed in the pituitary glands of aged and EV-treated animals (Marian et al., 1981; Carriere et al., 1988). The number of pituitary LHRH receptors is

largely regulated by the endogenous LHRH pattern (Clayton and Catt, 1981; Katt et al., 1985), hence, the disturbances in LH observed in aged and estradiol-treated animals most probably reflect an aberrant hypothalamic LHRH signal. In fact, electrical stimulation of the preoptic area was found to restore LH surges in EV-treated and aged rats (Clemens et al., 1969).

Increased levels of plasma and pituitary prolactin have been observed in old acyclic animals (Chuknyiska et al., 1986; Demarest et al., 1985; Estes and Simpkins, 1982; Huanget al., 1976; Steger, 1981; Mobbs et al., 1984; Flurkey et al., 1982; Wise et al., 1982; Larson and Wise, 1991) and animals exposed to estradiol for long periods of time (Brawer et al., 1975; Giok, 1961; Mobbs et al., 1984; Brawer et al., 1983). The incidence of lactotrope adenomas has been shown to increase in some species from 9 month to 24 months of age (Felicio et al., 1980; Zurcher et al., 1982). Likewise, in young animals repeatedly treated with high doses of estradiol, incidence of prolactinomas rose dramatically (Brawer et al., 1978). Importantly, the incidence of prolactin-secreting tumors (Felicio et al., 1983; Takahashi and Kawashima, 1983; Mobbs et al., 1984) was shown to be delayed by early ovariectomy and males of equivalent ages almost never develop hyperprolactenemia (Finch, 1978), therefore linking these endocrine changes to the permanent hypothalamic alterations induced by estradiol.

Together, these results demonstrate that the morphological and physiological endpoints of experimentally induced chronic estradiol exposure are similar to those occurring naturally in the aged female animal and suggest that the hypothalamic changes underlying both may be similar.

# ALTERATIONS IN SPECIFIC HYPOTHALAMIC CIRCUITRY AND FUNCTION

In recent years, the specific population of hypothalamic neurons targeted by the neurotoxic effects of estradiol has been identified. Quantitative immunocytochemical experiments revealed that an 8-week exposure to tonic estradiol levels resulted in the disappearance of greater than 60% of all arcuate beta-endorphin immunoreactive neurons (Desjardins et al., 1993). The mean number of beta-endorphin neurons in controls was 5500 whereas in estradiol-treated animals this number was reduced to 2000 neurons. The distribution of beta-endorphin neurons in normal animals parallels that of degenerated foci described previously in light and electron microscopic studies of estradiol toxicity (Brawer et al., 1978; Brawer et al., 1980).

That the decrease in the number of beta-endorphin neurons resulted from actual cell loss and not merely from decreases in beta-endorphin expression was demonstrated using unbiased stereological measurements of total neuron numbers in the arcuate nucleus of control and long-term estradiol-treated animals. Controls were found to possess on average  $\approx$ 24,000 neurons whereas estradiol-treated animals had a mean of  $\approx$ 20,500, indicating an average loss of about 3500 neurons. The absolute number of neurons lost was found to be equivalent to the total number of beta-endorphin neurons lost as determined immunocytochemically (Desjardins *et al.*, 1993), thus providing evidence for selective estradiol-mediated destruction of beta-endorphin neurons. Accordingly, the morphological appearance of remaining beta-endorphin neurons in the estradiol-treated group was consistent with a degenerative process. These neurons were spiny in appearance and exhibited a reduced form factor (defined as  $4\pi \times \text{area} \div$ 

perimeter<sup>2</sup>) as compared to beta-endorphin neurons in control animals (Desjardins *et al.*, 1993).

Evidence for the selectivity of estradiol's effects on beta-endorphin neurons was further obtained in sections adjacent to those stained for beta-endorphin. As had been previously demonstrated by our group using electron microscopy (Piotte et al., 1985), we have confirmed that long-term estradiol exposure did not decrease the numbers of tyrosine hydroxylase-immunoreactive neurons (Desiardins et al., 1993). These results are in contrast to those of Sarkar and co-workers suggestive of dopamine neuron degeneration following chronic estradiol exposure (Sarkar et al., 1982). If anything, we and others have noted significant increases in hypothalamic tyrosine hydroxylase expression following long-term treatment with estradiol (Desiardins et al., 1993; Kohama et al., 1989b). In adjacent sections, immunoreactive numbers of somatostatin and neurotensin neurons were unchanged following estradiol treatment. Furthermore, hypothalamic neuropeptide-Y and met-enkephalin concentrations, as measured by RIA, were not decreased in estradiol-exposed rats despite significant drops in hypothalamic beta-endorphin concentrations in the same animals (Forman et al., 1985; Desiardins et al., 1990a). Taken together, these findings suggest that the neurotoxic effects of estradiol are specifically targeted to the beta-endorphin neuronal population (Designations et al., 1993).

The reason for the selective vulnerability of beta-endorphin neurons is not clear but is unlikely to result from direct estradiol-receptor mediated mechanisms because only 10–15% of all beta-endorphin neurons have been shown to possess estradiol receptors and adjacent populations unaffected by estradiol neurotoxicity such as tyrosine hydroxylase-, somatostatin-, neurotensin-, neuropeptide-Y- and met-enkephalin-immunoreactive neurons have been shown to harbour estradiol receptors (Sar, 1984; Morrell et al., 1985; Sar et al., 1990; Alexanderet al., 1991; Axelson et al., 1991).

We have recently demonstrated that free radical-mediated lipid peroxidation may be involved in the estradiol-induced degeneration of beta-endorphin neurons. Indeed, EV-injected rats co-treated with the potent antioxidant, vitamin E, failed to demonstrate decreases in hypothalamic beta-endorphin concentrations and displayed regular ovarian cycles and morphology as compared to EV-treated controls (Desjardins *et al.*, 1992b). Co-treatment with the synthetic antioxidant, U743-89F, a 21-aminosteroid, was also found to protect beta-endorphin neurons from estradiol-induced degeneration (Schipper *et al.*, 1994).

Several unique features of the arcuate nucleus support the notion that it may be the site of significant free-radical production (Schipper et al., 1990b). The arcuate nucleus of rodent and human brains (Schipper et al., 1991b) contains a unique variety of peroxidase-positive astrocytes that are highly sensitive to circulating estradiol levels (Brawer et al., 1978; Schipper et al., 1990a). Peroxidase-positive astrocytes have been shown, in culture, to generate o-semiquinone free radicals from catechol estrogens (2-, 4-hydroxy estradiol) (Schipper, 1991a). These are normally generated spontaneously in the brain from circulating estradiol (Liehr, 1990). The catalyst for free radical production may be transition metals, such as iron, chromium or copper which occur in astrocytic granules (McLaren et al., 1992; Schipper et al., 1990b). Estradiol has also been shown to cause massive induction of the mRNA coding for phospholipase C alpha in the arcuate nucleus (Mobbs et al., 1992). This enzyme has been implicated in the generation of large amounts of free radicals via prostaglandin and leukotriene metab-

olism. It therefore appears that estradiol may act as a stimulator and substrate of free radical production in the arcuate nucleus.

The identification of the neurons destroyed by chronic estradiol exposure provided a key element linking numerous observations on the senescence of the female reproductive system. It was known that hypothalamic opioid binding was increased in long-term estradiol-treated animals relative to controls (Wilkinson et al., 1983; Wilkinson et al., 1986). It had also been demonstrated that this increase in opioid binding occurred predominantly in the medial preoptic area (MPOA) and concerned mu, but not delta or kappa opioid binding sites (Desiardins et al., 1996). Several lines of evidence also suggested that this enhanced opioid binding in the region of LHRH neuronal cell bodies was responsible for the maintenance of acyclicity in estradiol-treated animals via an opioid induced suppression of LHRH and LH. Opioids, particularly those acting on mu opioid receptors, have well-established inhibitory effects on LHRH and LH release (Kalra and Kalra, 1983). In fact, it has been previously shown that mu, but not delta or kappa, opioid agonists microinjected into the medial preoptic nucleus induced the suppression of LH pulse amplitude (Leadem and Yagenova, 1987; Mallory, 1990), in the absence of changes in other pulse parameters. The inhibition of LH pulse amplitude is precisely what has been shown to occur following long-term exposure to estradiol (Grosser et al., 1986; McCarthy et al., 1990). Furthermore, it was shown that in EVtreated rats, treatment with naltrexone, the long-acting opioid antagonist, restored normal pituitary LHRH receptor numbers, reversed the cystic morphology of ovaries and restored normal ovarian cycles (Carrière et al., 1989). These observations suggested that mu opioid receptors in the vicinity of LHRH neurons were mediating a chronic inhibition of the hypothalamic-pituitary-ovarian axis. Because beta-endorphin neurons of the arcuate nucleus are known to send extensive projections to the MPOA (Mezey et al., 1978; Zaborsky et al., 1979), and synapse on LHRH neurons (Chen et al., 1989), we proposed that the loss of beta-endorphin afferents resulting from destruction of cell bodies in the arcuate nucleus was responsible for inducing a compensatory upregulation of mu opioid receptors in the MPOA (Desjardins et al., 1990b; Desjardins et al., 1992a; Desiardins et al., 1993).

To test this hypothesis, we examined the monosodium glutamate (MSG) treated rat. MSG given to neonates results in the destruction of 80-90% of all arcuate neurons (Olney, 1971) with consequent reductions in hypothalamic beta-endorphin concentrations (Krieger et al., 1979). In this model, significant increases mu opioid binding were observed in the MPOA as compared to sham-treated controls (Designations et al., 1992a). This increase in opioid binding was further shown to be inversely proportional to arcuate beta-endorphin concentrations measured by RIA in the same animals. These findings thus provided support for the idea that a causal relationship existed between the loss of arcuate beta-endorphin neurons in estradiol-treated animals and the upregulation of mu opioid receptors in the MPOA. Critical to this idea is the fact that other relevant opioid ligands, such as met-enkephalin, are not decreased following arcuate neuron destruction and may act on mu opioid receptors, especially under conditions of upregulation (Paterson et al., 1984). These opioid peptides, localized in the MPOA thereby provide a continuing source of opioid receptor stimulation in spite of the destruction of arcuate beta-endorphin neurons. Indeed, functional opioid supersensitivity paralleling opioid receptor upregulation has been shown to occur following arcuate nucleus destruction in mice (Simantov and Amir, 1983).

## A POSSIBLE MODEL OF HYPOTHALAMIC AGING

We have therefore proposed that estradiol exposure occurring experimentally or during aging causes the destruction of beta-endorphin neurons in the arcuate nucleus. while sparing other nearby populations. The resulting loss of beta-endorphin innervation to the MPOA induces a chronic increase in mu opioid receptors in this region. Increased mu opioid receptors either on LHRH cell bodies or on cells that regulate LHRH neurons, then mediate the chronic suppression of LH by binding other local endogenous opioids such as met-enkephalin. The disturbances in LH release then produce and/or maintain the acyclic, polycystic condition observed in estradiol-treated and aged animals. In support of this scheme, we have shown that treatments which prevented the disappearance of beta-endorphin neurons also prevented the pituitary and ovarian changes responsible for acyclicity and anovulation (Designations et al., 1992b). To our knowledge, this is the first proposal of a mechanism contributing to the arrest of cyclicity which accounts for the long-standing observation of estradiol neurotoxicity in the arcuate nucleus. Our hypothesis is supported by evidence in aged rats indicating significant decrements in hypothalamic beta-endorphin concentrations (Gambert et al., 1980; Forman et al., 1981; Barden et al., 1981; Wardlaw et al., 1982; Dorsa et al., 1984; Rogers et al., 1985), diminutions in POMC mRNA (Nelson et al., 1988: Wise, 1991) and decreases in beta-endorphin-immunoreactive neurons (Miller et al., 1991) in addition to evidence suggestive of opioid supersensitivity in aged rats (Field and Kuhn, 1989) and monkeys (Murphy and Lipton, 1983).

The hypothesis of beta-endorphin neuron degeneration followed by opioid supersensitivity as an underlying mechanism for reproductive senescence seems to provide a more parsimonious explanation for the multiplicity of endocrine changes observed in aged mammals than do current contrasting views of hypothalamic aging such as those proposing decreased catecholamine drive from the mesecephalon or disturbed circadian regulation. For example, several studies in aged animals have shown that treatment with adrenergic agonists, dopamine receptor antagonists, or opioid antagonists (Clemens et al., 1969; Meites, 1988; Field and Kuhn, 1989) temporarily restore normal LH levels and/or reinstate estrous cyclicity in female rodents. These findings may be interpreted to suggest that any number of treatments that temporarily override the effects of chronic opioid supersensitivity (i.e., such as decreased noradrenaline turnover and increased dopamine and serotonin turnover in the MPOA (Gopalan et al., 1989a, 1989b)) are capable of restoring cycles for a limited period of time. However, this manipulation of events secondary to the neurotoxic lesion does not permanently restore cyclicity. In addition, anterior hypothalamic opioid supersensitivity in aged females may contribute to the development of hyperprolactemia and prolactin-secreting pituitary adenomas (Brawer et al., 1978; Wise, 1982; Finch et al., 1984; Mobbs et al., 1984; Larson and Wise, 1991;) because opioids stimulate prolactin release in rodents and primates (Leadem and Yagenova, 1987; Catlin et al., 1980). It is possible that the opioid supersensitivity exhibited by E<sub>2</sub>-treated animals and hypothesized in aged rats might also account for disturbances in feeding, metabolism, and temperature regulation often observed in these groups.

Reproductive senescence in females has often been used as a model of generalized senescence because it occurs at a period in life that is relatively devoid of other pa-

thologies and presents an opportunity to study a specific aging process in isolation. The implications of selective beta-endorphin cell loss as the mechanism underlying reproductive senescence in mammals are of evolutionary and social significance. On the one hand, they support theories of programmed aging that imply that individual aging is a programmed adaptation and thus conferred a unique advantage to placental mammals some 70 million years since their descent from a common ancestor (Finch, 1978). On the other hand, our demonstration that estradiol neurotoxicity occurs via the formation of free radicals also indirectly supports stochastic theories of aging such as the wear-and-tear and free radical theories of aging (Harman, 1962; Stehler, 1977).

If our hypothesis of steroid-induced loss of beta-endorphin hypothalamic neurons proves true in primates, it could provide a new explanation for the hypothalamic mechanisms underlying menopause. The suggestion that women might undergo an ovariandependent selective neuronal involution at mid-life (while no testicular-dependent aging syndrome occurs in males) must be viewed within the context of general aging theories. Steroid-dependent cell loss as a cause of specific system aging has been predicted by Finch's neuroendocrine theory of aging (Finch, 1978) and the pleiotropic view of aging adopted by Williams (1957). Indeed, steroid-induced hippocampal cell loss following chronic glucocorticoid exposure has already been well-documented and shown to result in the loss of hypothalamic-pituitary-adrenal axis homeostasis in aged rats (Sapolsky et al., 1986; Meaney et al., 1993). Vitamin E and other antioxidants are able to prevent the arrest of cyclicity in our models and have long been known to delay a variety of senescent changes occurring with age in rodents (Harman, 1962; Tappel, 1973), Vitamin E therapy has already been shown to decrease the incidence of coronary heart disease in women (Stampfer et al., 1993) and has been shown to be safe in moderate doses (Bendich and Machlin, 1988). It is therefore possible that in the future, palliative vitamin E therapy could at the same time protect us from heart attacks and other diseases resulting from artherosclerosis as well as delay the symptoms of menopause.

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