Pergamon

Experimental Gerontology, Vol. 30, Nos. 3/4, pp. 253–267, 1995

0531-5565/95 $9.50 + .00

0531-5565(94)00040-9

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

G. CLARISSA DESJARDINS,[1] ALAIN BEAUDET,[2,3] MICHAEL J. MEANEY[2,1] and JAMES R. BRAWER[3]

Departments of [2]Neurology and Neurosurgery, Montreal Neurological Institute, [3]Anatomy and Cell Biology and [1]Psychiatry, Douglas Hospital Research Center.

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 beta-endorphin 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

Correspondence to: Clarissa Desjardins, Advanced Bioconcept Inc., 1801 McGill College, Suite 720, Montreal, Quebec, H3A 2N4 Canada.

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; Huang*et 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 ≈24,000 neurons whereas estradiol-treated animals had a mean of ≈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^2) 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 (Desjardins *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 (Desjardins *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; Desjardins *et al.*, 1990a). Taken together, these findings suggest that the neurotoxic effects of estradiol are specifically targeted to the beta-endorphin neuronal population (Desjardins *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; Alexander*et 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 (Desjardins *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 EV-treated 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; Desjardins *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 (Desjardins *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 (Desjardins *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_2-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 ovarian-dependent 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.

REFERENCES

ADAMS, N.R. Pathological changes in the tissues of ewes with clover disease. *Aust. Vet. J.* **45,** 215, 1976.

ALEXANDER, M.J. and LEEMAN, S.E. A sexually differentiated estrogen-responsive population of neurotensin neurons in the arcuate nucleus of the rat. *Soc. Neurosci.* **176,** 1991.

ALLEN, L.G., HAHN, E., CATON, D., and KALRA, S.P. Evidence that a decrease in opioid tone on proestrus changes the episodic pattern of luteinizing hormone (LH) secretion: Implications in the preovulatory LH hypersecretion. *Endocrinology* **122,** 1004–1013, 1988.

ASCHEIM, P. Resultats fournis par la greffe heretrochrone des ovaires dans l'etude de la regulation hypothalamo-hypophyso-ovarienne de la ratte senile. *Gerontologia* **10,** 65–75, 1964.

ASCHEIM, P. Aging in the hypothalamic–hypophyseal ovarian axis in the rat. In: Everitt, A.V. and Burgess, J.A. (Editors), *Hypotalamus, Pituitary and Aging,* pp. 376–418, Charles C. Thomas, Springfield. 1976.

AXELSON, J.F., VAN LEEUWEN, F.W., SHANNON, W.J., and O'SHAUGHNESSY, K.A. Immunocytochemical localization of estrogen receptors within neurotensin neurons in the female rat. *Soc. Neurosci.* **176,** 1991.

BARDEN, N., DUPONT, A., LABRIE, F., MERAND, Y., ROULEAU, D., VAUCHY, H., and ROISSIER, J.R. Age-dependent changes in the β-endorphin content of discrete rat brain nuclei. *Brain Res.* **208,** 209–212, 1981.

BENDICH, A. and MACHLIN, L.J. Safety of oral intake of vitamin E. *Am. J. Clin. Nutr.* **5,** 612–9, 1988.

BLAKE, C.A., ELIAS, K.A., and HUFFMAN, L.J. Ovariectomy of young adult rats has a sparing effect on the suppressed ability of aged rats to release luteinizing hormone. *Biol. Reprod.* **28,** 575–585, 1983.

BRAWER, J.R., MUNOZ, M., and FAROOKHI, R. Development of the polycystic ovarian condition (PCO) in the estradiol valerate-treated rat. *Biol. Reprod.* **35,** 647–655, 1986.

BRAWER, J.R., NAFTOLIN, F., MARTIN, J., and SONNENSHEIN, C. Effects of a single injection of estradiol valerate on the hypthalamic arcuate nucleus and on reproductive function in the female rat. *Endocrinology* **103,** 501–512, 1978.

BRAWER, J.R., SCHIPPER, H., and NAFTOLIN, F. Ovary-dependent changes in the hypothalamic arcuate nucleus. *Endocrinology* **107,** 274–279, 1980.

BRAWER, J.R., SCHIPPER, H., and ROBAIRE, B. Effects of long-term androgen and estradiol exposure on the hypothalamus. *Endocrinology* **112,** 194–199, 1983.

CAMPBELL, C.S. and SCHWARTZ, N.B. The impact of constant light on the estrous cycle of the rat. *Endocrinology* **106,** 1230–1238, 1980.

CARRIÈRE, P.D., BRAWER, J.R., and FAROOKHI, R. Pituitary gonadotropin-releasing hormone receptor content in rats with polycystic ovaries. *Biol. Reprod.* **38,** 562–567, 1988.

CARRIÈRE, P.D., FAROOKHI, R., and BRAWER, J.R. The role of aberrant hypothalamic opiatergic function in generating polycystic ovaries in the rat. *Can. J. Physiol. Pharmacol.* **67,** 896–901, 1989.

CASANEUVA, F., COCCI, D., LOCATELLI, V., FLAUTO, C., ZAMBOTTI, F. BESTETTI, G., ROSSI, G.L., and MULLER, E. Defective central nervous system dopaminergic function in rats with estrogen-induced pituitary tumors, as assessed by plasma prolactin concentrations. *Endocrinology* **110,** 590–599, 1982.

CATLIN, D.H., POLAND, R.E., GORELICK, D.A., GERNER, R.H., HUI, K.K., RUBIN, R.I., LI, C.H. Intravenous infusion of beta-endorphin increases serum prolactin, but not growth hormone or cortisol in depressed subjects and withdrawing methadone addicts. *J. Clin. Endocrinol. Metab.* **50,** 1021–1025, 1980.

CHEN, W., WITKIN, J., and SILVERMAN, A. Beta-endorphin and synaptic input to gonadotropin-releasing hormone neurosecretory cells in the male rat. *J. Comp. Neurol.* **286,** 85–95, 1989.

CHUKNYISKA, R.S., BLACKMAN, M.R., HYMER. W.C., and ROTH, G.S. Age-related alterations in the number and function of pituitary lactotropic cells from intact and ovariectomized rats. *Endocrinology* **118,** 1856–1862, 1986.

CLAYTON, R.N. and CATT, K.J. Gonadotropin-releasing hormone receptors: Characterization, physiological regulation, and relationship to reproductive function. *Endocr. Rev.* **2,** 186–209, 1981.

CLEMENS, J.A., AMENOMORI, Y., JENKINS, T., and MEITES, J. Effects of hypothalamic stimulation, hormones and drugs on ovarian function in old female rats. *Proc. Soc. Exp. Biol. Med.* **132,** 561–563, 1969.

COOPER, R.L., ROBERTS, B., ROGERS, D.C., SEAY, S.G., and CONN, P.M. Endocrine status vs. chronologic age as predictors of altered luteinizing hormone seretion in the "aging" rat. *Endocrinology* **114,** 391–396, 1984.

DEMAREST, K.T., MOORE, K.E., and RIEGLE, J.D. Adenohypophyseal dopamine content and prolactin secretion in the aged male and female rat. *Endocrinology* **116,** 1316–23, 1985.

DESJARDINS, G.C., BEAUDET, A., and BRAWER, J.R. Alterations in opioid parameters in the hypothalamus of rats with estradiol-induced polycystic ovarian disease. *Endocrinology* **127,** 2969–2976, 1990b.

DESJARDINS, G.C., BRAWER, J.R., and BEAUDET, A. Distribution of mu, delta and kappa opioid receptors in the hypothalamus of the rat. *Brain Res.* **536,** 114–123, 1990a.

DESJARDINS, G.C., BRAWER, J., and BEAUDET, A. Monosodium glutamate-induced reductions in hypothalamic beta-endorphin content result in mu opioid receptor upregulation in the medial preoptic area. *Neuroendocrinology* **56,** 378–384, 1992a.

DESJARDINS, C.G., BEAUDET, A., SCHIPPER, H.M., and BRAWER, J. Vitamin E protects beta-endorphin neurons from estradiol neurotoxicity. *Endocrinology* **131,** 2481–2482, 1992b.

DESJARDINS, G.C., BRAWER, J.R., and BEAUDET, A. Estradiol is selectively neurotoxic to hypothalamic beta-endorphin neurons. *Endocrinology* **132,** 86–93, 1993.

DORSA, D.M., SMITH, E.R., and DAVIDSON, J.M. Immunoreactive beta-endorphin and LHRH levels in the brains of aged male rats with impaired sex behavior. *Neurobiol. Aging* **5,** 115–120, 1984.

DROUVA, S., EPELBAUM, J., TAPIA-ARANCIBIA, L., LAPLANTE, E., and KORDON, C. Opiate receptors modulate LHRH and SRIF release from mediobasal hypothalamic neurons. *Neuroendocrinology* **32,** 163–167, 1981.

ELLEFSON, R. Porphyrinogens, porphyrins, and the porphyrias. *Mayo Clin. Proc.* **57,** 454–458, 1982.

ESTES, K.S. and SIMPKINS, J.W. Resumption of pulsatile LH release after alpha-adrenergic stimulation in aging constant estrous rats. *Endocrinology* **111,** 1778–1784, 1982.

ESTES, K.S., SIMPKINS, J.W., and CHEN, C.L. Alteration in pulsatile release of LH in aging female rats. *Proc. Soc. Exp. Biol. Med.* **162,** 384–387, 1980.

FAROOKHI, R., HEMMINGS, R., and BRAWER, J.R. Unilateral ovariectomy restores ovulatory cyclicity in rats with a polycystic ovarian condition. *Biol. Reprod.* **32,** 530–540, 1985.

FELICIO, L.S., NELSON, J.F., and FINCH, C.E. Spontaneous pituitary tumorigenesis and plasma estradiol in aging female C57BL/6J mice. *Exp. Gerontol.* **15,** 139–143, 1980.

FELICIO, L.S., NELSON, J.F., GOSDEN, R.G., and FINCH, C.E. Restoration of ovulatory cycles by young ovarian grafts in aging mice: Potentiation by long-term ovariectomy decreases with age. *Proc. Natl. Acad. Sci.* **80,** 6076–6080, 1983.

FELICIO, L.S., NELSON, J.F., and FINCH, C.E. Prolongation and cessation of estrous cycles in aging C57BL/6J female mice is caused by ovarian steroids, not intrinsic pituitary aging. *Biol. Reprod.* **34,** 849–858, 1986.

FERIN, M., VAN VUGT, D., and WARDLAW, S. The hypothalamic control of the menstrual cycle and and the role of endogenous opioid peptides. *Rec. Prog. Horm. Res.* **40,** 441–485, 1984.

FIELD, E.A. and KUHN, C.M. Opiate antagonist treatment reinstates estrous cycles in middle-aged persistent estrous rats. *Biol. Reprod.* **40,** 714–719, 1989.

FINCH, C.E. Genotypic influences in female reproductive senescence in rodents. In: Bergma, D. and Harrison, D.E. (Editors), pp. 335–354, *Genetic effects on Aging,* Birth defects: Original article Series 14, Alan R. Liss, New York, 1978.

FINCH, C.E. *Longevity, Senescence and the Genome, Chicago University Press, Chicago, 1990.*

FINCH, C.E., FELICIO, L.S., MOBBS, C.V., and NELSON, J.F. Ovarian and steroidal influences on neuroendocrine aging processes in female rodents. *Endocrine Rev.* **5,** 467–497, 1984.

FLURKEY, K., GEE, D.M., SINHA, Y.N., WISNER, JR., J.R., and FINCH, C.E. Age effects on luteinizing hormone, progesterone and prolactin in proestrous and acyclic C57BL/6J mice. *Biol. Reprod.* **26,** 835–846, 1982.

FORMAN, L.J., MARQUIS, D.E., and STEVENS, R. The effect of chronic estrogen treatment on immunoreactive beta-endorphin levels in intact female rats. *Proc. Soc. Exp. Biol. Med.* **179,** 365–372, 1985.

FORMAN, L.J., SONNTAG, W.E., VAN VUGT, D.A., and MEITES, J. Immunoreactive β-endorphin in the plasma pituitary and hypothalamus of young and old male rats. *Neurobiol. Aging* **2,** 281–284, 1981.

GAMBERT, S.R., GARTHWAITE, T.L., PONTZER, C.H., and HAGEN, T.C. Age-related changes in central nervous system beta-endorphin and ACTH. *Science* **213,** 1282, 1980.

GARCIA-SEGURA, L.M., BAETENS, D., and NAFTOLIN, F. Synaptic remodelling in the arcuate nucleus after injection of estradiol valerate in adult female rats. *Brain Res.* **366,** 131–136, 1986.

GEE, D.M., FLURKEY, K., and FINCH, C.E. Aging and the regulation of luteinizing hormone in C57BL/6J mice: Impaired elevations after ovariectomy and spontaneous elevations at advanced ages. *Biol. Reprod.* **28,** 598–607, 1983.

GIOK, K.H. *An Experimental Study of Pituitary Tumors: Genesis, Cytology and Hormone Content.* Springer-Verlag, Berlin, 1961.

GOPALAN, C., GILAMORE, D.P., BROWN, C.H., and WILSON, A. Effects of opiates on biogenic amine turnover in specific hypothalamic areas on the afternoon of pro-estrus in the rat-I. Catecholamines. *Biogenic Amines* **6,** 597–606, 1989a.

GOPALAN, C., GILAMORE, D.P., BROWN, C.H., and WILSON, A. Effects of opiates on biogenic amine turnover in specific hypothalamic areas on the afternoon of pro-estrus in the rat-II. Serotonin. *Biogenic Amines* **6,** 607–614, 1989b.

GRAY, G.D. and WEXLER, B.C. Estrogen and testosterone sensitivity of middle-aged female rats in the regulation of LH. *Exp. Gerontol.* **15,** 201–207, 1980.

GROSSER, P., ROBAIRE, B., FAROOKHI, R., and BRAWER, J. Plasma patterns of LH, FSH and prolactin in rats with a polycystic ovarian condition induced by estradiol valerate. *J. Endocrinol.* **114,** 33–39, 1986.

HARMAN, S.M. Role of free radicals in mutation, cancer, aging and maintenance of life. *Free Rad. Res.* **16,** 752–763, 1962.

HARMAN, S.M. and TALBERT, G.B. Reproductive aging. In: Finch, C.E. and Schneider, E.L. (Editors), *Handbook of the Biology of Aging, 2nd ed.* pp. 457–510, Van Nostrand, New York, 1985.

HEMMINGS, R., FAROOKHI, R., and BRAWER, J. Pituitary and ovarian responses to luteinizing hormone releasing hormone (LHRH) in a rat with polycystic ovarian syndrome (PCO). *Biol. Reprod.* **29,** 239–248, 1983.

HOWLAND, B.E. Reduced gonadotropin release in repsonse to progesterone or gonadotropin releasing hormone (GnRH) in old female rats. *Life Sci.* **19,** 219–224, 1976.

HSU, H.K. and PENG, M.T. Hypothalamic neuron number of old female rats. *Gerontol.* **24,** 434–440, 1978.

HUANG, H.H., MASHALL, S., and MEITES, J. Capacity of old vs. young female rats to secrete LH, FSH and prolactin. *Biol. Reprod.* **14,** 538–543, 1976.

HUANG, H.H., STEGER, R.W., BRUNI, J.F., and MEITES, J. Patterns of sex steroid and gonadotropin secretion in aged female rats. *Endocrinology* **103,** 1855–1859, 1978.

JACOBSEN, W. and KALRA, S.P. Decreases in mediobasal hypothalamic and preoptic (^{3}H)-naloxone binding are associated with the progesterone-induced luteinizing hormone surge. *Endocrinology* **51,** 337–344, 1989.

KALRA, S.P. and KALRA, P.S. Neural regulation of luteinizing hormone secretion in the rat. *Endocrine Rev.* **4,** 311–351, 1983.

KALRA, S.P. and LEADEM, C.A. Control of luteinizing hormone secretion by endogenous opioid peptides. In: *Opioid Modulation of Endocrine Function,* G. Delitalia, M. Motta, M. Serio (Editors), pp. 171–185, 1984.

KALRA, S.P., SIMPKINS, J.W., and CHEN, S.L. The opioid-catecholamine modulation of gonadotropin and prolactin secretion. In: *Functional Correlates of Hormones Receptors in Reproduction.* pp. 135–149, Elsevier, North Holland, 1980a.

KATT, J.A., DUNCAN, J.A., HERBON, L., BARKIN, A., and MASHALL, J.C. The frequency of gonadotrpin-releasing hormone stimulation determines the number of pituitary gonadotropin-releasing hormone receptors. *Endocrinology* **116,** 2113–2115, 1985.

KOHAMA, S.G., ANDERSON, C.P., and FINCH, C.E. Progesterone implants extend the capacity for 4 day estrous cycles in aging C57BL/6J mice and protect against acyclicity induced by estradiol. *Biol. Reprod.* **41,** 233–240, 1989a.

KOHAMA, S.G., ANDERSON, C.P., OSTERBURG, H.H., MAY, P.C., and FINCH, C.E. Oral administration of estradiol to young C57BL/6J mice induces age-like neuroendocrine dysfunctions in the regulation of estrous cycles. *Biol. Reprod.* **41,** 227–232, 1989b.

KRIEGER, D., LIOTTA, A., NICHOLSEN, G., and KIZER, J. Brain ACTH and endorphin reduced in rats with monosodium glutamate-induced arcuate nuclear lesions. *Nature* **278,** 562–563, 1979.

KROHN, P.L. Transplantation in Aging. In: *Topics in the Biology of Aging,* Krohn, P.L. (Editor), pp. 125–148, Wiley, New York, 1966.

LARSON, G.H. and WISE, P.M. Age-related alterations in prolactin secretion by individual cells as assessed by the reverse hemolytic plaque assay. *Biol. Reprod.* **44,** 648–655, 1991.

LEADEM, C.A. and YAGENOVA, S.V. Effects of specific activation of mu-, delta- and kappa-opioid receptors on the secretion of luteinizing hormone and prolactin in the ovariectomized rat. *Neuroendocrinology* **45,** 109–117, 1987.

LIEHR, J.G. and ROY, D. Free radical generation by redox cycling of estrogens. *Free Rad. Biol. Med.* **8,** 415–423, 1990.

LU, J.K.H. Changes in ovarian function and gonadotropin and prolactin secretion in aging female rats. In: *Neuroendocrinology of Aging,* Meites, J. (Editor), pp. 103–122, Plenum Press, New York, 1983.

MACHADO-SALAS, J., SCHEIBEL, M.E., and SCHEIBEL, A.B. Morphological changes in the hypothalamus of the old mouse. *Exp. Neurol.* **57,** 102, 1977.

MALLORY, D.S. and GALLO, R.V. Medial preoptic-anterior hypothalamic LHRH relrease is inhibited by a mu-opioid agonist in the ovariectomized rat. *Brain Res. Bull.* **25,** 251–257, 1990.

MARIAN, J., COOPER, R.L., and CONN, P.M. Regulation of the rat pituitary gonadotropin-releasing hormone receptor. *Mol. Pharmacol.* **19,** 399–405, 1981.

McCARTHY, G.F., FAROOKHI, R., and BRAWER, J.R. Plasma gonadotropin pattern characterizing the development of polycystic ovaries in the estradiol valerate-treated rat. *Can. J. Physiol. Pharmacol.* **68,** 28–33, 1990.

McLAREN, J., SCHIPPER, H.M., and BRAWER, J.R. Iron content correlates with peroxidase activity in cysteamine-induced astrocytic organelles. *J. Histochem. Cytochem.* **40,** 1887–1897, 1992.

McNEIL, T.H. Neural structure and aging. *Rev. Biol. Res. Aging* **1,** 163, 1983.

MEANEY, M.J., BODNOFF, S.R., O'DONNELL, D., ROWE, W., SARRIEAU, A., ROSE, G.M., POIR-

IER, J., and SECKL, J.R. Glucocorticoids as regulators of neuron survival and repair in the aged brain. In: *Restorative Neurology* Vol. 6., Cuello, A.C. (Editor), pp. 123–146, Elsevier, Amsterdam, 1993.

MEITES, J. Neuroendocrine basis of aging in the rat. In: *Regulation of Neuroendocrine Aging,* Everitt, A.V., Walton, J.R. (Editors), pp. 37–50, Karger, Basel, 1988.

MERVIS, R. Cytomorphological alterations in the aging animals brain with emphasis on Golgi studies. In: *Aging and Cell Structure,* Vol. 1, Johson, Jr., J.E. (Editor), p. 143, Plenum Press, New York, 1981.

MEZEY, E., KISS, J.Z., MUELLER, G.P., ESKAY, R., O'DONOHUE, T.L., MILLER, A.E., and RIEGLE, G.D. Hypothalamic LH-releasing activity in young and aged intact and gonadectomized rats. *Exp. Aging Res.* **4,** 145–155, 1978.

MILLER, A.E. and RIEGLE, G.D. Temporal changes in serum progesterone in aging female rats. *Endocrinology* **106,** 1597–1583, 1980.

MILLER, M.M., JOSHI, D., BILLIAR, R.B., and NELSON, J.F. Loss during aging of beta-endorphin neurons in the hypothalamus of female C57BL/6J mice. *Neurobiol. Aging* **12,** 239–244, 1991.

MOBBS, C.V. and FINCH, C.E. E2-induced impairments as a mechanism in reproductive senescence of female C57BL/6J mice. *J. Gerontol.* **47,** B48–B51, 1992.

MOBBS, C.V., FLURKEY, K., GEE, D.M., YAMAMOTO, K., SINHA, Y.N., and FINCH, C.E. Estradiol-induced anovulatory syndrome in the female C57BL/6J mice: Age-like neuroendocrine, but not ovarian impairments. *Biol. Reprod.* **30** 556, 1984.

MOBBS, C.V., KAPLITT, M., KOW, L.M., and PFAFF, D.W. PLC-alpha: A common mediator of the action of estrogen and other hormones? *Mol. Cell Endocrinol.* **80,** C187–C191, 1992.

MORRELL, J., McGINTY, F., and PFAFF, D.W. A subset of beta-endorphin or dynorphin-containing neurons in the medial basal hypothalamus accumulate estradiol. *Neuroendocrinology* **41,** 417–426, 1985.

MURPHY, M.T. and LIPTON, J.M. Beta-endorphin: Effect on thermoregulation in aged monkeys. *Neurobiol. Aging* **4,** 187–190, 1983.

NAFTOLIN, F., GARCIA-SEGURA, L.M., KEEFE, D., LERANTH, C., MACLUSKY, N.J., and BRAWER, J.R. Estrogen effects on the synaptology and neural membranes of the rat hypothalamic arcuate nucleus. *Biol. Reprod.* **42,** 21–28, 1990.

NASS, T.E., LAPOLT, P.S., JUDD, H.L., and LU, J.K.H. Alterations in ovarian steroid and gonadotrophin secretion preceding the cessation of regular oestrous cycles in ageing female rats. *J. Endocr.* **100,** 43–50, 1984.

NELSON, F.F., FELICIO, L.S., OSTERBURG, H.H., and FINCH, C.E. Altered profiles of estradiol and progesterone associated with prolonged estrous cycles and persistent vaginal cornification in aging C57BL/6J mice. *Biol. Reprod.* **24,** 784–794, 1980.

NELSON, J.F., FELICIO, L.S., RANDALL, P.K., SIMS, C., and FINCH, C.E. A longitudinal study of estrous cyclicity in aging C57BL/6J mice: I. Cycle frequency, length and vaginal cytology. *Biol. Reprod.* **27,** 327–339, 1982.

NELSON, J.F. and FELICIO, L.S. Reproductive aging in the female: An etiological perspective updated. *Rev. Biol. Res. Aging* **3,** 359–381, 1985.

NELSON, J.F., BENDER, M., and SCHACHTNER, B.S. Age-related changes in proopiomelanocortin meessenger ribonucleic acid levels in hypothalamus and pituitary of female C57BL/6J mice. *Endocrinology* **123,** 340–344, 1988.

OLNEY, J. Glutamate-induced neuronal necrosis in the infant mouse hypothalamus. *J. Neuropath. Exp. Neurol.* **30,** 75–90, 1971.

PAGE, R.D. and BUTCHER, R.L. Follicular and plasma patterns of steroids in yound and old rats during normal and prolonged estrous cycles. *Biol. Reprod.* **27,** 383–392, 1982.

PATERSON, S.J., ROBSON, L.E., and KOSTERLITZ, H.W. Opioid receptors. *Peptides* **6,** 147–189, 1984.

PENG, M.-T. and HUANG, H.H. Aging of hypothalamic–pituitary–ovarian function in the rat. *Fertil. Steril.* **23,** 535–542, 1972.

PIOTTE, M., JOH, T.H., and BRAWER. J.R. The fine structural organization of tyrosine hydroxylase immunoreactive neurons in the rat arcuate nucleus. *J. Comp. Neurol.* **239,** 44–53, 1985.

PROUD, V., HSIA, Y., and WOLF, B. Disorders of amino acid metabolism. In: *Basic Neurochemistry,* 4th ed., Siegel, G., Agranoff, B., Albers, R., and Molinoff, P. (Editors), pp. 733–763, Raven Press, New York, 1989.

ROGERS, J., SHOEMAKER, W.J., MORGAN, D.G., and FINCH, C.E. Senescent changes in tissue weight and immunoreactive β-endorphin, enkephalin, and vasopressin in eight regions of C57BL/6J mouse brain and pituitary. *Neurobiol. Aging* **6,** 1–9, 1985.

SAPOLSKY, R.M., KREY, L.C., and MCEWEN, B.S. The neuroendocrinology of stress and aging: The glucocorticoid cascade. *Endocr. Rev.* **7,** 284–301, 1986.

SAR, M. Estradiol is concentrated in tyrosine hydroxylase-containing neurons of the hypothalamus. *Science* **223,** 938–940, 1984.

SAR, M., SAHU, A., CROWLEY, W.R., and KARLA, S.P. Localization of neuropeptide-Y imunoreactivity in estradiol-concentrating cells in the hypothalamus. *Endocrinology* **127,** 2752–2756, 1990.

SARKAR, D.K., GOTTSCHALL, P.E., and MEITES, J. Damage to hypothalamic dopaminergic neurons is associated with development of prolactin-secreting pituitary tumors. *Science* **218,** 684–686, 1982.

SCHIPPER, H.M. Gomori-positive astrocytes: Biological properties and implications for neurologic and neuroendocrine disorders. *GLIA* **4,** 365–377, 1991b.

SCHIPPER, H.M., BRAWER, J., DESJARDINS, G.C., BEAUDET, A., and BRAWER, J. The 21-aminosteriod antioxidant, U743-89F, prevents estradiol-induced depletion of hypothalamic beta-endorphin neurons in adult female rats. *Brain Res.* **652,** 161–163, 1994.

SCHIPPER, H.M., BRAWER, J.R., NELSON, J.F., FELICIO. L.S., and FINCH, C.E. Role of the gonads in histologic aging of the hypothalamic arcuate nucleus. *Biol. Reprod.* **25,** 413–419, 1981.

SCHIPPER, H.M., KOTAKE, Y., and JANZEN, E. Catechol oxidation by peroxidase-positive astrocytes in primary culture: An electron spin resonance study. *J. Neuroscience* **11,** 2170–2176, 1991a.

SCHIPPER, H.M., LECHAN, R.M., and REICHLIN, S. Glial peroxidase activity in the hypothalamic arcuate nucleus: Effects of estradiol-valerate induced persistent estrus. *Brain Res.* **507,** 200–207, 1990b.

SCHIPPER, H.M., PIOTTE, M., and BRAWER, J. Effects of progestins on the age-related accumulation of astrocytic granules in the hypothalamic arcuate nucleus. *Brain Res.* **527,** 176–179, 1990a.

SCHULSTER, A., FAROOKHI, R., and BRAWER, J.R. Polycystic ovarian condition in estradiol valerate-treated rats: Spontaneous changes in characteristic endocrine features. *Biol. Reproduc.* **29,** 239–248, 1984.

SHAAR, C.J., EUKER, J.S., RIEGLE, G.D., and MEITES, J. Effects of castration and gonadal steroids on serum luteinizing hormone and prolactin in old and young rats. *J. Endocrinol.* **66,** 45–51, 1975.

SHIVERS, B.D., HARLAN, R.E., MORRELL, J.I., and PFAFF, D.W. Absence of estradiol concentration in cell nuclei of LHRH-immunoreactive neurons. *Nature* **304,** 345–347, 1983.

SIMANTOV, R. and AMIR, S. Regulation of opiate receptors in mouse brain: Arcuate nuclear lesion induces receptor upregulation and supersensitivity to opiates. *Brain Res.* **262,** 168–171, 1983.

SIMANTOV, R., KUHAR, M.J., and UHL, G.R. Opioid peptide enkephalin: Immunohistochemical mapping in rat central nervous system. *Proc. Natl. Acad. Sci. USA* **74,** 2167–2171, 1977.

SIMARD, M., BRAWER, J.R., and FAROOKHI, R. An intractable ovary-dependent impairment in the hypothalamo–pituitary axis of the estradiol-valerate induced polycystic ovarian condition in the rat. *Biol. Reprod.* **36,** 1229–1237, 1987.

STAMPFER, M.J., HENNEKENS, C.H., MANSON, J.E., GOLDITZ, G.A., ROSNER, B., and WILLET, W.C. Vitamin E consumption and the risk of coronary disease in women. *N. Engl. J. Med.* **328,** 1444–1449, 1993.

STEGER, R.W. Age-related changes in the control of prolactin secretion in the female rat. *Neurobiol. Aging* **2,** 119–123, 1981.

STEGER, R.W., HUANG, H.H., CHAMBERLAIN, D.S., and MEITES, J. Changes in control of gonadotropin secretion in the transition period between regular cycles and constant estrus in aging female rats. *Biol. Reprod.* **22,** 595–603, 1980.

STEHLER, B.L. *Time, Cells and Aging,* Academic Press, New York, 1977.

TAKAHASHI, S. and KAWASHIMA, S.S. Age-related changes in prolactin cells in male and female rats of the Wistar/Tw strain. *J. Sci. Hiroshima Univ. ser. B,* div.1, **31,** 185–195, 1983.

TAKAHASHI, S., KAWASHIMA, S.S., and WAKABAYASHI, S.K. Effects of gonadectomy and chlorpromazine treatment on prolactin, LH and FSH secretion in young and old rats of both sexes. *Exp. Gerontol.* **15,** 185–194, 1980.

TAPPEL, A.L. Lipid peroxidation damage to cell components. *Fed. Proc.* **32,** 1870–1874, 1973.

TELFORD, N., MOBBS, C.V., SINHA, Y.N., and FINCH, C.E. The increase of anterior pituitary dopamine in aging C57BL/6J female mice is caused by ovarian steroids, not intrinsic pituitary aging. *Neuroendocrinology* **43,** 135–142, 1986.

THUNG, P.J. The relation between amyloid and ageing in comparative pathology. *Gerontologia* **1** 234–254, 1956.

TREOLAR, A.E. Menstrual activity and premenopause. *Maturitas* **3,** 249–264, 1981.

VAN VUGT, D.A., AYLSWORTH, C.F., SYLVESTER, P.W., LEUNG, F.C., and MEITES, J. Evidence

for hypothalamic noradrenergic involvement in naloxone-induced stimulation of luteinizing hormone release. *Neuroendocrinology* **33,** 261–264, 1981.

VAN VUGT, D.A., LAM, N.Y., and FERIN, M. Reduced frequency of pulsatile luteinizing hormone secretion in the luteal phase of the rhesus monkey. Involvement of endogenous opiates. *Endocrinology* **115,** 1095–1101, 1984.

WARDLAW, S.L., THORON, L., and FRANTZ, A.G. Effect of sex steroids on brain beta-endorphin. *Brain Res.* **245,** 327–331, 1982.

WATKINS, B.E., MEITES, J., and RIEGLE, G.D. Age-related changes in pituitary responsiveness to LHRH in the female rat. *Endocrinology* **97,** 543–548, 1975.

WEILAND, N.G. and WISE, P.M. Aging progressively alters the diurnal rhythms and decreases the density of alpha-1-adrenergic receptors in selected hypothalamic regions. *Endocrinology* **126,** 2392–2397, 1990.

WIKLUND, J.A. and GORSKI, J. Genetic differences in estrogen-induced deoxyribonucleic acid synthesis in the rat pituitary: Correlations with pituitary tumor susceptibility. *Endocrinology* **111,** 1140–1149, 1982.

WILKINSON, M., BHANOT, R., WILKINSON, D.A., and BRAWER, J.R. Prolonged estrogen treatment induces changes in opiate, benzodiazepine and beta-adrenergic binding sites in female rat hypothalamus. *Brain Res. Bull.* **11,** 279–281, 1983.

WILKINSON, C.W. and DORSA, D.D. The effects of aging on molecular forms of beta- and gamma-endorphins in the rat hypothalamus. *Neuroendocrinology* **43,** 124–131, 1986.

WILLIAMS, G.C. Pleiotropy, natural selection, and the evolution of senescence. *Evolution* **11,** 398–411, 1957.

WISE, P.M. Alterations in the proestrous pattern of median eminence LHRH, serum, LH, FSH, estradiol and progesterone concentrations in middle-aged rats. *Life Sci.* **31,** 165–173, 1982.

WISE, P.M., SCARBROUGH, K., LARSON, G.H., LLOYD, J.M., WEILAND, N.G., and CHIU, S. Neuroendocrine influences on aging of the female reproductive system. *Front. Neuroendocrinol.* **12,** 323–356, 1991.

ZABORSKY, L. and MAKARA, G.B. Intrahypothalamic connections: An electron microscopic study of the rat. *Exp. Brain Res.* **34,** 201–215, 1979.

ZURCHER, C., VAN ZWIETEN, M.J., SOLLEVELD, H.A., VAN BEZOOIJEN, C.F.A., and HOLLANDER, C.F. Possible influence of multiple pathologic changes in aging rats on studies of organ aging, with emphasis on the liver. In: *Liver and Aging: 1982: Liver and Drugs,* Kitani, K. (Editor), pp. 19–36, Elsevier, Amsterdam, 1982.