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Sex Steroid Signaling During Aging

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A plethora of changes occur at genetic, molecular, cellular, tissue, organ and organism levels during different stages of the lifespan. Consequently, adaptability of the organism to its environment decreases, making it more susceptible to develop a variety of disabilities and diseases which lead to an increased likelihood of death. Among several factors causing these conditions, one important contributing factor is alteration in the level and action of hormones, their receptors and responses with advancing age. Hormones which play a key role in aging include growth hormone, insulin, melatonin, thyroxine, dehydroepiandrosterone (DHEA), estrogen and testosterone. Out of these, the involvement of sex steroids (DHEA, estrogen and testosterone) is of special significance because they exert a multitude of functions in both reproductive and non-reproductive tissues. Particularly, the use of estrogen in postmenopausal women has generated more debates among the public and attracted the attention of clinicians and scientists. Though hormonal interventions show beneficial effects, they are associated with risks of harmful consequences and therefore hormonal prescriptions should be made with caution. These investigations require rigorous evaluation. A recent report based on US study suggests more harmful than beneficial effects of long-term treatment with sex steroids. In this article, an overview is presented for the role of sex steroids in aging and especially on the recent progress in hormone replacement therapy. It is expected that a better understanding of the hormonal changes and interventions during aging will help to develop a strategy to delay or prevent a number of age-dependent diseases and make old age healthy and happy.

Key Words: Aging, Hormone signaling, Sex steroid, DHEA, Estrogen, Androgen, Diseases, Hormone Replacement Therapy

Introduction

Aging is a universal biological phenomenon, but our understanding of why and how we age remains limited. It refers to a progressive loss of physiological functions, decline in fertility, decreased ability to respond to a wide range of stresses, increased risk of age-associated diseases and disorders, and more likelihood of mortality. There has been a global increase in the life expectancy of humans and a consequent rise in the proportion of elderly people in the total population as a result of general health awareness, improved hygiene, immunization, and advancements in medical treatment of diseases. India has the second largest elderly population in the world. With the increase in elderly population, there is also high prevalence of age-associated health problems such as arthritis, osteoporosis, diabetes, cancer, Alzheimer's disease (AD), dementia and cognitive dysfunction, depression, Parkinson's disease and cardiovascular disease (including heart attack and stroke).

Many diseases commonly encountered in the aged people can be assigned to age-related alterations in the levels of hormones, their receptors and coregulators and the signal transduction pathways. The important hormones which play a key role during aging include growth hormone, insulin, melatonin, thyroxine, dehydroepiandrosterone (DHEA), testosterone, estrogen and androgen. In particular, the role of sex steroid hormones, especially estrogen and androgen, in this context is very important. This is also supported by the

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observation that there is an inverse relationship between reproductive potential and longevity. For example, the species with large reproductive potential and early onset of reproduction have short lifespan whereas those with small reproductive potential and late onset of reproduction have long lifespan (Finch 1990). This would be expected if available metabolic resources are shared between investment in reproduction and investment in the preservation of the adult body.

Over the past several decades, sex steroid hormones have been known to be involved mostly in reproduction. Even the standard physiology text books provide the impression that androgens regulate only male-specific and estrogens only female-specific reproductive processes. However, recent research findings have made it clear that the sex steroid hormones have several functions other than regulation of sexual and reproductive behavior. In addition to their well known influence on bone and heart, these hormones exert a wide variety of effects on the brain (Thakur 1999). In particular, estrogens offer protection against age-dependent diseases such as osteoporosis and coronary artery disease, and likewise have an effect on memory, cognition and progression of some forms of dementia. Though involvement of estrogen in brain development and function has long been known, only recently its role in the age-dependent deterioration of mental function has attracted more careful attention.

After accumulating information on age-related alterations in molecules and molecular pathways, researchers are presently exploring different possibilities of developing new strategies to intervene in the age-associated changes so that the problems of old age can be postponed, quality of life can be improved and a healthy lifespan can be achieved. These approaches include gene therapy, hormonal replacement, stress management, anti-oxidant (vitamin A, C, E) treatment, calorie restriction and small molecule (carnosine) intervention. Such anti-aging approaches have been employed by different workers using different model systems (Yu 1999, Thakur 2002). Out of these approaches, hormone replacement therapy (HRT) is discussed here and it is based on the principle that a major causal factor for most of the age-dependent diseases is imbalance in the level and responsiveness of hormones. Hence it is expected that by exploring the hormonal changes during aging, means can be found to prevent or alleviate some of the most debilitating diseases of old age. However, several workers have noted the harmful effects of HRT, particularly when the treatment is prolonged (Lacey et al. 2002, Rossouw et al. 2002). Therefore, extensive investigation needs to be carried out before recommending the hormonal treatment for diseases of the elderly.

This article reviews the progress that has been made during the past decades in the area of sex steroid hormones and aging. Mainly the following major issues have been addressed: What are the changes in the level of sex steroids, their receptors and other factors involved in the signal transduction? How do these changes influence different physiological processes and lead to age-associated diseases and disorders? Can HRT delay or prevent aging and extend healthy lifespan? What are the harmful side effects of HRT? The observations made in the author's laboratory and reported from other researchers in this context are discussed below. Despite considerable progress achieved in recent years, many questions remain unanswered. It is likely that advancements in the molecular biology techniques will help in filling these gaps in the existing knowledge and in developing strategies that will enable everyone to live a healthy life free of diseases and disabilities of old age.

Sex Steroids Involved in Aging and their Mechanism of Action

Sex steroid hormones which play important role during the lifespan of an organism include DHEA, estrogen and testosterone. They mediate their action through genomic and non-genomic pathways (Thakur 1995, Sharma 1999). There are specific intracellular receptors to which these hormones bind with great specificity and affinity. Consequently, many proteins associated with the inactive receptor (such as heat shock protein HSP) are dissociated. Now the receptor becomes activated and forms dimer. Then the hormone-receptor complex binds to specific hormone response elements (HRE) located in the promoter of target genes. This is followed by recruitment of a

number of coregulators and interaction with basal transcription factors (like TATA-binding protein TBP and TBP-associated factors TAFs) to modulate the transcriptional activity of specific gene (figure 1). In addition to this genomic pathway, in some cases, the hormone exerts its action at the cell membrane level through the non-genomic pathway. As a result the level and activity of different gene products alter and this causes changes in cellular functions.

Several studies have shown changes in the level of hormone and its receptor, other factors involved in signal trasduction and responsiveness with advancing age (Kanungo 1980, Thakur 1988, Roth 1995, Timiras et al. 1995). All these changes in molecules or molecular events finally influence the hormone action leading to alterations in physiological activities. Age changes in some important steroid hormones are mentioned below.

DHEA

It is a steroid hormone produced by the adrenal gland and acts as a precursor to the sex hormones estrogen and testosterone. It is estimated that over 50% of total androgen in adult men and approximately 75% before menopause and close to 100% of estrogen after menopause in women are derived from peripheral conversion of DHEA and its sulfate (DHEAS) (Labrie et al. 1995). The level and receptor of this hormone drop drastically with increasing age. In normal subjects, the serum concentrations of DHEA and its sulfate are highest in the third decade of life, after which their concentrations decrease gradually so that by the age

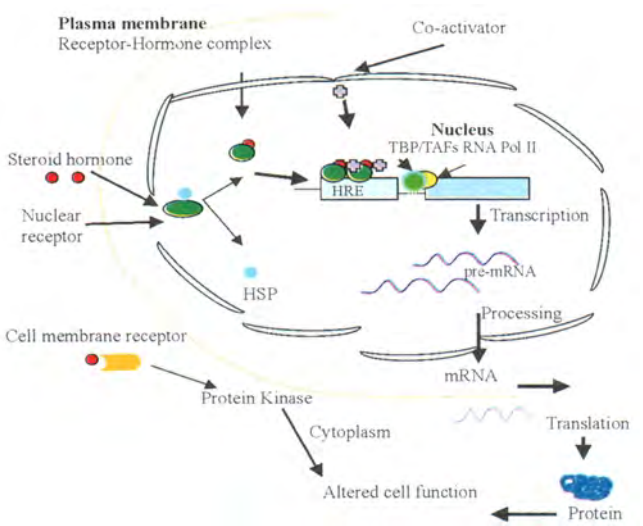

Figure1 Mechanism of action of sex steroid hormone

of 70-80 years the values are about 20% of peak values in men and 30% of peak values in women (Labrie et al. 1997). This condition is called "adrenopause" and is caused by a selective decrease in the number of functional zona reticularis cells in the adrenal cortex (Labrie et al. 1995). Ravaglia et al. (1996) reported a strong interrelation of DHEAS with functional activities of 90-106 years old healthy people. Among the oldest men, those with the highest functional activities had the highest DHEAS levels and individuals with poor functional activities had the lowest DHEAS levels. Although the decline of DHEAS levels persists into advanced age with a sexually dimorphic pattern, cortisol levels in men and women show a parallel linear increase with aging (Laughlin & Barrett-Connor 2000).

In addition to DHEA, there are other hormones which play important role in longevity and aging of lower organisms. For example, there is a 10-fold difference between the lifespan of female worker bees (*Apis mellifica*) which have rapid senescence and lifespan of months, and queens of the same genotype which show much slower senescence during the lifespan of many years with active egg production. This is due to exposure of larvae to nutrients and juvenile hormones. Similarly, in female octopus, the secretion of optic gland has profound effects on its behavior and probably also causes its senescence and death (Wodinsky 1978). The female octopus lays eggs only once in its lifetime, broods them and simultaneously decreases its food intake, and dies soon after the young ones hatch. If, however, the optic glands are removed after spawning, it stops brooding, resumes feeding, continues to grow and its lifespan is extended. Which factors of the optic gland are responsible for such responses is not known, but it is clear that hormones are involved in determining the duration of lifespan.

Estrogen

The most remarkable and rapidly occurring change in women around the age of 50 is menopause. During this phase, the cycling estradiol production is replaced by very low and constant level of estradiol. For many years, it was believed that the menopause results from the exhaustion of ovarian follicles. But later research has provided the evidence that both ovary and brain are key pacemakers in menopause (Wise et al. 1996). By the

time a woman is 65 years old, the ovary is virtually devoid of follicles and is no longer the primary site of estrogen or progesterone synthesis. Also, inhibin, a glycoprotein hormone that is synthesized by the granulosa and luteal cells of the ovary and that selectively suppresses follicle stimulating hormone (FSH) secretion, becomes undetectable in the blood. In response, the anterior pituitary gland secretes copious amounts of both of the gonadotropins FSH and luteinizing hormone (LH). The level of FSH increases by the time women are 45 to 50 years, when they are still menstruating, whereas the level of LH increases later, when women are postmenopausal. An understanding of the factors that bring about these hormonal changes during the reproductive life will help to develop strategies for alleviating the negative aspects of menopause (Wise et al. 1996).

The low serum levels of estrogen in old female rats correlate well with the low level of its receptor (ER) in the brain (Kanungo et al. 1975). Particularly, the mouse brain cortex expresses ER, the size of which is larger than that of uterine ER and the expression of which decreases drastically at the old age. The expression of ER is downregulated by estrogen (Asaithambi et al. 1997). The presence of low levels of estrogen and ER in the old age is responsible for reproductive aging in females, characterized by irregular estrus cycles and a decline in fertility, pseudopregnancies and sexual behavior (Thakur 1988). ER exists in two forms – ERα and ERβ. The proportion of these receptors varies in different tissues and with age. The level of ERα is relatively higher than that of ERβ in the mouse brain cortex and the amount of both receptors decrease at the old age (Sharma & Thakur, manuscript submitted). Age-related decrements in ERα levels cause reduced responsiveness of hippocampal synapses to estrogen in aged rats (Adams et al. 2002).

ER, through which estrogen mediates its action, shows age-related changes in its structure and properties. Following the digestion of ER with proteolytic enzymes and the analysis of cleaved products by SDS-PAGE, similar pattern is observed between young and old. However, in vitro transformation by RNase, urea and ATP shows that the degree of ER transformation is lower in old than young. Furthermore, the transformed ER from old age is less capable of binding to DNA than that from young age. Thus these results show that the conformation of ER probably does not change with age, but the degree of transformation and the ability of transformed receptor to bind to DNA decrease with age (Thakur & Kaur 1991). Despite the similarity in different physico-chemical properties, the number of estrogen binding sites and the retention time of ER complexes in nuclei and the ability of these complexes to bind to DNA decrease in the uterus of old age (Kaur & Thakur 1991). Post-translational modification of nuclear proteins by phosphorylation enhances the binding of ER complex by two-fold in the nuclei from young rats but reduces this to half in nuclei from old (Kaur & Thakur 1990).

In addition to age-related changes in ER expression and biochemical characteristics, response to estradiol also varies with the age. For instance, in the rat brain, estradiol stimulates the phosphorylation and methylation of histones and non-histone chromosomal proteins in the adult but not in the old (Thakur & Kanungo 1981). It enhances acetylation of non-histone chromosomal proteins only in immature rats. Nuclei purified from the brain after acetylation in the presence or absence of estradiol and incubated with (^{3}H)UTP show that transcription is higher after estradiol treatment in immature and adult rats (Thakur et al. 1978, Kanungo & Thakur 1979). Both testosterone and estradiol modulate the synthesis and phosphorylation of brain cortex proteins in age- and sex-dependent manner (Mukherjee et al. 1999). Administration of estrogen induces the synthesis of liver proteins significantly in young rats but shows no remarkable effect in old. Interestingly, there is about two-fold increase in the synthesis of a specific cytosolic protein of 45 kD, after estrogen injection in young but not in old female rats. In contrast, DNA methylation is reduced by estrogen to greater extent in young than old rats. The HPLC data further reveal that estrogen lowers the level of 5-methyl cytosine by 8% in young but shows no effect in the old. Such age-dependent changes in the covalent modifications of chromosomal proteins and DNA brought by estrogen in the rat uterus and brain attribute to alterations in cellular functions during aging (Thakur & Kaur 1992, Thakur et al.

1993). These findings further establish the reduced responsiveness of target tissues to estrogen in old age (Thakur & Kaur 1993).

Testosterone

Serum levels of total and bioavailable testosterone decrease slowly and subtly with age in men (Morley et al. 1993). More than 60% of healthy men over 65 years of age have free testosterone levels below the normal values of men aged 30 to 35 (Tenover 1994). Although all men are not destined to become hypogonadal as they age, the prevalence of androgen deficiency in the older male is significant. This condition is known as "andropause" and is characterized by a decrease in testicular Leydig cells number and in their secretory capacity, as well as by an age-related decrease in episodic and stimulated gonadotropin secretion (Harman & Tsitouras 1980).

Also the level, structure, expression and other characteristics of androgen receptor (AR), through which testosterone acts, change with age. The findings of Mukherjee et al. (1996) suggest a possible protective role of testosterone in the cleavage of chromatin of the liver by endogenous nucleases during the aging of mice. Testosterone induces the amplification of exons 4 and 5 of AR gene in the brain cortex of aged female mice (Thakur et al. 2000a). The amount and synthesis of AR decreases but phosphorylation of AR increases in the brain cortex with advancing age of mice and they are regulated by testosterone and estradiol in age- and sex-specific manner (Thakur et al. 2000b). The rat ventral prostate, which has been used extensively as a model for hormone-dependent prostate cancer, regresses following androgen ablation due to the selective loss of secretory epithelial cells that undergo apoptosis. The rate of regression significantly slows down after castration in older animals, suggesting a decline in hormone responsiveness with advancing age (Morrissey et al. 2002). The reduced responsiveness to testosterone in old age is also attributed to a decrease in the level of testosterone and its receptor. This has an impact on sexual function, muscle mass, bone, prostate and central nervous system, and leads to the impairment of sexual functions, cognition and memory and ultimately the appearance of a variety of age-associated disorders (Lamberts et al. 1997).

Sex Hormone-related Diseases in Elderly

In general, the hormonal responsiveness decreases with advancing age and this leads to a variety of diseases like dementia, osteoporosis, cardiovascular disease, diabetes and cancer (table 1). These diseases are more prevalent in old age. In addition, the elderly people have many physiological disturbances due to alterations in hormone action. Such hormonal conditions are described below.

Estrogen

The levels of endogenous sex steroid hormones are significantly related to bone density in older women and men (Greendale et al. 1997). Individual variation in age-related bone loss may be partially accounted for by alterations in sex steroid levels with aging. It is observed that hypertension is significantly delayed in females with functional ovaries. This protection is lost on ovariectomy and is restored on estrogen replacement (Roberts et al. 2001). Zhu et al. (2002) have reported that ERβ-deficient mice develop sustained systolic and diastolic hypertension as they age, indicating an essential role for ERβ in the regulation of blood pressure. This is also supported by the observation that an association is found between ERβ gene polymorphism and systemic blood pressure in postmenopausal Japanese women

Table 1 *Old age diseases associated with defects in hormones, receptors and their mechanism of action*

Somatopause (decline in GH/IGF-1)
Menopause (decline in estrogen)
Andropause (decline in tetosterone)
Adrenopause (decline in DHEA and DHEAS)
decreased sexual activity
deficits in memory
cognitive impairment
depression
anxiety
insomnia
bone loss
osteoporosis
diabetes
atherosclerosis
cardiovascular disease
cancer of different kinds (breast, prostate)

(Ogawa et al. 2000). Older women also have low level of thyroxine and this is associated with greater risk of cognitive impairment (Volpato et al. 2002).

Testosterone

Many physiological changes associated with aging such as decreased sexual function, depression, anxiety, irritability, insomnia, weakness, impotency, poor memory, reduced muscle and bone mass, osteoporosis and muscle wasting are attributed to androgen deficiency (Sternbach 1998). Numerous studies of large populations of healthy men have shown a marked rise in the incidence of impotence to over 50% in men aged 60 to 70. During aging in humans, the reduction of adrenal androgen secretion is accompanied by a host of neuroendocrine-metabolic dysfunctions that include decline in the growth hormone–insulin growth factor-1 (GH-IGF-1) system, thyroid function, immune competence, fragmentation of sleep and neuronal loss (Yen 2001). Epidemiological studies indicate a close relationship between high IGF-1 levels and cancer development.

Many studies show a significant positive correlation between testosterone or GH levels and muscle mass and bone mineral density, but a negative correlation between testosterone or GH levels and abdominal fat mass. There is a decline in muscle mass by 20%-40% between the age of 25 and 75, a doubling of fat mass and a decrease in bone mineral density by 0.3% per year after age 35. These age-associated changes have important functional and metabolic consequences. The beneficial effects of hormone substitution on body composition suggest that testosterone and GH are at least two co-determinants of the observed age-associated changes (Vermeulen 2002).

Furthermore, testosterone is found to inhibit vascular cell adhesion molecule-1 mRNA and protein expression in human umbilical vein endothelial cells by its conversion to estradiol via aromatase (Mukherjee et al. 2002). Thus testosterone may have beneficial effects in atherosclerosis which is more prevalent in postmenopausal women. Studies of Goodman-Gruen and Barrett-Connor (2000) suggest an inverse association between androgens and type 2 diabetes in men and a positive association between androgens and type 2 diabetes in women. Diabetes is also associated with lower levels of cognitive function and greater cognitive decline among older women (Gregg et al. 2000).

Anti-aging Effects of Hormones

Among various approaches employed to reverse the undesirable effects of aging, the hormone replacement therapy (HRT) has been in use for a long time. HRT for postmenopausal women has been studied and discussed for many years. But the idea of male HRT is a relatively recent development because of the interest in preventing age-related dysfunction and improving quality of life among increasing populations of old men. The logic behind this approach has been that since the level of various hormones decreases during aging, supplying these hormones externally may compensate for this loss and rejuvenate the system (Everitt & Meites 1989). Various therapeutic procedures have been followed to replace the lost hormones, such as gland transplantation, secretory-cell injections and hormone administrations. Whereas many of these approaches have not been successful, others have been refined to some extent and are still in use.

The effects of different hormones on the lifespan and vigor of animals have been studied by several workers. Posterior pituitary hormones (except GH) and estrogen are reported to maintain and prolong the lifespan and hence are believed to act as "life maintaining hormones". For example, as early as 1889, Brown-Sequard administered a suspension of the testis of a dog to himself and reported that it rejuvenated him at age 72. However, this finding has not been corroborated later. Recently, it has been demonstrated in soil nematode (*C elegans*) that the germ-line stem cells affect lifespan by influencing the production of or the response to a steroid hormone which promotes longevity. By killing the germ-line precursor cells, z2 and z3, the nematode lifespan is extended by ~60% (Arantes-Oliveira et al. 2002). On the other hand, GH, thyroxine, testosterone and adrenal corticoids are reported to decrease the lifespan and hence are believed to act as "aging hormones" (Everitt & Delbridge 1976).

When hormones, whose levels decline with age, are supplemented exogenously, they show anti-aging effects. They increase longevity and help the

organism to recover many functions which were lost or reduced with advancing age. Such anti-aging effects of a wide variety of hormones, vitamins and other agents are mainly due to their effects on maintenance or recovery of functional activity. However, some of these hormones are reported to have serious side effects. For example, steroids may cause kidney damage and brittle bone. A recent study conducted in US reveals that longer duration of estrogen plus progestin treatment does more harm than benefits (Rossouw et al. 2002). Therefore, it is essential to examine the harmful side effects of hormones before their supplementation to cure chronic diseases of old age. The main sex steroid hormones which show anti-aging effects are described below.

DHEA

The secretion and blood levels of DHEA and DHEAS decrease profoundly with age. The question arises whether administration of the steroid to compensate for the decline counteracts defects associated with aging. There are experimental evidences which show that DHEA exerts a beneficial influence on some aging-associated deficits in rodents. It is well established that aging is associated with a decline in reproductive functions in rats. In order to evaluate the effect of DHEA on gonadotropin releasing hormone (GnRH) gene expression which is involved in reproductive functions, Li et al. (1997) studied the effect of short-term administration of DHEA to young and aged rats of both sexes. In the aged animals, the mRNA levels declined by 10% than those observed in the young rats. DHEA completely restores the mRNA levels when compared to those detected in young male animals. In female rats, the hybridization signal decreases by 28% in old. However, DHEA increases GnRH mRNA levels by 11% in young and 33% in aged female rats, thus completely reversing the influence of aging. These results indicate that the decrease in GnRH expression, which is responsible for the loss of reproductive functions in aged rats, can be totally reversed by a short-term administration of DHEA which restores the GnRH neuronal activity. They also suggest that DHEA might play a role in the prevention and/or improvement of some deficits associated with aging through stimulation of GnRH biosynthesis.

Animal studies in rodents, which have very low circulating DHEAS levels, further suggest that DHEA administration prevents obesity, diabetes mellitus, cancer and heart disease, while enhancing immune function. Thus DHEA is a multifunctional hormone with immunoenhancing, antidiabetic, antiobesity, anticancer, neurotrophic, memory-enhancing and anti-aging effects (Labrie et al. 1995, Yen 2001). These results suggest that DHEA prolongs lifespan and might be an "elixir of youth". Experimental evidences suggest that DHEA treatment might prevent aging and therefore elderly people take DHEA as a food supplement in order to remain biologically young (Johnson et al. 2002). In a study conducted by Baulieu et al.(2000), 280 healthy individuals (60-79 years old men and women) were orally given DHEA 50 mg or placebo daily for a year in a double-blind, placebo-controlled study. The results show improvement of bone turnover and skin status and the decrease of osteoclastic activity in women over 70 years. A significant increase in most libido parameters is also found in these older women. Interestingly, a number of biological indices confirmed the lack of harmful consequences of this 50 mg/day DHEA administration over one year, indicating that this kind of replacement therapy normalizes some effects of aging. Thus when taken as a dietary supplement, DHEA claims to improve mood and memory, counteract stress hormones, preserve muscle tone, and restore sexual vigor. However, high doses of DHEA may confer a great risk for developing prostate and breast cancer.

Estrogen

One third of the average human female lifespan is postmenopausal, during which the level of estrogen is very low. Some women supplement this period of reduced estrogen production with estrogen replacement therapy (ERT). Many epidemiological studies have examined the long-term (5 to 10 years) effects of postmenopausal estrogen deprivation and of ERT. Some results suggest beneficial effects whereas others show serious side effects. Particularly longer duration of the treatment is more harmful. In some cases, ERT has been found as the most effective method for preventing osteoporotic

bone loss and fractures in postmenopausal women. It reduces the risk of fatal and nonfatal myocardial infarction, heart disease and stroke by 20-40%. Apart from relieving hot flashes and mood changes, reducing skin and reproductive tract atrophy (such as dryness of the vagina and urinary incontinence), and preventing changes in body composition, estrogen/progesterone replacement therapy delays atherosclerosis, loss of bone mass, and impairment of cognitive function.

Estrogen plays a very important role in brain aging (Thakur 1999). It acts as a potent growth and protective factor. In the developing nervous system of rodents and primates, sex steroids exert a trophic influence by stimulating axonal and dendritic growth, modulating synapse formation and neurotransmitter activity. At later stages, estradiol treatment is associated with enhancement of short memory, anti-depressive effect, improvement of cerebral blood flow, direct stimulation of neuron, development of gliacyte and suppression of apolipoprotein (apo) E4. In the mouse brain, estrogen upregulates apoE which has been implicated in neuronal protection and repair (Levin-Allerhand et al. 2001).

It is observed that women receiving estrogen replacement are 40% less likely to have AD and related dementias than women who do not receive estrogen. Overall the literature suggests that estrogen can help to reduce the incidence or severity of cognitive decline which is associated with aging and neurodegenerative diseases. ERT improves cognitive function and reduces the risk of AD-type senile dementia in women. Interestingly, ER has been mapped to brain regions known to be highly susceptible to AD-type changes in humans. It is co-localized with low-affinity nerve growth factor (NGF) receptors in cholinergic neurons of the rat basal forebrain suggesting that estrogen-NGF interaction may be important for survival of cholinergic neurons. The level of amyloid precursor protein (APP) mRNA is higher in the brain of old than adult mice and it is downregulated by estrogen, indicating the involvement of estrogen in AD (Asaithambi et al. 1999). Estrogen treatment is also associated with diminution of brain amyloid beta peptide (Aβ) levels. This suggests that modulation of Aβ metabolism may be one of the ways by which ERT prevents or delays the onset of AD in postmenopausal women (Peranceaka et al. 2000). There are ample evidences from epidemiological and clinical studies suggesting that estrogen has beneficial and protective role in AD (Thakur 2000).

In a population of older women, lifetime HRT exposure has been found to be associated with improved global cognition (Carlson et al. 2001). Estradiol is reported to protect verbal memory and possibly also frontal lobe mediated functions in older women. In contrast to the positive findings in women, endogenous sex steroids do not appear to be closely linked to better cognition in older men (Wolf & Kirschbaum 2002). Alkayed et al. (2000) have observed that young adult female rats sustain smaller infarcts after experimental stroke than age-matched males. This sex-difference disappears after surgical ovariectomy or after reproductive senescence in middle-aged females. However, it can be restored by estrogen treatment, suggesting a role for HRT in stroke injury prevention in postmenopausal women. Postmenopausal women experience permanent hypoestrogenicity and suffer from increased risk of brain injury associated with neurodegenerative diseases such as stroke and AD. However, ERT appears to decrease the risk and severity of neurodegenerative conditions. Studies using rats have shown that estrogen exerts similar effects and can enhance cell survival and induce synaptic plasticity (Wise & Dubal 2000).

ERT alleviates many postmenopausal symptoms but this benefit is not without the risks of harmful side effects. Particularly, longer duration of the hormonal treatment increases the incidence of breast, uterine and ovarian cancers in women and it rises with increasing years of use (Lacey et al. 2002). Before 1980s estrogen alone was the dominant hormone for the relief of menopausal symptoms and prevention of osteoporosis. It was used for longer duration in case of chronic conditions, especially heart disease. When this treatment was found to be associated with the increased risk of endometrial cancer, progestin was also combined with estrogen for treatment of women with an intact uterus. However, the recently published results of Women's Health Intiative (WHI) have made the clinicians to reconsider this use of HRT (Rossouw et al 2002). WHI is the first randomized

primary prevention trial planned for 8.5 years. In this study 16,608 healthy postmenopausal women with intact uterus were given conjugated equine estrogen (0.625 mg/day) plus medroxyprogesterone acetate (2.5 mg/day). A part of this study has been terminated after 5.2 years when it was observed that the volunteers developed coronary heart disease (CHD) with invasive breast cancer. In addition, there were reports about stroke, pulmonary embolism, endometrial cancer, colorectal cancer, hip fracture and death due to other causes. Thus the overall health risks exceeded benefits from use of combined estrogen plus progestin. Another part of this study with estrogen alone is continuing and will be completed by March 2005. Furthermore, Enserink (2002) also reports that as the quality of epidemiological research improves, predictions that HRT would cut heart disease fades, whereas risks of cancer become clearer.

Keeping in view the harmful side effects of hormonal treatment, a new class of compounds called selective estrogen receptor modulators (SERM) is being considered as possible alternative to ERT. With the recognition that SERM have differential tissue-dependent effects on ER function, there is recent interest in the effects of raloxifene, tamoxifen and other modulators on cognition and other functions (Yaffe 2001). The raloxifene is particularly interesting because, like estrogen, it improves lipid metabolism and reduces bone loss, without adverse effects on the breast or uterus. Lacreuse et al. (2002) tested the effect of raloxifene and ERT on cognitive function of rhesus monkeys. Their findings indicate that estradiol, not raloxifene, enhances some aspects of spatial working memory in aged monkeys. Tamoxifen is known to suppress the growth of ER-positive breast cancer cells. Long-term treatment of menopausal breast cancer patients with tamoxifen lowers the incidence of new breast cancer by 40%. In addition, the number of cardiovascular incidents decreases by 70% and the age-related decrease in bone mineral density is partially prevented (Love 1992).

Testosterone

Over the past several decades, there has been an increasing interest in evaluating whether testosterone therapy might be beneficial for older men in preventing or reversing some aspects of aging (Tenover 1999). The major androgen target organs of interest with regard to beneficial effects of male HRT include bone, muscle, adipose tissue, cardiovascular system and central nervous system (libido and mood). Muscle weakness, anemia, lowered bone mass, and mood disturbances rapidly normalize in mid-adult hypogonadal men during testosterone replacement therapy (Brodsky et al. 1996). Hypogonadal men also show the development of osteoporosis, but testosterone replacement increases bone density. Testosterone replacement in hypogonadal men also enhances skeletal muscle mass by stimulating the rate of muscle protein synthesis (Brodsky et al. 1996). At the same time, male HRT has potential adverse effects on target organ such as the prostate. Sih et al. (1997) examined effects of testosterone administration in hypogonadal men who were above 50 years of age or older. The men received either placebo or 200 mg testosterone cypionate by intramuscular injections twice weekly for 12 months. They showed greater increase in grip strength and haemoglobin levels and a significant decrease in leptin levels. Leptin is the product of obese (*ob*) gene whose expression is suppressed by testosterone. It is postulated to regulate weight and adipose tissue mass by signaling satiety or hunger and energy expenditure or conservation. There are no significant changes in serum prostate-specific antigen (PSA) levels or digital rectal examination of the prostate during one year treatment. Testosterone supplementation also improves working memory in older men (Janowsky et al. 2000). The results of Cherrier et al. (2001) suggest that short-term testosterone administration enhances cognitive function in healthy older men. Transdermal testosterone treatment also helps to improve cognition (Kenny et al. 2002). The results of Barrett-Connor et al. (1999) suggest that testosterone treatment might improve depressed mood in older men who have low levels of bioavailable testosterone. However, a clinical trial is necessary to test this hypothesis.

Testosterone treatment might be beneficial, but older men receiving testosterone must be carefully monitored because of its potential risks. Much long-term studies and a substantially large sample of older men are required to consider the long term

course for the development of detectable prostate cancer or symptomatic benign prostatic enlargement. Testosterone replacement therapy may offer hypogonadal men benefit, but long term studies on its efficacy and safety are lacking. These investigations have to be pursued before advocating for testosterone treatment.

Future Prospectives

A number of age-associated changes occur in the level of sex steroids, their receptors and other factors involved in hormone action and responses which in turn influence the specific cellular functions. In majority of cases, the level and responsiveness of hormones decline with age leading to various diseases and disabilities. Some of these changes can be reversed after replacement of hormone, but this may be associated with serious side effects. Several questions remain unanswered regarding the wider applicability of hormone replacement therapy. The development of synthetic hormones with variable biological actions in different organs will be of great help. Extensive studies need to be conducted for understanding the effects of hormones on the aging of a wide range of species before the usefulness of hormones for human beings can be guaranteed.

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