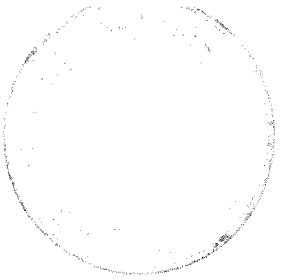


Chapter 1

Introduction

The Fountain of Youth was the legendary spring that could reputedly restore the youth of anyone who drinks its water. Tales of such water have been recounted across the world for thousands of years. Human beings have endlessly been trying to discover or invent a formula which would always keep them healthy, energetic and young. Dehydroepiandrosterone (DHEA) is the result of one such pursuit. For some not very clear reasons, DHEA is believed to be an anti-aging hormone effective in preventing and reversing many of the debilitating changes in emotional and physical well-being we associate with aging. Hence DHEA is considered as today's Fountain of Youth.

The world population is expanding rapidly, at the start of the 21st century, it has reached 6 billion. Importantly, it is expected that the biggest increases will occur in the aging population, i.e. those over the age of 65. It is predicted that the world population over the age of 65 will double in the next 50 years (Sehulman and Lunenfeld, 2002; Diczfalusy, 1998). With an increase in the number of aging people, it is desired that there is delayed symptoms of aging, memory loss and loss of libido.

Both human and animal studies have shown that there is a decline in growth hormone, sex hormone and DHEA-S with aging (Roshan, Nader and Orlander, 1999). This has popularised the hormone replacement therapy. A number of androgen preparations have been marketed for men and women, with varying levels of evidence to support their efficacy (Sonia and Susan, 2003) (Fig. 1)

Fig. 1

In the USA, DHEA is widely available without prescription (Hinson and Raven, 1999). The daily administration of 50 mg DHEA for 4 months restored DHEA-S to levels usually found in young men, but did not lead to a significant improvement in well-being or mood (Arlt et al., 2001). By contrast, an identical measure showed significant improvements in well being and mood in women with adrenal insufficiency (Arlt et al., 1999a). It is unlikely that sex accounts for this difference, as a recent study described positive effects of DHEA replacement on well being and mood in adrenal in sufficiency regardless of sex (Hunt et al., 2000). DHEA may have the potential to improve impaired sexuality and mood, but does not further enhance normal performance (Arlt et al., 1999a). The advantage of DHEA over other androgenic compounds is that DHEA, at physiological doses, is converted into androgens and/or estrogens only in the specific intracrine target tissues that possess the appropriate physiological enzymatic machinery, thus limiting the action of the sex steroids to those tissues possessing the tissue-specific profile of expression of the genes responsible for their formation, while leaving the other tissues unaffected and thus minimizing the potential side effects observed with androgens or estrogens administered systemically (Labrie et al., 2003). DHEA hormonal replacement therapy is expected to lengthen human life by arresting physiological degeneration changes and prevention of age-related clinical disorders (Zdrojewicz and Kesik, 2001). There are still controversies regarding the benefits and risks of replacement therapy and so more elaborate research is required to elucidate its effects.

Adrenal Glands and DHEA

In mammals, the adrenal glands (also known as suprarenal glands) are star-shaped endocrine glands present on top of the kidneys. Each adrenal gland is separated into two distinct structures, the adrenal cortex and medulla, both of which produce hormones (Fig. 2). The cortex mainly produces cortisol, aldosterone, and androgens, while the medulla chiefly produces epinephrine and norepinephrine.

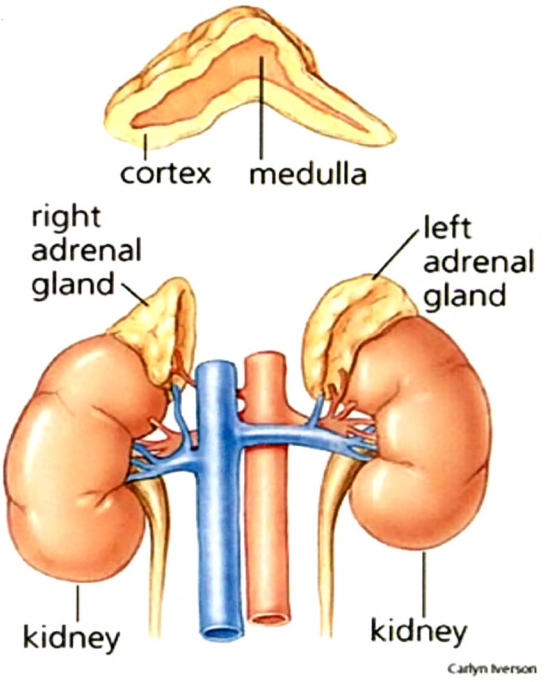

cortex
medulla
right adrenal gland
left adrenal gland
kidney
kidney
Carlyn Iverson

Fig. 2

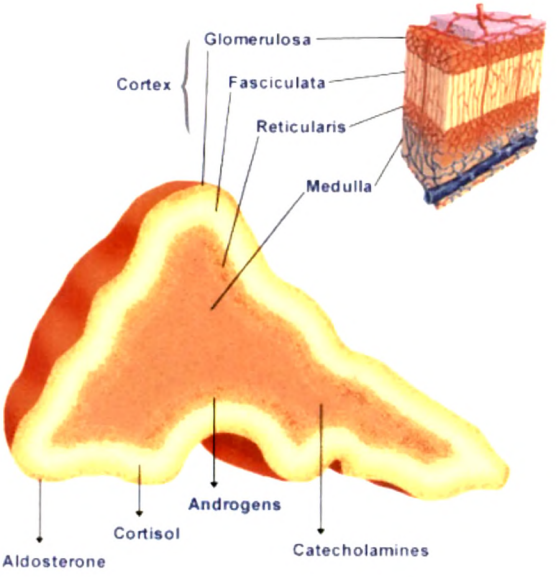

Glomerulosa
Cortex
Fasciculata
Reticularis
Medulla
Androgens
Cortisol
Aldosterone
Catecholamines

Fig. 3

The adrenal cortex comprises three zones, or layers (Fig. 3).

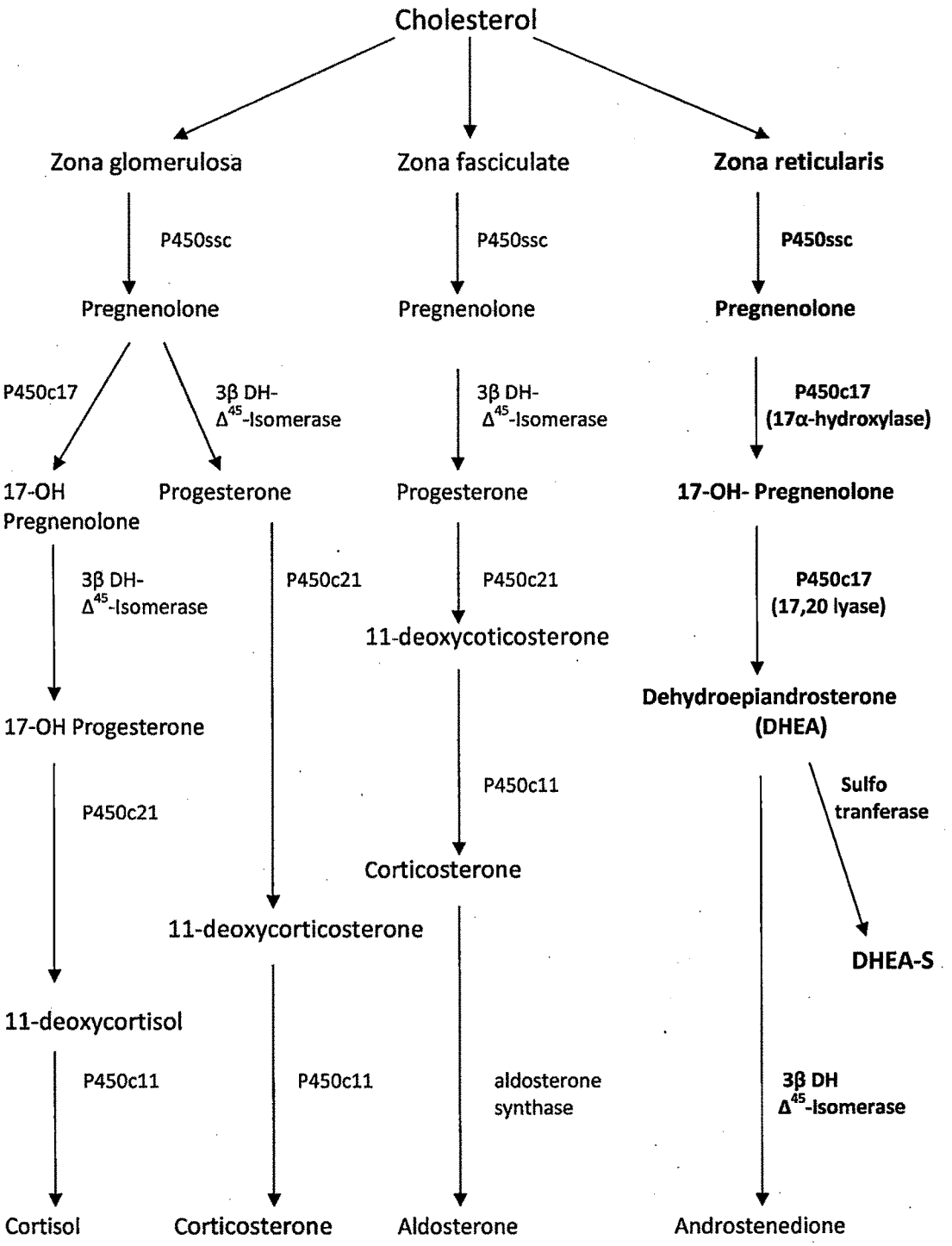
Zona glomerulosa (ZG): It is the outermost layer and the main site for production of mineralocorticoids, mainly aldosterone, which is largely responsible for the long-term regulation of blood pressure.

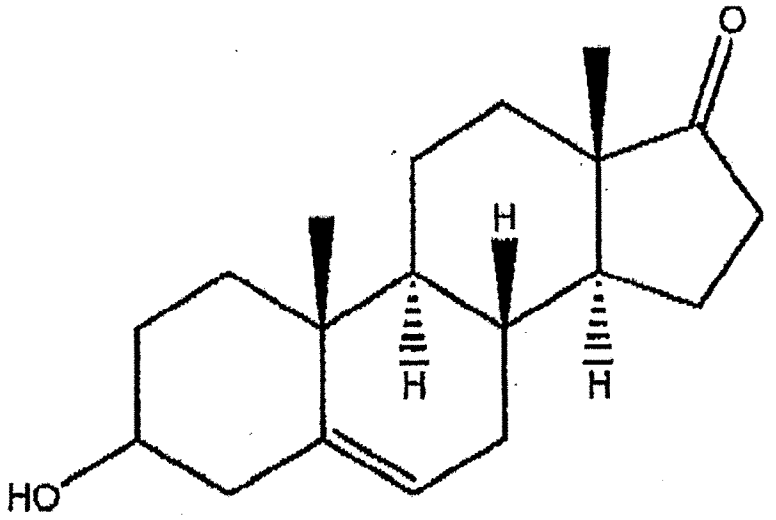
Zona fasciculate (ZF): It is situated between the glomerulosa and reticularis and responsible for producing glucocorticoids, chiefly cortisol in humans. It secretes a basal level of cortisol but can also produce bursts of the hormone in response to adrenocorticotropic hormone (ACTH) from the anterior pituitary.

Zona reticularis (ZR): The inner most cortical layer, it produces androgens, mainly **dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEA-S)** in humans.

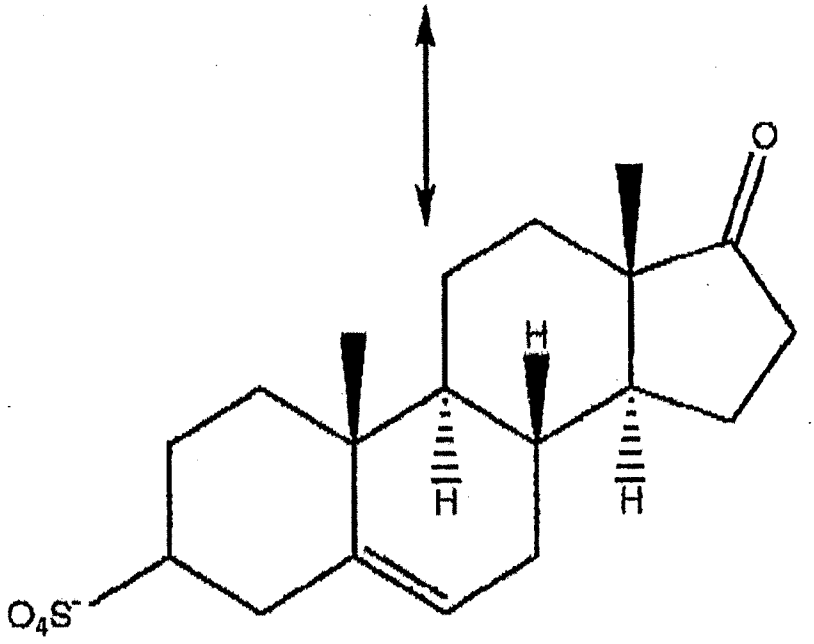
The adrenal medulla is the core of the adrenal gland, and is surrounded by the adrenal cortex. It mainly synthesises and secretes epinephrine and norepinephrine (Fig. 3).

Fig. 4: Synthesis of steroid hormones in adrenal gland





dehydroepiandrosterone



dehydroepiandrosterone sulphate

Fig.5

Table 1: Plasma levels and metabolic clearance rate (MCR) of steroid hormones:

Steroid hormones	**Production**	**Plasma concentration**	**MCR**
	µg/day	**ng/ml**	**l/d**
Cortisol	15,000	50-160	200
Aldosterone	100	0.07	1,500
Progesterone			
Proliferative phase	4,200	0.3-1.5	-
Secretory phase	42,000	3-20	2,800
Testostrerone			
Men	7,000	7	1,000
Women	200	0.3	700
Androstenedione			
Men	2,000	1.2	2,300
Women	4,000	1.6	2,000
Dehydroepiandrosterone	**5,000**	**5**	**1,600**
Dehydroepiandrosterone- sulphate	**15,000**	**2,500**	**6**
Estradiol			
Proliferative phase	40	0.06	600
24 hr before ovulation	-	0.6	-
Secretory phase	200	0.2	800
Estrone			
Proliferative phase	60	0.06	
Secretory phase	120	0.1	

(Kelly, 1990)

Biological Role of steroid hormones

1) Corticosterone:

- As Glucocorticoid in rats
  - ✓ To maintain blood glucose level
- Precursor for Aldosterone synthesis

2) Cortisol:

- Glucocorticoid in humans

  Major functions:
  - ✓ To maintain blood glucose level
  - ✓ Increase protein catabolism in muscle, bone, skin etc., anabolism in liver
  - ✓ Changes fat distribution

  In fetus:
  - ✓ Important for lung maturation and alveolar surfactant
  - ✓ Glycogenesis and hepatic maturation

3) Deoxycorticosterone:

- Mineralocorticosteroid action
- Precursor for corticosterone synthesis

4) Aldosterone:

- Mineralocorticoid
- Essential for controlling the electrolyte metabolism by kidney
  - ✓ Conservation of sodium
  - ✓ Excretion of potassium

5) Androstenedion:

- Precursor for synthesis of testosterone and estrogens

6) Dehydroepiandrosterone/ Dehydroepiandrosterone sulphate:

- Precursor for synthesis of androstendione

DHEA-S is secreted in very large amounts (3.5 to 20 mg daily) only by adrenal (Burger, 2002). Its serum concentration is the highest of all steroid hormones, although its physiological role is poorly understood. The daily production rate of DHEA is 6 to 8 mg, 50% being secreted by the ZR in both genders. In women 20% is secreted by the ovarian theca cells, while the remaining amount is derived from circulating DHEA-S catalyzed by steroid sulfatase whereas in men the testes secrete 5% of DHEA-S and 10-25% of DHEA (Longcope, 1996; 1986). In addition, DHEA also can be produced intracellularly form DHEA-S (Fig. 5). 17-hydroxypregnenolone in blood is a minor precursor of DHEA (Strott, Bermudez and Lipsett, 1970). DHEA and DHEA-S secretions are mainly modulated by adrenocorticotropic hormone (ACTH) (Buvat, 2003). DHEA is rapidly cleared form the blood at a rate of ~2000 L/day, whereas DHEA-S has a clearance of 5-20 L/day. Thus, DHEA has a short half life of 15-30 min., whereas the half life of DHEA-S is 7-10 hr. On a molar basis, circulating concentrations of DHEA-S are approximately 250 and 500 times higher than those of DHEA in women and men respectively. Renal excretion accounts for 51% - 73% of the elimination of DHEA-S and its metabolites (Wattana, 2004).

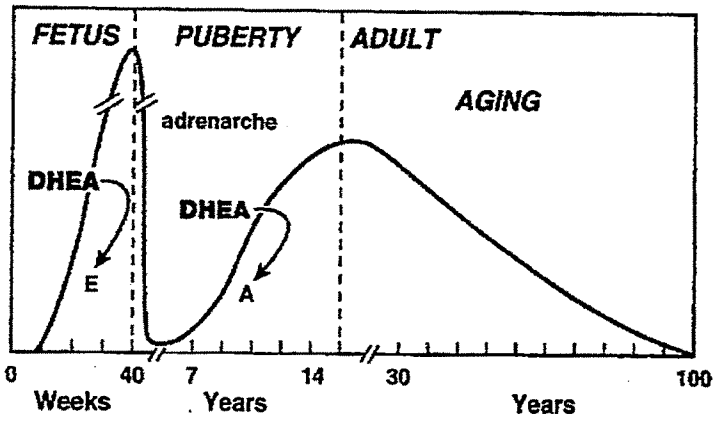


Fig. 6

Plasma concentrations of DHEA shows an age related pattern, during early neonatal and post natal stages (Fig. 6) (Hinson and Raven, 1999).

Dehydroepiandrosterone (DHEA) is produced principally as the 3-sulfoconjugate DHEA-S during intrauterine life. The fetal adrenal expresses large amount of DHEA sulphotransferase and minimal amounts of 3β-hydroxysteroid dehydrogenase, at least until very near gestation. This pattern of enzyme expression favors substantial secretion of DHEA/DHEA-S with minimal cortisol produced. The DHEA/DHEA-S serves as the major precursor for placental estrogen formation in human pregnancy. At birth the human adrenal undergoes reorganization where by the large, inner fetal zone regresses and DHEA/DHEA-S production is diminished. Just prior to gonadal maturation the human adrenal undergoes morphological and functional changes (adrenarche) that gives rise to a prominent zona reticularis (ZR) (Parker, 1999) that is characterized by the presence of DHEA sulfotransferase, the absence of 3β-hydroxysteroid dehydrogenase, and an enhancement of DHEA/DHEA-S production (Parker, 1999; Parker et al.,1997). During young adulthood, adrenal synthesis of DHEA/DHAE-S is usually high, but these bind androgen receptors with low affinity (Handa R, 2000) and thus are considered weak androgens. The role of adrenal androgens, particularly DHEA and DHEA-S, in normal physiology has become, in recent years, the subject of intense investigation.

Aging and Adrenal DHEA

Aging has been shown to be associated with striking reduction in circulating levels of adrenal androgens such as DHEA and DHEA-S in human (Parker et al., 1997). The functional alterations in the adrenal steroidogenic pathway may accompany structural changes in the adrenal cortex during aging (Neville and O'Hare, 1982). Parker et al. (1997) showed that the average total cortical thickness of the young men was slightly higher than that of the older men. The thickness of the ZR in the older men was reduced by one third compared to that of the young men. Moreover the percent of the cortical thickness attributed to cell of the ZR was significantly lower in older men than in the young men. The median percent of cortex identifiable as ZR in young men was 37% while in older men, it was only 24%, and the thickness of the outer two cortical zones was slightly greater in the old men than in the young men. Finally, the

proportion of outer cortical zone thickness to that of the ZR was strikingly higher in the older men than in the young men (Parker et al., 1997). This supports the decline of DHEA-S in contrast with the maintenance of cortisol biosynthesis in aging (Peter, 2002). It has been demonstrated that the deficiency in adrenal androgen production in women is restricted to the Δ^5-pathway steroid products (DHEA and DHEA-S), where as there is no reduction in the capacity of the adrenal to produce androstenedione or cortisol (Parker et al., 2000). In the 50-60 yr old group, serum DHEA decreases by 74% and 70% from peak values in 20-30 yr old men and women respectively (Fernand, 1997). Some investigators have proposed that aging is associated with the reduced efficiency of the 17-20 lyase activity of the enzyme 17-hydroxylase which causes reduction in the conversion of C21 steroids to C19 steroids (Natawa et al., 2002; Parker et al., 1997).

Possible Mechanism of action of DHEA

Most known effects of hormones have a prerequisite binding to a receptor. As the receptors for DHEA and DHEA-S are well not identified, it was believed that they have no hormonal. Hence, they were considered as pro-hormones, exerting indirect androgenic and estrogenic effects following peripheral conversion in to small amounts of testosterone and estradiol (Buvat, 2003). There are three possible mechanisms of action of DHEA. One is that the DHEA could have a specific receptor. The second is the conversion of DHEA. It is possible that DHEA is converted into a more active sex steroid such as testosterone or estradiol in peripheral tissue, and thereafter is bound to androgen receptor or estrogen receptor. This mechanism is called as intracrine action (Natawa, 2002). It is known that daily administration of 25 mg DHEA increased the serum DHEA, DHEA-S, androstenedione, and estradiol levels of the subjects of the older group to the same level as that of younger subjects (Yamada et al., 2007). Also chronic DHEA administration is capable of modifying circulating levels of androgens and progestins in both early and late postmenopausal women by modulating the age-related changes in adrenal function (Genazzani et al., 2006). The last possibility is that

last possibility is that the hydrophobic DHEA molecule may alter the cell function after binding to some macro molecules such as enzyme proteins (Natawa, 2002).

These concepts are changing due to the recent identification of a putative specific DHEA receptor on the plasma membrane of bovine aorotic endothelial cells. This receptor is functionally coupled with the G protein family (Liu and Dillon, 2002). Williams et al. (2002) have strongly supported the existence of a DHEA specific receptor, in human vascular smooth muscle cells (VSMC) involving ERK 1 signalling pathways. DHEA inhibits in vitro VSMC proliferation through a mechanism independent of its transformation into estrogens since this effect is unaffected by anti-estrogens and anti-androgens (Williams et al., 2002). DHEA and DHEA-S binds γ-aminobutyric acid ($GABA_A$) receptors and modulate neurosecretion mediated by NMDA (specific type of glutamate receptor) receptors (Nathalie and Synthia, 1998). DHEA has direct effect on gonadotropin-releasing hormone (GnRH) transcription that appears to be unique from those observed after conversion to other steroidogenic compounds (Hong, Shuo-Yen and Denise, 2003). DHEA promoted proliferation and inhibited apoptosis of osteoblasts significantly, via mitogen-activated protein kinase signalling pathway independent of either androgen receptor or estrogen receptor, suggesting that it may exert roles via a DHEA-specific receptor directly, not by way of conversion to androgens or estrogens (Wang et al., 2007). Injection of DHEA-S produces mechanical allodynia and that the development of this mechanical allodynia is mediated by sigma-1 and $GABA_A$ receptors (Yoon et al., 2009).

DHEA as neurosteroid

Neurosteroids play an important role in mammalian physiology, including that of humans. Stress situations may alter the physiological functions regulated by these neurosreroids (Torres and Ortega, 2003). DHEA has other special feature of belonging to the neurosteroid family. Colette et al. showed the presence of DHEA-S in brain in amounts exceeding that in plasma and in organs such as liver, kidney, spleen, testis and adrenal. Free DHEA was close to the levels in plasma, posterior brain, liver, spleen,

and kidney, but its level was around 0.42 ng/g in anterior brain (Corpechot et al., 1981). Pregnenolone, DHEA and their sulphate derivatives increase significantly in plasma and brain after corticotropin-releasing hormone (CRH) and adrenocorticotropin hormone (ACTH) administration in males and females. Zwain and Yen (1999) showed that astrocytes and neurons express cytochrome P450 enzyme 17α-hydroxylase ($P_{450c}17$) and synthesize DHEA from pregnenolone. Astrocytes also have the capacity to metabolize DHEA into sex steroid hormones (Ismailh and Samuel, 1999). It is logical to assume that DHEA synthesis evolves in two steps from cholesterol as described for steroidogenic glands, principally catalyzed by two different cytochrome P450 enzymes (Fig. 4).

Indeed Pregnenolone (PREG) was found in the brain, at concentrations about 1 order of magnitude larger than those of DHEA, as might be expected from a precursor to product relationship (Corpéchot et al., 1983). An alternate pathway for DHEA synthesis has been suggested by treating rat and human brain cells with ferrous sulfate and beta-amyloid (A beta) (Brown et al., 2003). The biosynthesis of 7α-hydroxyl-DHEA and/or δ-5-androsteine-3β, 17β-diol is likely to regulate DHEA cerebral concentrations of DHEA activity in the aging brain including in Alzheimer's disease (Weill-Engerer et al., 2003). During neurogenesis in the developing cortex DHEA and DHEA-S regulate the survival of neural precursors and progeny through the serine-threonine protein kinase (AKt) signalling pathway (Lei et al., 2002). DHEA such as 17β-estradiol is active in preventing the catecholamine-depleting effect of MPTP (1-methyl-4-phenyl-1, 2, 3, 6 -tetra hydro-pyridine) and involves in neuroprotection of striatal dopamine (DA) neurons (D'Astous et al., 2003). Several findings suggest that insulin like growth factor-1 (IGF-1) is involved in the action of DHEA. DHEA resulted in a significant reduction in IGF-1 receptor levels. This effect was dose dependent and restricted to the hypothalamus. The effects of DHEA on the hypothalamic IGF-1 system may be highly relevant to the control and maintenance of hypothalamic neuroendocrine function (Ribeiro and Garcia-Segura, 2002).

DHEA-S is effective in promoting recovery of function of both cognitive and sensory motor performance after experimental traumatic brain injury in rats (Hoffman et al.,

2003). The long-term cognitive and behavioral effects induced by mild traumatic brain injury may be improved by a repeated weekly treatment with DHEA-S (Milman et al., 2008). The activity of DHEA, and possibly of other neurosteroids, on the mechanism of cognition and memory in aging is also exerted through the restriction of defective protein kinase C (PKC) signal transduction machinery (Racchi et al., 2001). DHEA modulated the activity of Na^+K^+ ATPase and also protected the age-related loss of membrane integrity and functions. Exogenous DHEA might be beneficial in terms of neuroprotection against age-related loss of Na^+K^+ ATPase mediated brain functions like learning and memory (Taha et al., 2008). Chronic treatment with DHEA enhances cognitive function in middle-aged senescent rats (Chen et al., 2008).

DHEA and DHEA-S exert multiple effects in rodent and human brain. Treating adult male rats with subcutaneous pellets of DHEA increased the number of newly formed cells in the dentate gyrus of the hippocampus, and also antagonized the suppression by corticosterone. Subcutaneous DHEA pellets stimulated neurogenesis in a small number of older rats. It regulates neurogenesis in the hippocampus and modulates the inhibitory effect of increased corticoids on both the formation of new neurons and their survival (Karishma and Herbert, 2002). An inhibitory effect of DHEA on monoamine oxidase (MAO) activity may be involved in the antidepressant and neuroprotective effects of the steroid. Since MAO inhibition reduces neurodegeneration in clinical trials for Parkinson's disease, DHEA may be useful to treat depression and to prevent neuronal death in this disorder (Pérez-Neri, Montes and Ríos, 2009; Kumar et al., 2008). Pinnock et al. 2009 showed antidepressant activity by altered neurogenesis of DHEA in combination with fluoxetine. DHEA can induce the division and differentiation of neuronal-competent bone marrow mesenchymal stem cells (MSCs) into neurons in vitro and should provide an improved basis for new treatments using MSCs of a wide variety of neurological diseases (Shiri et al., 2009). DHEA might act as a neurotrophic signal derived from keratinocytes to promote axonal outgrowth from a subpopulation of sensory neurons (Ulmann et al., 2009). DHEA affects neural crest-derived cell survival by multiple pro-survival signalling pathways comprising an integrated system of non-genomic and genomic mechanisms (Charalampopoulos, Margioris and Gravanis, 2008). DHEA-S inhibits persistent sodium

currents via the activation of sigma-1 receptor-Gi protein-protein kinase C-coupled signalling pathway, and the main functional consequence of this effect of DHEA-S is presumably to protect neurons under ischemia (Cheng et al., 2008).

DHEA and different tissues

Effect of DHEA-S on the heart may depend on hormonal milieu. In premenopausal women, DHEA-S might have endrogenic effects and might enhance fibroblast proliferation; while during the postmenopausal period it is dominantly osteogenic in action, with anti-proliferative effect on cardiac cells (Szathmari, Treszl and Vasarhelyi, 2003). DHEA can directly attenuate collagen type I synthesis at the transcriptional level in vivo and in vitro in cardiac fibroblasts (Iwasaki et al., 2005). DHEA-S may play a role in vascular remodelling in cardiovascular disease. DHEA decreased proliferation and increased vascular smooth muscle cell apoptosis in vitro and in vivo, reducing vascular remodelling while sparing healthy tissues after oral intake (Bonnet et al., 2009). DHEA-S exhibited inhibitory effects on vascular smooth muscle cell (VSMC) proliferation and migration activities, inducing G1 cell cycle arrest with up regulation of one of the cyclin dependent kinase (CDK) inhibitors p16(INK4a) and apoptosis with activating peroxisome proliferator-activated receptor (PPAR)-α in VSMCs (li et al., 2009). Rabbits fed high-fat diet supplemented with DHEA showed a partial reduction of oxidative stress and inflammatory state. Cardiac necrosis, shift of heavy-chain myosin isoforms, and cardiac functionality, were also partially counteracted in these animals (Aragno et al., 2009).

DHEA may protect hepatic tissue against oxidative injury in obstructive jaundice by decreasing malondialdehyde concentration and increasing superoxide dismutase activity and total glutathione concentration (Celebi et al., 2004). DHEA leads to activation of the cAMP/ protein kinase A (PKA) signalling system in the modulation of lipid metabolism by repressing sterol regulatory element-binding protein-1 (SREBP-1) in cultured primary chicken hepatocytes (Tang et al., 2009). Pharmacological administration of dehydroepiandrostenone (DHEA, 300 mg/kg) for 2 weeks

significantly increased the activity of hepatic peroxisomal beta-oxidation of fatty acids in male F-344 rats (Khan, 2008). Supraphysiological concentration of DHEA can substantially influence gene expression of the peroxisome proliferator-activated receptors (PPARs) signalling machinery at both transcriptional and posttranscriptional levels in human hepatoma HepG2 cells (Poczatková et al., 2007).

DHEA induces significant modifications in Adipose tissue fatty acid composition in vivo, mainly in unsaturated fatty acids, and changes occurred in a tissue-dependent manner. These changes may be related to the capacity of DHEA to lower serum insulin levels (de Heredia et al., 2009). DHEA treatment decreases body weight and adiposity in old female rats fed a high-fat diet, leading to an improvement of the homeostatic model assessment (HOMA) index for insulin sensitivity, with decreasing circulating insulin levels, and preventing the age-associated decline of visceral-adipose adiponectin expression (Sánchez et al., 2008). Treatment with DHEA for 5 days reduced the triglyceride content and monolayer of adipocytes. DHEA down regulates adiposity through the reduction of PPAR γ in adipocytes (Kajita et al., 2003). DHEA treatment may improve glucose tolerance through a PI 3-kinase-PKCzeta pathway and down regulates adiposity in OLETF rats (Ishizuka et al., 2007). DHEA-S stimulates lipolysis in subcutaneous fat in women and in visceral fat in men (Hernández-Morante et al., 2008).

DHEA acts directly on rat zona glomerulosa (ZG) cells to diminish aldosterone secretion and rat zona fasciculata-reticularis (ZFR) cells to diminish corticosterone secretion by inhibition of a post-cAMP pathway and decreases functions of steroidogenic enzymes after P450(scc) as well as steroidogenic acute regulatory (StAR) protein expression (Chang, Wun and Wang, 2008a,b).

Dehydroepiandrosterone (DHEA) pre-treatment alters renal interleukin 1β (IL-1β), interleukin 6 (IL-6) and vascular endothelial growth factor (VEGF) synthesis in ischaemia/reperfusion (I/R)-induced kidney damage (Vannay et al., 2009). DHEA may have a beneficial effect on renal tissue against oxidative damage due to I/R injury by

preventing decreases in some antioxidant enzyme activities and reduced pro-oxidant state and oxidative damage (Aksoy et al., 2004; Aragno et al., 2003).

Topical application of DHEA tends to improve skin tone by counteracting papery appearance of skin and epidermal atrophy, a characteristic feature of hormone-related skin aging (Nouveau et al., 2008). DHEA could exert an anti-aging effect in skin through stimulation of collagen biosynthesis, improved structural organization of the dermis while modulating keratinocyte metabolism (Calvo et al., 2008).

Conversion of DHEA to estrone in osteoblast by P_{450}AROM (aromatase cytochrome P_{450}), which is positively regulated by glucocorticoid and 1, 25-(OH) (2) D (3) is important in maintenance of bone mineral density.(Takayanagi et al., 2002).

DHEA and disease

DHEA showed anti-atherosclerotic effect without converting into estrogen (Cheng, Hu and Ruan, 2009). Atherosclerotic lesions in the aortic sinus showed a 45% reduction in area with DHEA treatment versus untreated mice by inhibition of macrophage infiltration (Yamakawa et al., 2009). In rodents, DHEA is metabolized to (among others) androstene-3β,7β,17β-triol (AET), which retains potent anti-inflammatory activity. 17α-ethynyl-5-androstene-3β,7β,17β-triol (HE3286) is a novel, metabolically stabilized, orally bio available derivative of AET. A single dose of daily oral treatment with HE3286 (40 mg/kg), beginning at onset of collagen-induced arthritis, significantly decreased disease by reducing joint inflammation, erosion, and synovial proliferation in the DBA mouse model (Offner et al., 2009). DHEA can inhibit the expression of molecules involved in the inflammatory process in endothelial cells activated with Interferon-gamma (IFN-γ). Therefore, DHEA may be considered as a potential preventive intervention for atherosclerosis (Li, Xia and Wang, 2009). DHEA- fatty acyl ester-enriched high-density lipoprotein (HDL) was a stronger vasodilator than native HDL, and vascular relaxation was in part mediated by nitric oxide synthase, suggesting

that DHEA- fatty acyl esters may improve HDL's antiatherogenic function (Paatela et al., 2009).

DHEA has no toxic effect on chondrocytes up to 100μM concentration and ability to modulate the imbalance between metalloproteinase and tissue inhibitor of metalloproteinase 1 during osteoarthritis at the transcriptional level, which suggest that it has a protective role against articular cartilage lose (Jo et al., 2003). mRNA levels of cysteine proteinases/cystatin C system and urokinase plasminogen activator/plasminogen activator inhibitor-1 (uPA/PAI-1) system, closely related to the progression of osteoarthritis in articular cartilage are suppressed by DHEA especially in the early and medium stages of osteoarthritis (Bao et al., 2009).

DHEA would be a potential chemo preventive agent against colon cancer because it decreases the number of azoxymethane (AOM) induced abnormal cryptic foci, which is a possible precursor to adenoma and cancer in a murine model (Kazutaka et al., 2003). DHEA inhibits bone marrow and leukaemia cell growth by reducing food intake in mice (Catalina et al., 2003). It strongly inhibits the proliferation of cervical cancer cells (Girón et al. 2009). Endogenous DHEA metabolites also have an anti-proliferative action that is not induced by inhibiting Glucose-6-phosphate dehydrogenase or HMG-CoA reductase activity alone. These non-androgenic DHEA metabolites may serve as chemo-preventive or anti-proliferative agents (Yoshida et al., 2003).

DHEA can be an alternative drug against Trypanosoma cruzi infection (Caetano et al., 2009; Santos et al., 2007). Treatment with DHEA in vivo and in vitro inhibits reproduction, growth and viability of tapeworm Taenia crassiceps metacestodes and so can be use to prevent murine cysticercosis (Vargas-Villavicencio, Larralde and Morales-Montor, 2008).

DHEA-S levels were significantly associated with decreased malaria parasite density, even after adjustment for age (Leenstra et al., 2003). DHEA and DHEA-S, as well as their analogue, 16 alpha- bromoepiandrosterone (EPI) exerted anti malarial activities against two Chloroquine-sensitive Plasmodium falciparum strains. Both EPI and

DHEA/DHEA-S are potent inhibitor of glucose-6-phosphate dehydrogenase (G-6-PD), and G-6-PD deficiency is known to exert anti-malarial protection via enhanced opsonization and phagocytosis of rings the early form of the parasite. Enhanced ring phagocytosis due to the exposure of negatively charged membrane phospholipids may explain the anti-malarial activity of EPI (Ayi et al., 2002).

Local daily intra-vaginal DHEA administration at doses of 3.25-13 mg was able to rapidly and efficiently achieve correction of all signs and symptoms of vaginal atrophy and improve sexual function and caused no or minimal changes in serum sex steroid levels, which remain within the normal postmenopausal range. This treatment could thus avoid the risks of all estrogen formulations (Labrie et al., 2009).

DHEA treatment improves the oxidative imbalance induced by hyperglycemia, down regulates the tumour necrosis factor TNF-α/TNF-α receptor system, and prevents advanced glycation end product formation, suggesting a beneficial effect on the onset and/or progression of chronic complications in type 2 diabetic patients (Brignardello et al., 2007). DHEA reduces oxidative stress and the consequent increase of lipoxygenase pathway products induced by experimental diabetes in rat kidney and also by reducing the inflammatory response to oxidative stress, DHEA treatment might delay the progression of diabetic nephropathy (Aragno et al., 2001).

DHEA and immune system

DHEA administration leads to an increased survival following a septic challenge. The immune-enhancing effect of DHEA is accompanied by a reduction of tumour necrosis factor (TNF)-α release and an improved activity of T-cell immunity. DHEA administration may, therefore, be beneficial in systemic inflammation (Oberbeck et al., 2001). DHEA can improve the severity of experimental autoimmune neuritis (EAN) by suppressing the proliferation of auto reactive T cell and expression of pro-inflammatory cytokines (Tan et al., 2009). DHEA helps in airway hyper responsiveness by suppressing interferon-gamma (IFN-γ), interleukin (IL)-5, and IL-10 and so it may be

a useful therapy for asthma (Choi et al., 2008). 1 mg/kg DHEA-S twice daily for 5 weeks increases the responsiveness of young pigs to antigenic challenge (Burdick et al., 2009). The combination of low doses of BCG and DHEA had an additive effect in suppressing the development of airway hypersensitivity (Cui et al., 2008). DHEA treatment decreased the mortality rate of mice during polymicrobial sepsis. This was accompanied by improved cellular immune functions and an increased heat shock response (HSP-70) of lungs and spleen (Oberbeck et al., 2007). DHEA inhibits the expression of molecules involved in the inflammatory process in endothelial cells activated with oxidized low density lipoproteins (López-Marure et al., 2007).

DHEA and other hormones

DHEA and other steroids

The decline of plasma DHEA and maintenance of glucocorticoid levels with increasing age contribute to excess body fat accumulation, hyperglycemia, hyperlipidaemia, hyperinsulinaemia and cancer. Metabolically DHEA antagonizes the action of insulin and glucocorticoid by partitioning energy away from fat synthesis and toward oxidation (Berdanier et al.,, 1993). 11β-Hydroxysteroid dehydrogenase (11β-HSD) type 1 and type 2 catalyze the inter conversion of inactive and active glucocorticoids. Impaired regulation of these enzymes has been associated with obesity, diabetes, hypertension, and cardiovascular disease. DHEA induces a shift from 11β-HSD1 to 11β-HSD2 expression, increasing conversion from active to inactive glucocorticoids. This provides a possible explanation for the antiglucocorticoid effects of DHEA (Balazs et al., 2008). The enhancement of corticosterone release was attenuated significantly in the presence of different concentration of DHEA. DHEA decreased corticosterone release following addition of deoxy corticosterone suggesting that DHEA might decrease 11β hydroxylase activity. Similarly DHEA decreased corticosterone release after addition of progesterone, suggesting that DHEA might decrease 21 hydroxylase and 11β hydroxylase activities. DHEA also significantly increased the basal release of pregnenolone. These suggest that DHEA might decrease 3β HSD activities. DHEA inhibits corticosterone secretion by inhibiting the signalling pathway downstream to

cAMP, by diminishing steroidogenic enzyme activity downstream from P_{450scc} and by diminishing stAR protein expression (Chang et al., 2003). Many others have proposed a beneficial action of DHEA on the overall well-being. This beneficial role could be related to a double action of DHEA: a direct effect provided by its transformation in to sexual hormones and an indirect one by its competition with cortisol, of which the synthesis and consequently the activity decrease (Boudarene, Legros and Timsit-Berthier, 2002). Patricia et al. 2003 demonstrated that DHEA administration resulted in a decrease in plasma cortisol concentrations in healthy older individuals. This again suggests the antiglucocorticoid activity of DHEA. One possible explanation for the cortisol lowering effect of DHEA is that DHEA and/or DHEA-S may have an inhibitory effect on the hypothalamic pituitary-adrenal axis (Patricia et al., 2003). Arlt et al. (1999) showed that in elderly men a dose of 50mg DHEA, which restores serum DHEA and DHEA-S to youthful levels in healthy men, induces significant increase in serum estrogens E1 and E2 concentrations, whereas total testosterone T and dihydrotestotserone DHT, the main circulating androgens in men, remain unaffected. This is in contrast with the results of previous study on pharmacokinetics and bio conversion of DHEA in women showing a significant increase in serum androgens but only a slight increase in serum E1 and no change in serum E2 after oral DHEA administration (Arlt et al., 1998). This DHEA induced increase in estrogenic activity may contribute to beneficial effects of DHEA in men. It also supports the view of DHEA as a sexually dimorphic hormone that changes the circulating androgens/estrogen ratio in a gender-dependent fashion (Arlt et al., 1999). Oral ingestion of DHEA on a chronic basis in the rat increase serum DHEA-S and total testosterone without any evident change in prostate weight or histology (Rhoden et al., 2003).

DHEA and insulin

Insulin could have an independent regulatory effect on DHEA-S secretion but glucose metabolism is not related. DHEA-S levels showed a significant positive relationship with insulin (Rowydam, 2003). Insulin may reduce serum DHEA and DHEA-S both by inhibition of production and stimulation of clearance of these steroids (John, John and

William., 1992). Despite the experimental evidence for insulin infusion producing a reduction in serum DHEA-S and some effect of meals on the observed DHEA-S concentration, there were no associations between insulin and DHEA-S at the population level. Variations in DHEA-S levels are due to age, sex, obesity, and substantial polygenic genetic influence (Nestler et al., 2002). Low concentrations of these endogenous androgens have been linked with insulin resistance, which is an important upstream factor for metabolic abnormalities such as hyperglycemia, hypertension, or hyperlipidemia, and increased cardiovascular risk. DHEA improves the insulin resistance caused by aging or obesity. In humans the serum DHEA concentration was shown to be associated with hyperinsulenemia in diabetes. DHEA increased not only insulin sensitivity due to the effects in the liver and muscle, but also insulin secretion (Kawano et al., 2003; Aoki et al., 2003; Fukui et al., 2007).

DHEA and thyroid hormone

In hyperthyroidism, the serum DHEA-S concentration was normal. In hypothyroidism, the serum concentrations of both DHEA and DHEA-S were significantly decreased. The serum concentration of pregnenolone the precursor of DHEA and DHEA-S was increased in hyperthyroidism and decreased in hypothyroidism. The correlation was strong between DHEA-S and total T_4 (Bassi et al., 1980). In the hyperthyroid condition, the rate of clearance of blood constitutes would be increased more than in the euthyroid condition. These differences might partly explain the normal DHEA and increase DHEA-S in hyperthyroidism (Tagawa et al., 2000). The balance of the conversion between DHEA-S and DHEA in the hyperthyroid state favoured DHEA-S. Similar to cortisol, the DHEA response in the CRH test in hyperthyroidism seemed to be insufficiently compensated for by increased ACTH, although the DHEA response to low-dose of ACTH was similar in the hyperthyroid and euthyroid states. Increased DHEA-S might play some role in the pathological states in many organs in hyperthyroidism (Yamakita et al., 2001). DHEA regulates the expression of malic enzyme gene in the euthyroid rats by the rate of gene transcription in dose dependent manner. Thyroid hormone is absolutely required for the induction of hepatic malic

enzyme activity by DHEA. The degree of induction appears to be related to the level of thyroid hormone in the blood since ≈ 2 fold stimulation by DHEA was detected in liver of T_3 treated hypothyroid rats, where as an 8-9 fold increase was observed when euthyroid rats were treated with DHEA. Treatment of euthyroid rats with both hormones alters the malic enzyme gene transcription in an additive fashion (Min Kyung et al., 1989).

Other effects of DHEA

Alexaki et al. 2009 showed protective role of DHEA against apoptosis in keratinocytes, using non-cancerous immortalized human HaCaT cells. DHEA transmits its signal via specific G protein-coupled, membrane binding sites and inhibits apoptosis, through prevention of mitochondrial disruption and altered balance of Bcl-2 proteins.

DHEA caused a time dependent and concentration dependent decrease in cytochrome P_{450} 1A1 (CYP 1A1) mRNA levels indicating that DHEA inhibits CYP1A1 expression by decreasing CYP1A1 mRNA stability (Henry and Grace, 1999). DHEA was also able to significantly reduce the basal expression of CYP 1A2 but not CYP 1B1 (Henry et al., 2003).

DHEA administration significantly increased the levels of brown adipose tissue (BAT) uncoupling protein UCP-1 and UCP-3 mRNA expression (Ryu et al., 2003).

DHEA produced a dose dependent receptor-mediated increase in the male macrophage cholesterol esters content. DHEA up regulated mRNA expression of the lipoprotein-processing enzymes acyl coenzyme A: cholesterol acyltransferase I and lysosomal acid lipase (Ng et al., 2003).

DHEA supplementation significantly reduced the plasma levels of plasminogen inhibitor type1 (Hiroaki et al., 2003).

DHEA is a potent uncompetitive inhibitor of mammalian glucose 6 phosphate dehydrogenase (G6PD), lowers NADPH levels and reduces NADPH dependent oxygen-free radical production (Arthur and Laura, 2004).

Administration of DHEA at 0.3% in the diet for 7 consecutive days increased liver NAD and NADP, but not NADH concentrations. This indicates the shift of the redox couple (NAD/NADH) towards oxidation in the DHEA treated rats (Julian et al., 2001).

Intravenous DHEA administration reduced the activation of leukocytes and improved red blood cell velocity and capillary perfusion in muscle flap microcirculation during ischemia reperfusion injury. This protective effect was most likely the result of delayed expression of Mac-1 integrin, L-selectine, and CD44 molecules on leukocytes (Ayhan et al., 2003).

Training and DHEA

Physical training decrease abdominal fat deposit improves muscular mass and affects favorably triglyceride and DHEA-S levels. Changes in triglycerides and DHEA were inversely related (Boudou et al., 2001). Exercise combined with DHEA administration before steroid treatment prevents steroid induced muscle atrophy (Choe and An, 2009)

Adverse effect of DHEA

High oral dose of DHEA (150-200 mg/day) has shown induction of acute manic episode in a 68 yr old man (Vacheron-Trystram et al., 2002). DHEA administration has beneficial effects on oestrogen-induced pituitary hyperplasia and hyper prolectinaemia. DHEA also induces diverse hormonal effects and a slight pituitary enlargement and this limits its use as a possible therapeutic drug (Suarez et al., 2002). Late promotion of breast cancer in postmenopausal women may be stimulated by

prolonged intake of DHEA, and the risk may be increased by the endocrine abnormality associated with pre-existing abdominal obesity. Caution is advised in the use of dietary supplements of DHEA particularly by obese postmenopausal women (Stoll, 1999).

Mice fed with pellet containing 0.6% DHEA for 3 months showed a significant neuronal loss in the cerebral cortex and hippocampus, a slightly decreased dopamine/dihydroxyphenylacetic acid ratio, as well as motor impairment. High concentrations of DHEA inhibit complex I of the mitochondrial respiratory chain and are neurotoxic in vitro and in vivo (Safiulina et al., 2006).

Forgoing reviews have highlighted the role of DHEA on well being and its therapeutic use for different diseases. Some studies have also showed adverse effects at higher doses. Some of the studies mentioned above showed similarity between the age related pattern of mitochondrial respiration rates and DHEA. These studies led us to raise the question that is this related? It is known that development is an energy requiring process; it is interesting to know if DHEA treatment helps in development and aging? In the present study effects of DHEA treatment have been examined on rats of different age groups at the sub-cellular level i.e. mitochondria mainly from brain and liver. As the work reported in this thesis mainly deals with the effect of DHEA on mitochondria and the electron transport chain a brief account of these is given bellow. A brief account of FoF1 ATPase, cytochromes, glutamate dehydrogenase (GDH), malate dehydrogenase (MDH) and succinate DCIP reductase (SDR) is also appended.

Mitochondria

In cell biology, a mitochondrion (plural mitochondria) is a membrane-enclosed organelle found in most eukaryotic cells. It ranges from 0.5 to 10 micrometers (μm) in diameter. Mitochondria are sometimes described as "cellular power plants" because

they generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy. In addition to supplying cellular energy, mitochondria are involved in a range of other processes, such as signaling, cellular differentiation, cell death, as well as the control of the cell cycle and cell growth (Henze and Martin, 2003; McBride, Neuspiel and Wasiak, 2006). Mitochondria have been implicated in several human diseases, including mitochondrial disorders (Gardner and Boles, 2005) and cardiac dysfunction, (Lesnefsky et al., 2001) and may play a role in the aging process.

The word mitochondrion comes from the Greek words mitos (thread) + chondrion (granule). Several characteristics make mitochondria unique. The number of mitochondria in a cell varies widely by organism and tissue type. Many cells have only a single mitochondrion, whereas others can contain several thousand mitochondria (Alberts et al., 1994; Voet, Judith and Charlotte, 2006). Mitochondrial proteins vary depending on the tissue and the species. In humans, 615 distinct types of proteins have been identified from cardiac mitochondria (Taylor et al., 2003) whereas in murine species, 940 proteins encoded by distinct genes have been reported (Zhang et al., 2008). The mitochondrial proteome is thought to be dynamically regulated (Zhang et al., 2008a). Although most of a cell's DNA is contained in the cell nucleus, the mitochondrion has its own independent genome. Further, its DNA shows substantial similarity to bacterial genomes (Andersson et al., 2003).

A mitochondrion contains outer and inner membranes composed of phospholipid bilayers and proteins (Alberts et al., 1994). The two membranes, however, have different properties. Because of this double-membrane organization, there are five distinct compartments within the mitochondrion. There is the outer mitochondrial membrane, the inter-membrane space (the space between the outer and inner membranes), the inner mitochondrial membrane, the crista space (formed by infoldings of the inner membrane), and the matrix (space within the inner membrane) (Fig. 7).

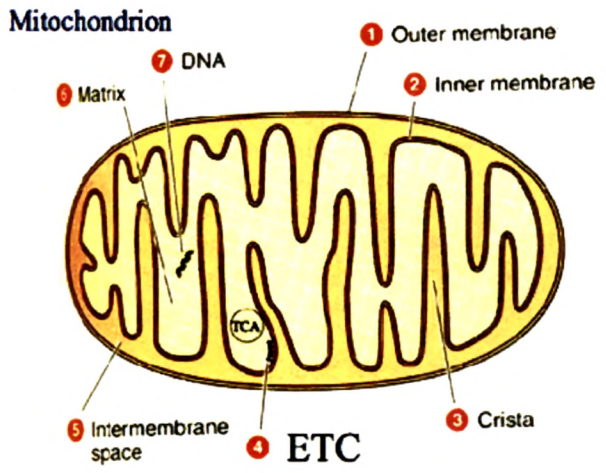


Fig.7

The most prominent roles of mitochondria are to produce ATP (i.e., phosphorylation of ADP) through respiration, and to regulate cellular metabolism (Voet, Judith and Charlotte, 2006). The central sets of reactions involved in ATP production are collectively known as the citric acid cycle, or the Krebs cycle. However, the mitochondrion has many other functions in addition to the production of ATP.

The end result of these pathways is the production of two kinds of energy-rich electron donors, NADH and succinate. Electrons from these donors are passed through an electron transport chain to oxygen, which is reduced to water. This is a multi-step redox process that occurs on the mitochondrial inner membrane. The enzymes that catalyze these reactions have the ability to simultaneously create a proton gradient across the membrane, producing a thermodynamically unlikely high-energy state with the potential to do work. Although electron transport occurs with great efficiency, a small percentage of electrons are prematurely leaked to oxygen, resulting in the formation of the toxic free-radical superoxide. Electron transport chain involves five different complexes (Fig.8).

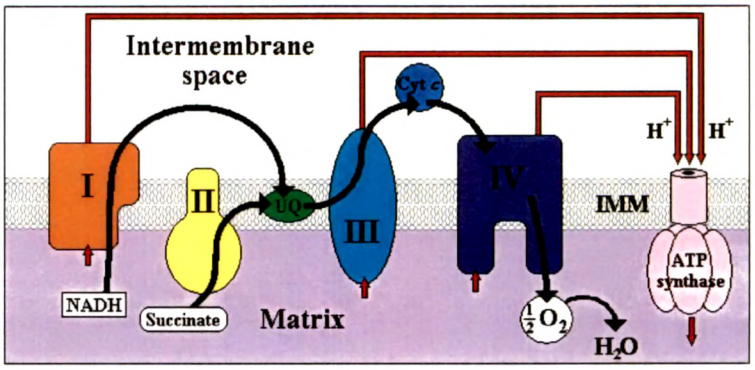


Fig.8

FoF$_1$ ATP

FoF$_1$ ATP or complex V which functions as ATP synthase *in situ* is the final enzyme in the oxidative phosphorylation pathway. This enzyme is found in all forms of life and functions in the same way in both prokaryotes and eukaryotes (Boyer, 1997). It is a massive protein complex with a mushroom-like shape (Fig.9). The mammalian enzyme complex contains 16 subunits and has a mass of approximately 600 kilodaltons (Rubinstein, Walker and Henderson, 2003). The portion embedded within the membrane is called Fo and contains a ring of 16 unlike subunits. These subunits are α3β3γ1δ1ε1 and probably factor B in the catalytic F1 domain; OSCP, a, b, c, d, e, f, g, F6 and A6L in Fo and stator; and the ATPase inhibitor protein, IF1, which binds reversibly to F1 to inhibit ATP hydrolysis (Noji and Yoshida, 2001; Pedersen and Amzel, 1993; Belogrudov and Hatefi, 2002). The stalk and the ball-shaped headpiece are called F1 and is the site of ATP synthesis. The ball-shaped complex at the end of the F1 portion contains six proteins of two different kinds (three alpha subunits and three β subunits), whereas the "stalk" consists of one protein: the γ subunit, with the tip of the stalk extending into the ball of α and β subunits (Leslie, Walker, 2000). Both α and β subunits bind nucleotides, but only the β subunits catalyze the ATP synthesis reaction. Reaching along the side of the F1 portion and back into the membrane is a long rod-like subunit that anchors α and β subunits into the base of the enzyme (Fig.9).

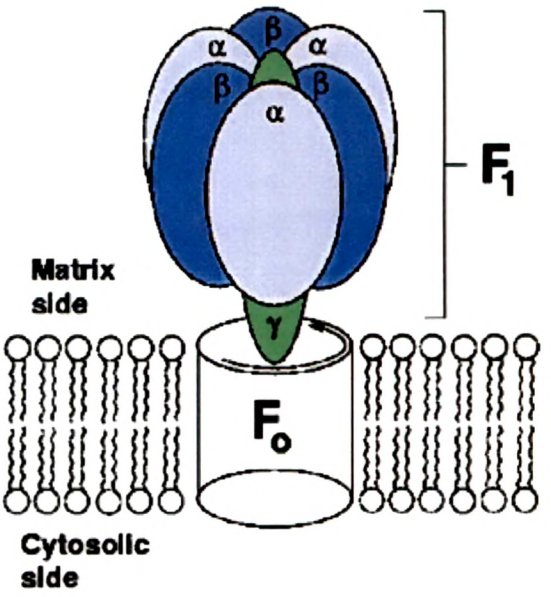


Fig.9

The enzyme uses the energy stored in a proton gradient across a membrane to drive the synthesis of ATP from ADP and phosphate (Pi). Estimates of the number of protons required to synthesise one ATP have ranged from three to four, with some suggesting cells can vary this ratio, to suit different conditions (Van Walraven et al., 1996; Yoshida, Muneyuki and Hisabori, 2001; Schemidt et al., 1998).

This phosphorylation reaction is an equilibrium, which can be shifted by altering the proton-motive force. In the absence of a proton-motive force, the ATP synthase reaction will run from right to left, hydrolyzing ATP and pumping protons out of the matrix across the membrane. However, when the proton-motive force is high, the reaction is forced to run in the opposite direction; it proceeds from left to right, allowing protons to flow down their concentration gradient and turning ADP into ATP (Boyer, 1997). Indeed, in the closely related vacuolar type H^+-ATPases, the same reaction is used to acidify cellular compartments, by pumping protons and hydrolysing ATP (Nelson et al., 2000; Noji and Yoshida, 2001; Capaldi and Aggeler, 2002; Dimroth, Kaim and Matthey, 2000; Gresser, Myers and Boyer, 1982).

Glutamate dehydrogenase

Glutamate dehydrogenase (GDH) is a mitochondrial matrix enzyme, with a key role in nitrogen and glutamate metabolism and energy homeostasis. GDH is expressed at high levels in liver, brain, pancreas and kidney, but not in muscle. In the pancreatic cells, GDH is thought to be involved in insulin secretion mechanisms. In nervous tissue, where glutamate is present in concentrations higher than in other tissues, GDH appears to function in both the synthesis and the catabolism of glutamate and perhaps in ammonia detoxification.

GDH is a hexamer. The monomer unit has:

1. N-terminal Glu-BD(Binding domain) that is composed mostly of β-strands.
2. NAD-BD - can bind either NAD+ or NADP+.
3. 48-residue antenna-like projection that extends from the top of each NAD-BD. The antenna consists of an ascending helix and a descending random coil strand that contains a small α-helix toward the C-terminal end of the strand. GDH catalyses the oxidative deamination of Glutamate to 2-oxoglutarate and free $NH4^+$ using either NAD+ or NADP+ as a co-factor (Banerjee et al., 2003; Smith et al., 2002; Smith et al., 2001).

Malate dehydrogenase

Malate dehydrogenase is present in two forms in the shuttle system: mitchondrial malate dehydrogenase and cytosolic dehydrogenase. The two malate dehydrogenases are differentiated by their location and structure, and catalyze their reaction in opposite directions in their respective processes.

First, in the cytosol, malate dehydogenase reacts with oxaloacetate and NADH to produce malate and NAD+. After malate reaches the mitochrondial matrix it is converted by mitochrondrial malate dehydrogenase into oxaloacetate, during which NAD+ is reduced with two electrons to form NADH and an H+ is released. The net effect of this shuttle is purely redox: NADH in the cytosol is oxidized to NAD+, and NAD+ in the matrix is reduced to NADH. The NAD+ in the cytosol can then be reduced again by another round of glycolysis, and the NADH in the matrix can be used to pass

electrons to the electron transport chain so that ATP can be synthesized (Banaszak and Bradshaw, 1975).

Succinate dehydrogenase

Succinate dehydrogenase or succinate-coenzyme Q reductase (SQR) or Complex II is an enzyme complex, bound to the inner mitochondrial membrane of mammalian mitochondria and many bacterial cells. It is the only enzyme that participates in both the citric acid cycle and the electron transport chain. Mammalian, mitochondrial, and many bacterial monomer SQRs are composed of four subunits: two hydrophilic and two hydrophobic. The first two subunits, a flavoprotein (SdhA) and an iron-sulfur protein (SdhB), are hydrophilic. The second two subunits are hydrophobic membrane anchor subunits, SdhC and SdhD (Oyedotun and Lemire, 2004; Yankovskaya et al., 2003).

Cytochromes

Cytochromes are, in general, membrane-bound hemoproteins that contain heme groups and carry out electron transport. They are found either as monomeric proteins (e.g., cytochrome c) or as subunits of bigger enzymatic complexes that catalyze redox reactions. They are found in the mitochondrial inner membrane and endoplasmic reticulum of eukaryotes, in the chloroplasts of plants, in photosynthetic microorganisms, and in bacteria. Cytochromes were initially described in 1884 by MacMunn as respiratory pigments (myohematin or histohematin). In the 1920s, Keilin rediscovered these respiratory pigments and named them the cytochromes, or "cellular pigments", and classified these heme proteins, on the basis of the position of their lowest energy absorption band in the reduced state, as cytochromes a (605 nm), b (~565 nm), and c (550 nm).

The heme group is a highly-conjugated ring system (which allows its electrons to be very mobile) surrounding a metal ion, which readily interconverts between the

oxidation states. For many cytochromes, the metal ion present is that of iron, which interconverts between Fe^{2+} (reduced) and Fe^{3+} (oxidised) states (electron-transfer processes) or between Fe^{2+} (reduced) and Fe^{3+} (formal, oxidised) states (oxidative processes). Cytochromes are, thus, capable of performing oxidation and reduction. Because the cytochromes (as well as other complexes) are held within membranes in an organized way, the redox reactions are carried out in the proper sequence for maximum efficiency (Reedy and Gibney, 2004).

Cytochrome c is a highly conserved protein across the entier spectrum of species i.e. they are found in plants, animals, and many unicellular organisms. This, along with its small size (molecular weight about 12,000 daltons), makes it useful in cladistic studies. Its primary structure consists of a chain of about 100 amino acids. Many higher order organisms possess a chain of 104 amino acids. It is loosely associated with the inner membrane of the mitochondrion. It belongs to the cytochrome c family of proteins. Cytochrome c is a highly soluble protein, unlike other cytochromes, with a solubility of about 100 g/L and is an essential component of the electron transport chain, where it carries one electron. It is capable of undergoing oxidation and reduction, but does not bind oxygen. It transfers electrons between Complexes III and IV. Cytochrome b is a component of respiratory chain complex III also known as the bc_1 complex or ubiquinol-cytochrome c reductase (Esposti et al., 1993). The enzyme cytochrome c oxidase or Complex IV is a large transmembrane protein complex found in bacteria and the mitochondrion. The complex is a large integral membrane protein composed of several metal prosthetic sites and 13 protein subunits in mammals. In mammals, ten subunits are nuclear in origin, and three are synthesized in the mitochondria. The complex contains two hemes, a cytochrome and cytochrome a3, and two copper centers, the CuA and CuB centers (Tsukihara et al., 1995).

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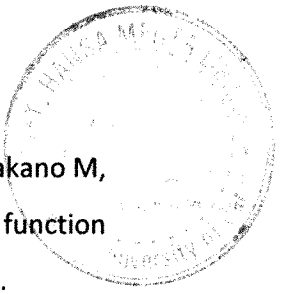

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