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Dehydroepiandrosterone (DHEA) and the Aging Brain: Flipping a Coin in the "Fountain of Youth"

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ABSTRACT

The physiological role of dehydroepiandrosterone (DHEA) and its sulphated ester DHEA(S) has been studied for nearly 2 decades and still eludes final clarification. The major interest in DHEA derives from its unique pattern of activity. Its levels exhibit a dramatic age-related decline that supports significant involvement of DHEA(S) in the aging process. Particularly relevant to the aging process is the functional decline that involves memory and cognitive abilities. DHEA is derived mainly from synthesis in the adrenal glands and gonads. It can also be detected in the brain where it is derived from a synthesis that is independent from peripheral steroid sources. For this reason DHEA and other steroid molecules have been named "neurosteroids." Pharmacological studies on animals provided evidence that neurosteroids could be involved in learning and memory processes because they can display memory-enhancing properties in aged rodents. However, human studies have reported contradictory results that so far do not directly support the use of DHEA in aging-related conditions. As such, it is important to remember that plasma levels of DHEA(S) may not reflect levels in the central nervous system (CNS), due to intrinsic ability of the brain to produce neurosteroids. Thus, the importance of neurosteroids in the memory process and in age-related cognitive impairment should not be dismissed. Furthermore, the fact that the compound is sold in most countries as a health food supplement is hampering the rigorous scientific evaluation of its potential. We will describe the effect of neurosteroids, in particular DHEA, on neurochemical mechanism involved in memory and learning. We will focus on a novel effect on a signal transduction mechanism involving a classical "cognitive kinase" such as protein kinase C. The final objective is to provide additional tools to understand the physiological role and therapeutic potentials of neurosteroids in normal and/or pathological aging, such as Alzheimer's disease.

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INTRODUCTION

Dehydroepiandrosterone (DHEA) and its sulphate ester [DHEA(S)] are the most abundant circulating hormones in humans. These molecules are synthesized mainly in the adrenal glands and the gonads. However, they can also be produced in the brain and in the peripheral nerves, either by metabolism of circulating hormones or by *de novo* synthesis from cholesterol (11,12,14,133,134). Due to the latter fact, they have been named "neurosteroids" (13). In the central nervous system, neurosteroids act via classic intracellular receptor-mediated mechanisms that regulate the transcription of specific genes. In addition to classic genomic steroidal actions, neurosteroids may modulate brain functions, acting as allosteric modulators of ion-gated and other neurotransmitter receptors (122), such as glutamate (42) and GABA receptors (84,85). In addition, they have shown putative effects as neuroprotective agents (66,67). Through these actions DHEA(S) may affect central functions such as memory and learning, protection of neurons against excitatory amino acid-induced neurotoxicity, and reduction of risk of age-related neurodegenerative disorders (163). In addition to the activities at the brain level, various other properties including metabolic, immunomodulating, and anticancer have been attributed to these steroids. The increasing interest in DHEA derives from the fact that its levels follow a peculiar age-related pattern. In particular, the levels of DHEA are very high in young adults and fall significantly with age (55,115), such that in some people, DHEA levels decline 95% during their lifetime. Because of these peculiar characteristics of DHEA, combined with its pleiotropic effects on many cellular and tissue functions, as well as correlation between DHEA levels and general good health, there is a growing interest in DHEA replacement therapy in the elderly (65). In some media DHEA has been named "the fountain of youth." Such claim is an exaggeration and the fact that DHEA is sold in most countries as a health food supplement is hampering the rigorous scientific evaluation of its potential.

CHEMISTRY AND BIOSYNTHETIC PATHWAYS

DHEA is a 19 carbon steroid hormone classified as an adrenal androgen. It is derived from the transformation of cholesterol into pregnenolone by an oxidative side chain cleavage reaction catalyzed by a specific enzyme located at the inner mitochondrial membrane. This cytochrome P450 side chain cleavage (cytochrome P450scc) removes the side-chain of 6 carbons from the cholesterol molecule. Subsequently the biosynthetic pathway involves the activity of a microsomal cytochrome P450 with 17α-hydroxylase: c17,20-lyase activity known as cytochrome P450c17, which converts pregnenolone (a 21 carbon steroid) into DHEA (Fig. 1). Finally, the conversion of DHEA into its sulphated derivative DHEA(S) is catalized by a phospho-adenosine-phosphosulphate-dependent hydroxysteroid sulfotransferase. DHEA(S) is metabolically interconvertible to DHEA through hydrolysis by a sulphatase.

The Pattern of DHEA(S) Biosynthesis and Production During Aging

The main site of production of circulating DHEA in humans is the adrenal cortex, which is histologically divided into three distinct zones producing different classes of steroid hormones. Adrenal androgens such as DHEA(S) are produced in the *zona reticularis*. Production of glucocorticoids occurs in the *zona fasciculata*, while mineral corticoids are derived from the outer layer, *zona glomerulosa* (1,59,118). In addition to adrenals, go-

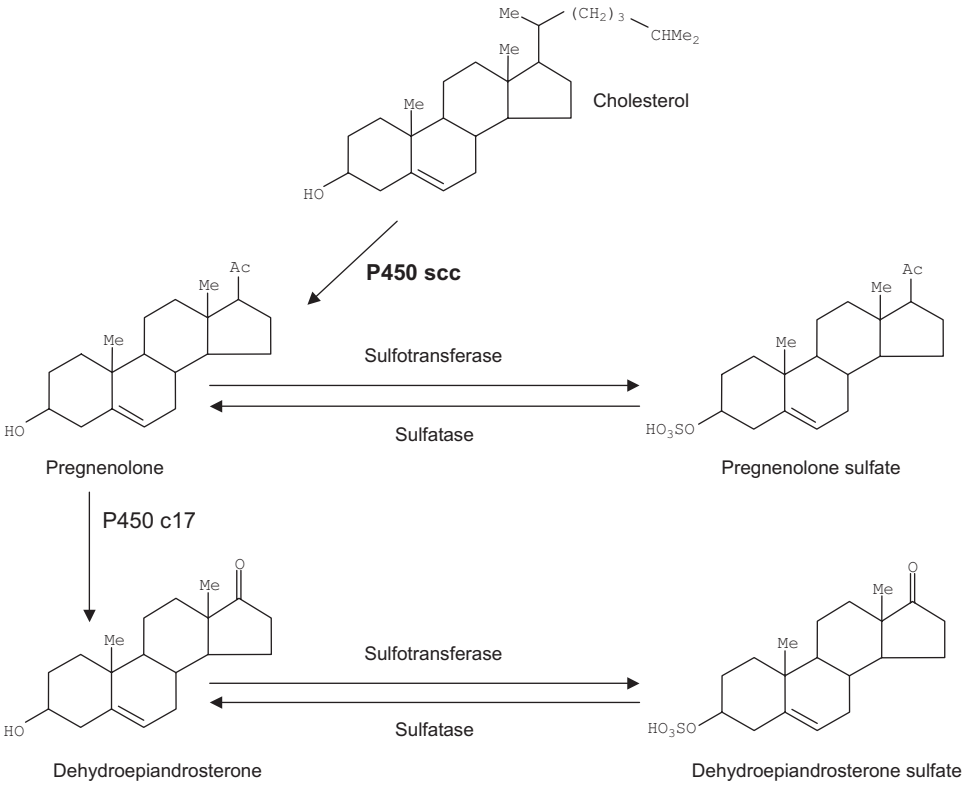

Fig. 1. Biosynthetic pathway of DHEA and DHEA(S). The enzymes involved are the cholesterol side chain cleaving cytochrome P450scc (P450scc), the 17α-hydroxylase/17,20 lyase cytochrome P450c17 (P450c17), hydroxysteroid sulfotransferase (sulfotransferase) and steroid sulfatase (sulfatase). Details of the synthetic pathway are found in the text.

nads (113,187) also produce DHEA in small amounts (10 to 20% of the circulating hormone). DHEA is well known for its peculiar developmental pattern of its production. It is produced at high levels by the adrenal glands in the fetus, and then its levels drop sharply after birth to levels that are virtually undetectable (40). The levels of DHEA show a significant increase in the years prior to puberty (the "adrenarche"), and reach a peak by the third decade of life (1,118). Peak levels of DHEA appear to be different in males and females, with the latter having lower circulating levels of the hormone. Following the peak in young adulthood its levels decline steadily by approximately 2% per year. The gender-based differences in the levels of DHEA indicate that the hormone levels in young women are 10 to 30% lower than in young men and are progressively decreasing with aging (154).

The pattern of age-related decline of adrenal DHEA(S) is in marked contrast with the pattern for adrenal glucocorticoids (GCs); their levels remain unchanged or slowly increase throughout the life-span (83,135). Alterations in DHEA production during aging may, therefore, be dependent on selective changes affecting the functional cells in the *zona reticularis*. Either the number of functional cells is reduced (45) or more complex functional alterations take place(118). A selective decrease in the expression and activity of CYP450c17 may also occur (82). It is particularly interesting to note that only humans and primates show a specific age-related pattern of DHEA(S) production. The DHEA levels

are very low in rats and mice (158), due to the absence or very low expression of the adrenal enzyme responsible for the conversion of pregnenolone into DHEA (CYP450c17). It is important to consider these differences when discussing the conflicting results reported for DHEA effects in rodents and humans. Nevertheless, rodents have been a valuable model to study the synthesis of steroids, such as DHEA, in the central nervous system.

Biosynthetic Pathways and Enzymes in the Central Nervous System

Since the pivotal observations of Baulieu and colleagues in the 1980s compelling evidence has accumulated on the biosynthesis and activity of steroids in the CNS. The original finding demonstrated that steroids, such as pregnenolone and dehydroepiandrosterone (DHEA), were present in tissues from the rodent nervous system (including brain and peripheral nerve), at concentrations exceeding their plasma levels. Furthermore, these levels remained in the CNS for a significant amount of time (36,37,87) even after the removal of peripheral steroid sources following castration or adrenalectomy. It is now clear that steroids can be synthesized *de novo* in the central or peripheral nervous systems and might accumulate in those structures independently of adrenal and gonadal sources. The term "neurosteroids" has been used to designate steroids directly synthesized from cholesterol in the nervous system (13).

Neurosteroidogenic enzymes include cytochrome P450 and non-P450 enzymes. The former is found mainly in mitochondria or microsomes. The synthesis of steroids and neurosteroids depends upon the tissue-specific and cell-specific synthesis of a particular array of steroidogenic enzymes. The central nervous system is no exception because steroidogenic enzymes are produced both in glia and in neurons. In addition, multiple glial cell types, such as astrocytes and oligodendrocytes, can differentially express some of these enzymes.

The immunohistochemical localization of cytochrome P-450 side chain cleavage (scc) in rat oligodendrocytes and the observation that the enzyme was biologically active were the first demonstrations that steroids can be synthesized within the CNS (74). Following this fundamental discovery, subsequent intense research efforts have unequivocally established the expression and localization of steroid synthetic enzymes in the nervous system of mammals and other animal species (138). For a comprehensive review on the complex biosynthetic pathways involved in the synthesis of all neurosteroids we refer the reader to recent more comprehensive reviews (99,100). Here we will discuss the findings related to the major enzymes involved in the synthesis of DHEA and DHEA(S), namely the cytochrome P450 enzymes: P450scc and P450c17 as well as sulfatases and sulfotransferases.

Cytochrome P450scc

The cleavage of the side chain of cholesterol leading to the formation of pregnenolone is catalyzed by an enzymatic complex composed of cytochrome P450scc (P450scc or CYP11A1 following CYP nomenclature) which possesses α-hydroxylase activity, adrenodoxin or ferredoxin, and adrenodoxin reductase, located at the inner mitochondrial membrane. Following the initial demonstration of the presence of P450scc in the human brain (75,76), the presence of the enzyme has been demonstrated in other vertebrates including amphibians (101,146) and birds (145,148). P450scc has been detected in most cell types of the nervous system such as type 1 astrocytes (98), oligodendrocytes (64), and neurons (54,149). In developing rodent embryos P450scc is specifically expressed in cell lineages derived from the neural crest and in sensory structures of the peripheral nervous system (PNS) (31). Ukena et al. have shown the presence of P450scc in Purkinje cells of neonatal

and adult rats, indicating that the gene is not only expressed in glial cells but also in neurons (149). Kimoto et al. reported the localization of cytochrome P450scc in pyramidal neurons and granule neurons of the rat hippocampus (68). They also observed the co-localization of P450scc with hydroxysteroid sulfotransferase. In addition, it was shown that the process of active neurosteroidogenesis was stimulated by glutamate NMDA agonists suggesting that in hippocampal neurons neurosteroid synthesis may be stimulated and regulated by glutamate-mediated synaptic communication (68). The distribution of P450scc mRNA seems to be similar in brains of female and male rats (31,98). Watzka et al. demonstrated that in human brain the levels of expression of P450scc mRNA are approximately 200 times lower in the temporal lobe, frontal lobe and hippocampus than in adrenal tissue (156). Remarkably, they observed that during childhood P450scc mRNA levels increased markedly in the temporal lobe reaching adult levels at puberty. In addition, P450scc mRNA is significantly higher in the temporal cortex and frontal lobe cortex of women compared to men suggesting an age and sex dependent expression of CYP11A1 mRNA in the human brain (19).

Cytochrome P450c17

The enzymatic system 17α-hydroxylase/17,20 lyase (cytochrome P-450c17) is responsible for the formation of C-19 steroids (DHEA) from C21 steroids (such as pregnenolone). This reaction is catalyzed by a single microsomal enzyme coupled to a cytochrome reductase, cytochrome P-450c17. This enzyme possesses both 17α-hydroxylase and 17,20 lyase activities. The existence of P-450c17 activity in the CNS of mammals was inferred from the early observation that the rat brain contains high levels of DHEA (36). In the subsequent years, however, the studies aimed at the direct demonstration of the presence of the enzyme in the brain remained unsuccessful for a long time (16,75,98). In 1994, the activity of a steroid 17α-hydroxylase enzyme was demonstrated in frog hypothalamic explants (101). This observation provided the first evidence for the presence of P-450c17 in the CNS, while subsequent work by Compagnone et al. (32) demonstrated the presence of P-450c17 in the rat embryo. Moreover, the enzyme has also been detected in the adult rat brain, however, with some contradictory results. Compagnone et al. (32) suggest that P-450c17 is expressed only in the peripheral nervous system whereas other studies have indicated its presence also in the brain of adult rodents (138). In addition, recent data suggest that astrocytes and neurons express P450c17 and synthesize DHEA from pregnenolone but that its formation is inhibited in the presence of microglia (170). Astrocytes have also the capacity to metabolize DHEA into sex steroid hormones (170, 171). It has been proposed that DHEA may be produced in the nervous system by a chemical reaction which does not require the presence of the P450c17 enzyme and which involves the formation of hydroperoxides. This hypothesis is based on the observation that DHEA is formed by rat glioma C6 cells. These cells do not express the cytochrome P450c17 after addition of $FeSO_4$ to the culture medium (25). This observation has been further extended by the same authors (26) using primary cultures of differentiating rat glial cells. They failed to detect P450c17 protein in astrocytes, but demonstrated its presence in oligodendrocytes. However, their results led to the proposal that in differentiating rat brain oligodendrocytes and astrocytes DHEA is formed via a P450c17-independent and oxidative stress-dependent alternative pathway. Kohchi et al. have investigated the relative contributions of astrocytes, oligodendrocytes, and neurons to neurosteroidogenesis (69). They showed that astrocytes express cytochrome P450 side-chain cleavage (P450scc) and 17α-hydroxylase/C17-20-lyase (P450c17) among other neurosteroidogenic enzymes, and are able to produce pregnenolone, DHEA and other androgens and estro-

gens. Oligodendrocytes do not appear to have P450c17 expression and are, therefore, unable to produce DHEA. Neurons express P450scc, P450c17 and may produce DHEA. However, the lack of the key enzyme (17β-HSD) does not allow the production of testosterone. It appears evident that neurosteroidogenesis may be accomplished by the coordinated contribution of different cell types in the brain. In addition, the expression of these enzymes not only varies among cell types but can also be found in multiple locations within the neuron. For example, P450c17 is found both in cell bodies and in fibers (axons or dendrites) extending from the cell bodies (32). Because those fibers might make connections with other neurons far from the cell body, there is a possibility that neurosteroids might be synthesized and released in areas distant from the cell body.

Sulfatase and Sulfotransferase

The effect of pregnenolone and DHEA differ according to their conjugation with sulfate. For example, DHEA and DHEA(S) exert different effects on neurite growth in cultures of mouse cerebral cortical neurons (33). Free steroids can readily pass the blood brain barrier while sulfoconjugated steroids can not, thus steroid sulfation is likely to take place in the brain. Sulfate conjugation of steroids is catalyzed by sulfotransferases or sulfokinases, a family of cytosolic enzymes which transfer the sulfate moiety from the universal donor molecule 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to a hydroxyl group of the steroid substrates. The cloning of human steroid sulfotransferase has revealed the existence of multiple isoforms expressed in a tissue-specific manner with different affinities for various steroid substrates. The major steroid sulfotransferase involved in the conjugation of DHEA is a member of the family of hydroxysteroid sulfotransferases (HST) that act on primary and secondary alcohols of hydroxy-steroids such as cholesterol, and DHEA (140,157).

Despite the evidence for its activity (116), detection of sulfotransferase in the mammalian brain has been problematic. More recently its activity has been directly demonstrated in rat brain homogenates (124). The mRNA coding for the steroid sulfatase, the enzyme which converts sulfated steroids to free steroids, has been detected in different regions of the embryonic rat brain by *in situ* hybridization (35). Using immunocytochemistry the enzyme has been localized in the frog brain (18). Finally, the presence and localization of dehydroepiandrosterone sulphotransferase has been demonstrated in the adult rat brain (2). In particular, the protein was detected in the hippocampus and dentate gyrus, and in the large neurons of the midbrain.

The enzyme responsible for the hydrolysis of sulfated steroids leading to the formation of unconjugated steroids is a sulfatase. In humans, the gene for steroid sulfatase was mapped on chromosome X (5). The rat sulfatase (81) and mouse sulfatase cDNA (132) have also been cloned. The presence of sulfatase mRNAs has been described in the cortex, hindbrain, and thalamus of mouse fetus during the last week of gestation (35). The sites of expression are similar to those of P450c17, suggesting that these two enzymes may have concerted actions in similar functional processes.

NEUROCHEMICAL ACTIVITIES OF DHEA AND RELATED NEUROSTEROIDS

The classical mode of endocrine action involves the secretion of the hormone into the bloodstream with activity occurring at distant target cells. However, steroids that are produced within the CNS may act locally by an autocrine/paracrine mechanism. It is im-

portant to note that this mechanism may be operative at hormone leves that are higher than the "physiological" concentrations of circulating steroid hormones. Steroids influence cellular functions traditionally by so-called "genomic actions" that involve interaction of steroids with their intracellular receptors and regulation of the transcription of hormone-sensitive genes.

In addition to classical genomic actions, neurosteroids are known to act in the CNS through a series of interactions with neurotransmitter receptors and other neurochemical pathways, all included in the definition of "membrane actions" or "non-genomic actions." Numerous membrane effects of steroids in the nervous system have been reported in the past decades. They include changes in neuronal excitability, release of neuropeptides and neurotransmitters and modulation of neurotransmitter receptors and ion channels (15,16, 133). For example, one of the best-documented effects of DHEA(S) in the CNS is related to the modulation of the $GABA_A$ receptor complex. It was originally shown (84) that DHEA and DHEA(S) (with DHEA(S) being 3–4 times more potent than DHEA) are negative modulators of the $GABA_A$ receptor since they non-competitively inhibit GABA-induced currents in cultured rat neurons (61,84). The $GABA_A$ receptor is a member of the ligand-gated ion channel family, and contains different subunits of the α, β, γ, and δ subtypes each including distinct binding sites for GABA, benzodiazepines, barbiturates and convulsants. It appears that, although no absolute specificity has been determined, the α and γ subunits are involved in the either positive or negative neuromodulation by steroids. The mechanism of modulation of GABAergic function by neurosteroids appears to be related to the alteration in the frequency and duration of opening of the receptor (56,57,85) and to the modulation of desensitization kinetics of the receptor (169). More details concerning the modulation of $GABA_A$ receptors by neurosteroids can be found elsewhere (73,121).

DHEA, DHEA(S) and pregnenolone sulfate have also modulatory activity on *N*-methyl-D-aspartate (NMDA) receptors (33,34). In particular, pregnenolone sulfate (117) acts as a negative modulator, whereas DHEA, pregnenolone and their sulfate esters are thought to be positive allosteric modulators of NMDA receptors. However, at variance with $GABA_A$ receptor interactions, the interaction of neurosteroids with the NMDA receptor has not been not conclusively documented (131).

DHEA(S) affects also sigma receptors. These receptors are present in high density in the CNS (155), and are thought to be important functional modulators of glutamatergic activity in the hippocampus (41,43,141,142,143). In fact, the first report of a modulatory role of DHEA(S) on the sigma receptor demonstrated that the norepinephrine release induced by NMDA in hippocampal slices was significantly enhanced by the addition of DHEA(S) at 30 nM or higher concentrations. (108). These findings have been replicated (20,93,94,108) in various systems and the overall data are consistent with the activity of DHEA(S) as a σ_1 receptor agonist. By this mechanism NMDA-induced neuronal excitability is likely to be potentiated (42). Several studies in rodents show that $GABA_A$ agonists impair learning and memory while $GABA_A$ antagonists enhance memory (29,62). It is also known that in humans benzodiazepines may impair cognition (46,71,86). On the other hand, σ receptor agonists enhance memory performance in young rodents and in rodent models of cognitive impairment (89,90,92–94,136). Finally, the NMDA receptor is involved in the development of long-term potentiation (63,127). These observations document the ability of DHEA(S) to modulate neurotransmitter receptors in the CNS that are primarily involved in learning and memory.

Effect of DHEA(S) on a "Cognitive" Signal Transduction Mechanism

In all animals the processes involved in cognition and memory include the fundamental cellular mechanisms of protein phosphorylation (103). Several studies have demonstrated the involvement of the calcium-phospholipid-dependent protein kinase C (PKC) in the long-term changes that reflect persistent biochemical and morphological alterations that characterize learning and memory mechanisms (21,63,119). PKC is a multigene family of enzymes with at least 12 different isoforms (44,114). The mechanism of its activation requires the translocation of PKC from the cytosol to different intracellular sites (70) where phosphorylation of specific substrates takes place (147). Recent studies (105,106,129) have emphasized the role of intracellular receptor proteins for activated C kinase (RACKs), in PKC compartmentalization. More interestingly, PKC-phosphorylating activity appears to be impaired in brain in senescent rodents (7,8,51,97,123). However, the important fact is that, in spite of strain-related differences in brain basal PKC activity, the translocation process appears to be the common component of defective functional machinery in the aging brain (8,9,51,70). The results obtained from the investigation of this matter have shown that the impaired PKC translocation associated with age-dependent cognitive impairment could be correlated to a reduced expression of the anchoring protein RACK-1 (9,105,106,120,130). Finally, also in Alzheimer's disease, a neurodegenerative condition significantly affecting cognition and memory, a reduced level of RACK-1 protein, can be observed in the brain of affected patients (10). In light of the observations outlined above it is particularly interesting to observe that DHEA(S) can affect these signal transduction mechanisms. In fact, we have shown that in aged rodents, treated with DHEA for two weeks, using subcutaneously implanted osmotic pumps, the effect of treatment was a complete restoration of RACK-1 levels to those found in young rats, both at the protein and mRNA levels (123). It is worth noticing that the same effect on RACK-1 protein was observed in the alveolar macrophages of aged rats (39). Corsini et al. have demonstrated (38) that age-associated immunological changes result, in part, from a decrease in the functional capacity of macrophages which is due to an impaired PKC translocation correlated to reduced RACK-1 expression. DHEA supplementation can return the RACK-1 levels to those observed in young animals and can also functionally rescue the macrophage-dependent immunological function in aging rat. Overall these data indicate that the effect of DHEA, and possibly of other neurosteroids, on the cognition and memory in aging is exerted through the restoration of defective PKC signal transduction machinery. In addition, the parallel effects on the immune system may bridge the pleiotropic effect of neurosteroid on different age-related deficits (Fig. 2).

PRECLINICAL STUDIES IN AGED ANIMALS

Besides the clear demonstration of the biological an neurochemical activities of DHEA(S) and other neurosteroids, key information on the cognitive effect of these molecules comes from animal studies. These have been conducted mostly in rodents and the key for the correct interpretation of the results is the identification of the behavioral test applied. These include avoidance-based paradigms, in which animals learn a behavior to avoid a noxious stimulus, and tests of spatial memory involving different types of mazes, for example the Y-maze and the Morris water-maze (95). The first to show that DHEA and DHEA(S) had memory-enhancing effects were Flood et al., who demonstrated the effect of DHEA on the avoidance based paradigm in either young or old mice (47,48,49,128).

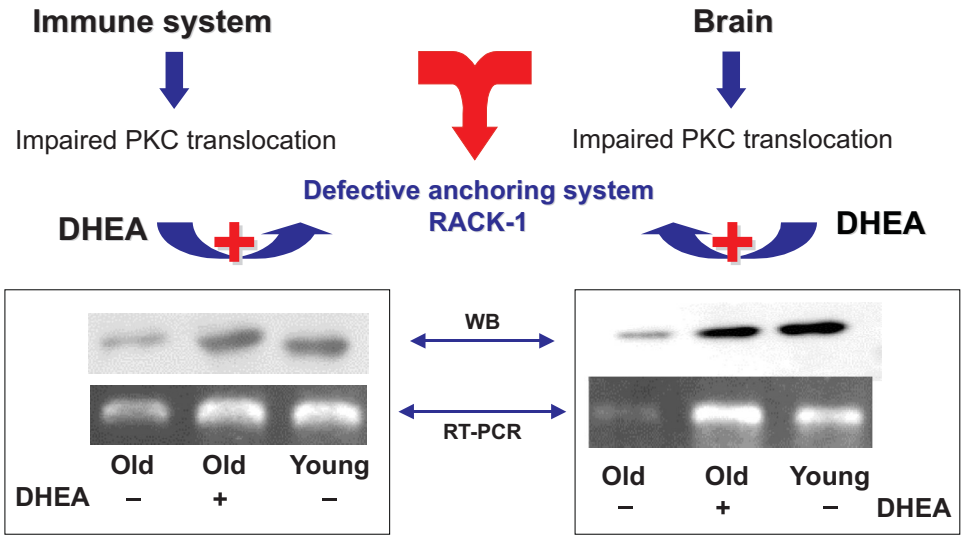

Fig. 2. Potential bridging of the immune system modulatory activity and the CNS cognitive effects of DHEA. Both systems in rodents, have age-related alterations in the functional PKC machinery due to reduced levels of the PKC anchoring protein RACK-1. In both systems DHEA replacement *in vivo* restores RACK-1 levels to those present in young animals. This results in restoration of functional activities in the immune system and may be one of the mechanisms underlying DHEA effect on cognition in rodents. Images are adapted from refs. 39, 123.

The memory-enhancing effect has been replicated in a paradigm of short-term working memory, assessed with a T-maze resulting in the observation that both DHEA and DHEA(S), administered i.p., enhance memory (96). In another series of experiments the administration of DHEAS, before or directly after training, enhanced memory although the same effect was not seen if the drug was given before retention testing. This finding suggested that DHEA(S) enhances the storage and/or consolidation of the learned material, but does not improve retrieval (128). The memory enhancing effect of DHEA(S) has also been confirmed in rats using behavioral tests: Morris water maze and Y maze (52).

In addition to DHEA(S), pregnenolone and its sulfate ester have been tested in a number of cognitive tasks in rodents. Levels of pregnenolone sulfate are selectively reduced within the hippocampus of 2-year-old rats (151), and correlate with memory deficits tested in Y-maze and Morris water-maze. The memory deficits of cognitively impaired aged rats could be transiently corrected after either intraperitoneal or bilateral intrahippocampal injections of pregnenolone sulfate, confirming the role of this steroid in memory (151). The chronic administration of pregnenolone sulfate in adult male rats was achieved by intracerebroventricular (i.c.v.) infusion using an osmotic minipump. The effect of steroid administration was then studied on memory performances in an Y-maze arm discrimination test. Since a "basal" level of steroids is continuously present in the nervous system, this approach was expected to more closely approximate the physiological conditions. Using a non-stressful spatial memory task in mice, the investigators demonstrated that by chronic infusion pregnenolone sulfate has memory-enhancing effects (72).

The initial studies by Flood et al. have been extended to a different memory paradigm by Melchior (96), and neurosteroids were shown to reduce the effect of amnestic treat-

ments. The use of the NMDA antagonist dizocilpine in rodents induces an amnestic cognition deficit that is antagonized by pregnenolone and its metabolites (30,88). In addition, while by subcutaneous administration scopolamine dose-dependently impairs learning during an appetite-reinforced visual discrimination task, by i.c.v. administration pregnenolone sulfate, dose-dependently blocked learning deficits induced by scopolamine and had an additive memory-enhancing effect (102). The ameliorating effect of DHEA(S) in mice treated with scopolamine can be blocked by the selective D_1 antagonist NE-100 (150). The beneficial effects of pregnenolone and DHEA(S) in mice treated with b-amyloid protein can be prevented by haloperidol (91), suggesting a significant role of δ receptors in the cognitive activities of these steroids.

Since the interconversion between the unsulfated and the sulfated forms of DHEA and pregnenolone is a reversible process, sorting the significance of the sulfation in the pro-amnestic process has been difficult. Steroid sulfatase inhibitors have been recently developed. The administration of the steroid sulfatase inhibitor p-O-(sulfamoyl)-N-tetradecanoyl tyramine (DU14) produced inhibition of steroid sulfatase activity and enhanced the reversal of amnesia by DHEA(S) (80). Another steroid sulfatase inhibitor, estrone-3-O-sulfamate (EMATE), potentiated the effect of DHEA(S) on memory retention such that lower doses of DHEA(S) were sufficient to obtain significant effects. EMATE, administered peripherally, potentiated the effect of centrally administered DHEA(S), but not of pregnenolone sulfate, on memory retention (50).

Consideration should be given to the dose-dependency of the effects of steroids such as DHEA(S). The dose needed to observe memory enhancement varied considerably between different studies making it difficult to unequivocally decide on the optimal dose-range for this steroid. Various routes of administration have been used. In fact, effective i.c.v. doses were often rather low while the doses given by peripheral routes (i.p. or s.c.) varied considerably, from 0.05 to 24 mg/kg. It should be noted that the commonly used replacement dose in elderly humans ranges from 0.5 to 1 mg/kg (see below). It is also of interest that all dose-response studies showed an inverted U-shaped pattern for the dose-response curve.

Summarizing the studies mentioned above, there is a substantial evidence in the literature supporting the role of neurosteroids in the maintenance of cognitive functions and possibly in the restoration of these functions in aged animals. The most difficult task now is to demonstrate similar results in humans and transfer the preclinical evidence into clinical efficacy.

CLINICAL STUDIES IN HUMANS

Since many animal studies convincingly demonstrated the beneficial effect of DHEA and DHEA(S) in preventing age-related memory deficits, many investigators have hypothesized the existence of a direct correlation between degenerative changes associated with human aging and the progressive deficit in circulating DHEA or DHEA(S). From these speculations the idea of a "fountain of youth" has emerged and DHEA has assumed a somewhat overstated role of a "miraculous drug" for old age (12). Given that most of the preclinical experimental studies have been conducted in rodents, it is necessary to remember that rats and mice have indeed very little circulating DHEA. Thus the extrapolation of data from rodents to humans is not straight forward.

In fact, several studies attempting to correlate DHEA and DHEA(S) plasma levels and cognitive functions in elderly humans produced conflicting results. Some studies reported

the association of cognitive deficits with either low (168) or high DHEA(S) levels (104, 110,111), while other studies did not find any correlation (6,22,27,28,78,107,110,125). Memory and cognitive performance in healthy elderly were assessed by neuropsychological test batteries evaluating visual memory (short- and long-term memory), verbal memory (immediate and delayed recall), spatial memory and attention/concentration test. The risk of cognitive decline in healthy elderly subjects was not correlated with the DHEA(S) levels measured many years before the cognitive testing (6). In addition, the longitudinal change in cognitive performance studied in a population of elderly women (65 to 80 years of age) was not correlated with the changes in plasma DHEA(S) levels (167). A cross-sectional study (27) as well as long- (107) and short-term (28) longitudinal studies in healthy aged men and women reported no association between plasma DHEA(S) levels and cognitive performance. Neither the rate of decline in serum DHEA(S) concentrations in men nor the mean DHEA(S) concentrations within individuals were related to memory status or memory decline. In summary, the data described suggest that in healthy elderly the levels of DHEA(S) do not seem to be correlated to cognitive performance. The cognitive performance of patients with Alzheimer's disease is often evaluated using the Mini-Mental State Examination test. As for healthy elderly subjects the correlation between the levels of DHEA(S) and cognitive performances are weak. Nevertheless, interesting results have been reported concerning the pattern of decline of DHEA and its sulfate form. A number of studies have indicated that in AD patients the levels of DHEA(S) are lower compared to age- and gender-matched elderly control individuals (77,112,137,144,168). These observations have been confirmed in a 3-year-long longitudinal study conducted in a 70 to 104 years old population (58). The results of this study clearly demonstrated that plasma DHEA(S) levels were significantly lower in Alzheimer patients as compared to control subjects. This finding suggests that low DHEA(S) plasma levels could be viewed as a risk factor for AD. Unexpectedly, a 6-month longitudinal study found that lower plasma DHEA levels, measured in men and women with mild-to-moderate Alzheimer's disease, correlate with a better memory performance at the beginning of the study (104). However, these levels were not predictive of the rate of cognitive decline over time.

Since so much medical and media attention has been drawn to the alleged benefits of DHEA(S) replacement in aging, with particular emphasis on cognition, it is important to consider in detail the results obtained so far in clinical trials of replacement therapy (see Table 1). The first single case study was conducted on a 47-year-old woman showing a life-long history of specific learning disabilities and deficient levels of circulating DHEA and DHEA(S). Chronic DHEA treatment resulted in normalization of the plasma levels of DHEA and DHEA(S) and resulted in the recovery of some memory functions (24). More recent clinical trials conducted by Wolf et al. did not show significant improvements. A single oral dose of DHEA (300 mg) had no effect on performance in several tests covering different aspects of memory, such as visual, verbal or declarative memory in healthy young (25-years old) individuals(160). In a larger double-blind, placebo-controlled trial involving 40 healthy elderly men and women (mean age, 69 years) psychological and physical well-being as well as cognitive performance were assessed using several questionnaires and neuropsychological tests. DHEA replacement (50 mg daily for two weeks) had no strong beneficial effect on any of the measured cognitive parameters with a non-significant tendency for women toward better performance in one of six cognitive tests (159). The same group could not demonstrate a beneficial effect of DHEA substitution on electrophysiological indices of central nervous system stimulus (161). Related to the pos-

TABLE 1. Summary of the main clinical studies conducted with DHEA

Method	Participants	Dosage regimen	Outcomes	Ref
Double-blind, cross-over, randomized, placebo-controlled	46 healthy subjects: 25 men, 15 women. Age range: 58–83.	DHEA: 50 mg/day for 6 weeks 2 weeks DHEA, 2 weeks wash-out, 2 weeks placebo	No significant effects on cognitive function. Men: 6 out of 9 tests of cognitive function better on DHEA (not significant); women: 4 out of 9 tests better on DHEA	159
Double-blind, cross-over, randomized, placebo-controlled	17 men. Age range: 59–81	DHEA: 50 mg/day for 5 weeks 2 weeks DHEA, 1 week wash-out, 2 weeks placebo	Neuropsychological tests and tests of event-related potentials (ERPs). No significant improvement of cognition: 4 out of 5 memory tests better on DHEA (not significant). Significantly larger amplitude of the P3 event-related potential on DHEA	161
Double-blind: Parallel groups Randomized stratified by age and body mass index	81 subjects: 38 men, 37 women Age range: 59–81	DHEA: 50 mg/day for 2 weeks; psychosocial laboratory stressor administered at end of 2-week period	Neuropsychological tests. Symbol cancellation test performance of DHEA group significantly better than of placebo group after stress	162
Randomized, double-blind, cross-over	46 men. Age range 62–76	50 mg DHEA daily for 13 weeks	Cognition assessed with tests of speed, attention and episodic memory. Well-being measured with questionnaires of mood and perceived health. No significant effects of DHEA observed on any of the trial outcomes	152
Double-blind, placebo-controlled study	280 healthy subjects (men/women). Age range 60–79 years	DHEA, 50 mg, daily for a year	Significant improvement of bone turnover, libido and skin status in women >70 years old. No data provided on cognition	17
Double-blind, randomized, cross-over treatment study	17 subjects (men and women) age range 45–63 years with midlife-onset dysthymia	3 weeks on 90 mg DHEA, 3 weeks on 450 mg DHEA, and 6 weeks on placebo	Neuropsychological tests related to depression and dysthymia scales. Robust effect of DHEA on mood (anhedonia, loss of energy, lack of motivation, emotional "numbness," sadness, inability to cope, and tendency to worry) No effect on cognitive function or sleep disturbance	23
Double-blind Placebo-controlled	22 patients with major depression. No medication on stabilized drug regimens	DHEA 90 mg/day for 6 weeks	DHEA was associated with a significantly greater decrease in Hamilton depression scale ratings than placebo	166

sible antiglucocorticoid action of DHEA, a beneficial effect could not be measured using cognitive tests following a standardized psychosocial laboratory stressor (Trier Social Stress Test; TSST) (162).

Depressed patients have been also treated with DHEA. In a study by Wolkowitz et al (164,165) six middle-aged and elderly patients with major depression and low basal plasma DHEA(S) levels were openly administered DHEA (30 to 90 mg/day for 4 weeks). The treatment adequately restored circulating plasma levels observed in younger healthy individuals. The authors observed a significant improvement in depression ratings, as well as in some aspects of memory performance. These improvements were directly related to increases in plasma levels of DHEA(S) and to increases in their ratios with plasma cortisol levels. The same authors (166) subsequently performed a double-blind, placebo-controlled study designed to assess possible antidepressant effects of DHEA. Twenty-two patients with major depression, taking antidepressant or not treated, were subjected to treatment with DHEA (up to 90 mg/day) for 6 weeks. Treatment with DHEA was associated with a significant improvement in depression scale ratings suggesting that it may have significant antidepressant effects in some patients with major depression. Similarly, a robust effect of DHEA on mood (anhedonia, loss of energy, lack of motivation, emotional "numbness," sadness, inability to cope, and tendency to worry) was observed in patients aged 45 to 63 years with mid-life-onset dysthymia when treated for 3 weeks with 90 mg/day of DHEA (23). These results have been included in a systematic review by Huppert and Van Niekerk for the Cochrane Database (60). The conclusion of the reviewer indicates that the data offer no support for an improvement in memory or other aspects of cognitive function following DHEA treatment in normal older people. Nevertheless, it is possible that any neuroprotective effect of DHEA(S) may only be evident in the long term trials. It is, therefore, necessary to undertake long-term trials with a sufficient number of participants to allow the detection of the effects of DHEA, if they exist. One of the largest trials recently completed (17) has studied the effect of a one year DHEA supplementation in 280 healthy individuals (60 to 79 years old) in a double-blind, placebo-controlled study. The data presented included significant improvement in aspects concerning bone turnover in women, libido parameters and skin status. No data have yet been presented for cognitive parameters. More recently van Niekerk et al. conducted a clinical trial in a non-clinical sample of 46 men aged 62 to 76 years where the subjects were treated in a randomized double-blind cross-over trial design with 50 mg DHEA daily for 13 weeks. A correlational analysis of baseline behavioral data with hormonal data (controlling for age) revealed a significant complex correlation between circadian DHEA and cortisol levels (including ratio) and confusion, anxiety, general mood disturbance and memory performances. However, the analysis of treatment effect revealed no significant effects of DHEA on any of the trial outcomes (152).

Dosage Regimens in Humans and Pharmacokinetic Studies

Several pharmacokinetic studies have established the appropriate dose range for DHEA replacement in aging. The various clinical trials (indicated above) have often used a variable dose of DHEA, ranging from 25 to up to 450 mg/day. The human dosage varied, however, less than in animal studies. This is probably because of the universal choice of the oral route in human trials. Arlt et al. (3) studied the pharmacokinetics and biotransformation of orally administered DHEA in 14 healthy male volunteers to define a suitable dose for DHEA substitution in elderly men. The levels of DHEA in the volunteers were

below 1500 ng/mL. 50 mg DHEA increased serum DHEA(S) to the mean levels of young adult men, whereas after 100 mg DHEA levels were supraphysiological. Arlt and coworkers also demonstrated that blood levels of androgens remained unchanged after DHEA administration, while the levels of estrogens significantly increased in a dose-dependent manner, but still within the upper normal range for men. Legrain et al. (79) utilized a pharmacokinetic study to choose the dosage regimen subsequently used in their clinical trial (17). DHEA tablets of 50 and 25 mg were orally administered daily to 24 healthy aging men and women for 8 days. The data obtained demonstrated the reestablishment of "young" levels of DHEA with both doses. Interestingly, the apparent terminal half-life of DHEA was longer than 20 h suggesting a significant back-hydrolysis of the large amount of DHEA(S) *in vivo*. Circulating testosterone and estrogens increased to levels within the normal young-adult levels as indicated previously in the study of Arlt et al. Other studies (4,53,109,153) substantially confirmed these data and concluded that the optimal human dose for DHEA replacement therapy is 50 mg/day or lower.

CONCLUSIONS

The data in the literature reviewed here provide evidence that DHEA, among other neurosteroids, have significant effects on the central nervous system. Among the several potential effects of DHEA are those involved in the modulation of cognitive performance. Preclinical and clinical research demonstrated a dichotomy between the measurable significant results obtained in animals, mostly rodents, and the vague and often not significant results obtained in humans. At this stage of the research, there is a limited evidence from controlled trials that DHEA enhances cognitive function in normal middle-aged or elderly people as well as in individuals with dementia. The theoretical grounds for a beneficial effect of DHEA on cognitive function in elderly people or people suffering from dementia are persuasive. The key point, however, is the design of flawless and complete studies toward a final clarification of the potential of DHEA in age-related cognitive decline and dementia. Although these studies do not support the idea that DHEA is a cognitive enhancer in healthy elderly, we need more research on demented individuals. In such research we may detect either an improvement in performance or a reduction in the rate of cognitive decline. A sufficiently large sample size is needed to detect changes if they are present and, in addition, long-term treatment is required. Short-term adverse effects have not been evidenced in clinical trials so far. Nevertheless, the use of longer-term treatments (more than 12 months) is particularly important to establish whether there are adverse long-term consequences of treatment. Finally, it will be important to evaluate effective dosage, acceptable route and duration of administration. Although most trials have indicated an optimal oral dosage regimen of 50 mg/day, this dose was not approved by any government agency. DHEA is being currently sold in health food stores, particularly in the USA. More attention should be given in the future studies to the pharmaceutical preparation and pharmacokinetics of DHEA formulations.

REFERENCES

1. Adams JB Control of secretion and function of C19-delta 5-steroids of the human adrenal gland. *Mol Cell Endocrinol* 1985;41:1–17.
2. Aldred S, Waring RH. Localisation of dehydroepiandrosterone sulphotransferase in adult rat brain. *Brain Res Bull* 1999;48:291–296.

3. Arlt W, Haas J, Callies F, et al. Biotransformation of oral dehydroepiandrosterone in elderly men: Significant increase in circulating estrogens. *J Clin Endocrinol Metab* 1999;84:2170–2176.
4. Arlt W, Callies F, Koehler I, et al. Dehydroepiandrosterone supplementation in healthy men with an age-related decline of dehydroepiandrosterone secretion. *J Clin Endocrinol Metab* 2001;86:4686–4692.
5. Ballabio A, Shapiro LJ. Steroid sulfatase deficiency and X-linked ichthyosis. In: Schriver CR, Beaudet AL, Sly WS, Valle D, Eds. *The metabolic and molecular bases of inherited disease*. New York: McGraw-Hill, 1995:2999–3022.
6. Barrett-Connor E, Edelstein SL. A prospective study of dehydroepiandrosterone sulfate and cognitive function in an older population: The Rancho Bernardo Study. *J Am Geriatr Soc* 1994;42:420–423.
7. Battaini F, Del Vesco R, Govoni S, Trabucchi M. Regulation of phorbol ester binding and protein kinase C activity in aged rat brain. *Neurobiol Aging* 1990;11:563–566.
8. Battaini F, Govoni S, Lucchi L, Ladisa V, Bergamaschi S, Trabucchi M. Age-related changes in brain protein kinase C, expression, activity, and translocation. *Drugs Dev* 1993;2:275–282.
9. Battaini F, Elkabes S, Bergamaschi S, et al. Protein kinase C activity, translocation, and conventional isoforms in aging rat brain. *Neurobiol Aging* 1995; 16:137–148.
10. Battaini F, Pascale A, Lucchi L, Pasinetti G. M, Govoni S. Protein kinase C anchoring deficit in postmortem brains of Alzheimer's disease patients. *Exp Neurol* 1999;159:559–564.
11. Baulieu EE. Neurosteroids: A novel function of the brain. *Psychoneuroendocrinology* 1998;23:963–987.
12. Baulieu EE. Dehydroepiandrosterone (DHEA): A fountain of youth? *J Clin Endocrinol Metab*ol 1996;81: 3147–3151.
13. Baulieu EE. Neurosteroids: Pregnenolone and dehydroepiandrosterone in the brain. In: Fuxe K, Agnati L, Eds. *Receptor interactions*. Basingstoke: Macmillan, 1987:89–104.
14. Baulieu EE, Robel P. Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) as neuroactive neurosteroids. *Proc Natl Acad Sci USA* 1998;95:4089–4091.
15. Baulieu EE, Robel P. Dehydroepiandrosterone and dehydroepiandrosterone sulfate as neuroactive neurosteroids. *J Endocrinol* 1996;150:S221–S239.
16. Baulieu EE, Robel P. Neurosteroids: A new brain function? *J Steroid Biochem Mol Biol* 1990;37:395–403.
17. Baulieu EE, Thomas G, Legrain S, et al. Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: Contribution of the DHEAge Study to a sociobiomedical issue. *Proc Natl Acad Sci USA* 2000;97:4279–4284.
18. Beaujean D, Mensah-Nyagan AG, Do-Rego JL, Luu-The V, Pelletier G, Vaudry H. Immunocytochemical localization and biological activity of hydroxysteroid sulfotransferase in the frog brain. *J Neurochem* 1999;72: 848–857.
19. Beyenburg S, Stoffel-Wagner B, Watzka M, et al. Expression of cytochrome P450scc mRNA in the hippocampus of patients with temporal lobe epilepsy. *Neuroreport* 1999;10:3067–3070.
20. Bergeron R, de Montigny C, Debonnel G. Potentiation of neuronal NMDA response induced by dehydroepiandrosterone and its suppression by progesterone: Effects mediated via sigma receptors. *J Neurosci* 1996; 16:1193–1202.
21. Bliss TV, Collingridge GL. A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature* 1993;361:31–39.
22. Birkenhager-Gillesse EG, Derksen J, Lagaay AM. Dehydroepiandrosterone sulphate (DHEAS) in the oldest old, aged 85 and over. *Ann NY Acad Sci* 1994;719:543–552.
23. Bloch M, Schmidt PJ, Danaceau MA, Adams LF, Rubinow D. Dehydroepiandrosterone treatment of midlife dysthymia. *Biol Psychiatry* 1999;45:1533–1541.
24. Bonnet KA, Brown RP. Cognitive effects of DHEA replacement therapy. In: Kalimi M, Regelson W, Eds. *The biologic role of dehydroepiandrosterone*. Berlin: Walter de Gruyter, 1990;65–79.
25. Cascio C, Prasad VV, Lin YY, Lieberman S, Papadopoulos V. Detection of P450c17-independent pathways for dehydroepiandrosterone (DHEA) biosynthesis in brain glial tumor cells. *Proc Natl Acad Sci USA* 1998; 95:2862–2867.
26. Cascio C, Brown RC, Liu Y, Han Z, Hales D, Papadopoulos V. Pathways of dehydroepiandrosterone formation in rat brain glia. *J Steroid Biochem Mol Biol* 2000;75:177–186.
27. Carlson LE, Sherwin BB. Steroid hormones, memory and mood in a healthy elderly population. *Psychoneuroendocrinology* 1998;23:583–603.
28. Carlson L, Sherwin B. Relationships among cortisol (CRT), dehydroepiandrosterone-sulfate (DHEAS), and memory in a longitudinal study of healthy elderly men and women. *Neurobiol Aging* 1999;20:315–324.
29. Castellano C, McGaugh J. Effects of post-training bicuculline and muscimol on retention: Lack of state dependency. *Behav Neural Biol* 1990;54:156–164.
30. Cheney DL, Uzunov D, Guidotti A. Pregnenolone sulfate antagonizes dizocilpine amnesia: Role for allopregnanolone. *Neuroreport* 1995;6:1697–1700.
31. Compagnone N, Bulfone A, Rubenstein J, Mellon S. Expression of the steroidogenic enzyme P450scc in the central and peripheral nervous systems during rodent embryogenesis. *Endocrinology* 1995;136:2689–2696.
32. Compagnone N, Bulfone A, Rubenstein J, Mellon S. Steroidogenic enzyme P450c17 is expressed in the embryonic central nervous system. *Endocrinology* 1995;136:5212–5223.

33. Compagnone N, Mellon S. Dehydroepiandrosterone: A potential signalling molecule for neocortical organization during development. *Proc Natl Acad Sci USA* 1998;95:4678–83.
34. Compagnone N, Mellon S. Neurosteroids: Biosynthesis and function of these novel neuromodulators. *Front Neuroendocrinol* 2000;21:1–56.
35. Compagnone N, Salido E, Shapiro L, Mellon S. Expression of steroid sulfatase during embryogenesis. *Endocrinology* 1997;138:4768–4773.
36. Corpechot C, Robel P, Axelson M, Sjovall J, Baulieu E. Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. *Proc Natl Acad Sci USA* 1981; 78:4704–4707.
37. Corpechot C, Synguelakis M, Talha S, et al. Pregnenolone and its sulfate ester in the rat brain. *Brain Res* 1983;270:119–125.
38. Corsini E, Battaini F, Lucchi L, et al. A defective protein kinase C anchoring system underlying age-associated impairment in TNF_{α} production in rat macrophages. *J Immunol* 1999;163:3468–3473.
39. Corsini E, Lucchi L, Meroni M, et al. *In vivo* dehydroepiandrosterone restores age-associated defects in the protein kinase C signal transduction pathway and related functional responses. *J Immunol* 2002;168: 1753–1758.
40. De Peretti E, Forest M. Pattern of plasma dehydroepiandrosterone sulfate levels in humans from birth to adulthood: Evidence for testicular production. *J Clin Endocrinol Metab* 1978;47:572–577.
41. Debonnel G. Current hypotheses on sigma receptors and their physiological role: Possible implications in psychiatry. *J Psychiatry Neurosci* 1993;18:157–172.
42. Debonnel G, Bergeron R, de Montigny C. Potentiation by dehydroepiandrosterone of the neuronal response to *N*-methyl-D-aspartate in the CA3 region of the rat dorsal hippocampus: An effect mediated via sigma receptors. *J Endocrinol* 1996;150: S33-S42.
43. Debonnel G, de Montigny C. Modulation of NMDA and dopaminergic neurotransmissions by sigma ligands: Possible implications for the treatment of psychiatric disorders. *Life Sci* 1996;58:721–734.
44. Dekker L. V, Parker P. J. Protein kinase C — a question of specificity. *Trends Biochem Sci* 1994;19:73–77.
45. Endoh A, Kristiansen S, Casson P, Buster J, Hornsby P. The *zona reticularis* is the site of biosynthesis of dehydroepiandrosterone and dehydroepiandrosterone sulfate in the adult human adrenal cortex resulting from its low expression of 3-beta-hydroxysteroid dehydrogenase. *J Clin Endocrinol Metab* 1996;81:3558–3565.
46. Engelhardt W, Friess K, Hartung E. Sold M, Dierks T. EEG and auditory evoked potential P300 compared with psychometric tests in assessing vigilance after benzodiazepine sedation and antagonism. *Br J Anaesth* 1992;69:75–80.
47. Flood J, Morley J, Roberts E. Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it. *Proc Natl Acad Sci USA* 1992;89:1567–1571.
48. Flood J, Roberts E. Dehydroepiandrosterone sulfate improves memory in aging mice. *Brain Res* 1988;448: 178–181.
49. Flood J, Smith G, Roberts E. Dehydroepiandrosterone and its sulfate enhance memory retention in mice. *Brain Res* 1988;447:269–278.
50. Flood J, Farr S, Johnson D, Li P, Morley J. Peripheral steroid sulfatase inhibition potentiates improvement of memory retention for hippocampally administered dehydroepiandrosterone sulfate but not pregnenolone sulfate. *Psychoneuroendocrinology* 1999;24:799–811.
51. Friedman E, Wang H. Effect of age on brain cortical protein kinase C and its mediation of 5-hydroxytryptamine release. *J Neurochem* 1989;52:187–192.
52. Frye C, Sturgis J. Neurosteroids affect spatial/reference, working, and long-term memory of female rats. *Neurobiol Learn Mem* 1995;64:83–96.
53. Frye R, Kroboth P, Kroboth F, et al. Sex differences in the pharmacokinetics of dehydroepiandrosterone (DHEA) after single- and multiple-dose administration in healthy older adults. *J Clin Pharmacol* 2000;40: 596–605.
54. Furukawa A, Miyatake A, Ohnishi T, Ichikawa Y. Steroidogenic acute regulatory protein (StAR) transcripts constitutively expressed in the adult rat central nervous system: Colocalization of StAR, cytochrome P-450scc (CYP XIA1), and 3-beta-hydroxysteroid dehydrogenase in the rat brain. *J Neurochem* 1998;71: 2231–2238.
55. Guazzo E, Kirkpatrick P, Goodyer I, Shiers H, Herbert J. Cortisol, dehydroepiandrosterone (DHEA), and DHEA sulfate in the cerebrospinal fluid of man: Relation to blood levels and the effects of age. *J Clin Endocrinol Metab* 1996;81:3951–3960.
56. Harrison N, Simmonds M. Modulation of the GABA receptor complex by a steroid anaesthetic. *Brain Res* 1984;323:287–292.
57. Harrison N, Vicini S, Barker J. A steroid anesthetic prolongs inhibitory postsynaptic currents in cultured rat hippocampal neurons. *J Neurosci* 1987;7:604–609.
58. Hillen T, Lun A, Reischies F, Borchelt M, Steinhagen-Thiessen E, Schaub R. DHEA-S plasma levels and incidence of Alzheimer's disease. *Biol Psychiatry* 2000;47:161–3.
59. Hornsby P. Biosynthesis of DHEAS by the human adrenal cortex and its age-related decline. *Ann NY Acad Sci* 1995;774:29–46.

60. Huppert F, Van Niekerk J. Dehydroepiandrosterone (DHEA) supplementation for cognitive function. *Cochrane Database Syst Rev* 2001;2:CD000304.
61. Imamura M, Prasad C. Modulation of GABA-gated chloride ion influx in brain by dehydroepiandrosterone and its metabolites. *Biochem Biophys Res Commun* 1998;243:771–775.
62. Introini-Collison I, Castellano C, McGaugh J. Interaction of GABAergic and beta-noradrenergic drugs in regulation of memory storage. *Behav Neural Biol* 1994;61:150–155.
63. Izquierdo I. Pharmacological evidence for a role of long-term potentiation in memory. *FASEB J* 1994;8: 1139–1145.
64. Jung-Testas I, Hu Z. Y, Baulieu E, Robel P. Neurosteroids: Biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells. *Endocrinology* 1989;125:2083–2091.
65. Khorram O. DHEA: A hormone with multiple effects. *Curr Opin Obstet Gynecol* 1996;8:351–354.
66. Kimonides V, Khatibi N, Svendsen C, Sofroniew M, Herbert J. Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) protect hippocampal neurons against excitatory amino acid-induced neurotoxicity. *Proc Natl Acad Sci USA* 1998;95:1852–1857.
67. Kimonides V, Spillantini M, Sofroniew M, Fawcett J, Herbert J. Dehydroepiandrosterone antagonizes the neurotoxic effects of corticosterone and translocation of stress-activated protein kinase 3 in hippocampal primary cultures. *Neuroscience* 1999;89:429–436.
68. Kimoto T, Tsurugizawa T, Ohta Y. Neurosteroid synthesis by cytochrome p450-containing systems localized in the rat brain hippocampal neurons: *N*-methyl-D-aspartate and calcium-dependent synthesis. *Endocrinology* 2001;142:3578–3589.
69. Kohchi C, Ukena K, Tsutsui K, et al. Age- and region-specific expressions of the messenger RNAs encoding for steroidogenic enzymes p450scc, P450c17 and 3beta-HSD in the postnatal rat brain. *Brain Res* 1998;801: 233–238.
70. Kraft AS, Anderson WB. Phorbol esters increase the amount of Ca^{2+}, phospholipid-dependent protein kinase associated with plasma membrane. *Nature* 1983;301:621–623.
71. Kulikowski J, McGlone F, Kranda K, Ott H. Are the amplitudes of visual evoked potentials sensitive indices of hangover effects after repeated doses of benzodiazepines? *Psychopharmacol Suppl* 1984;1:154–164.
72. Ladurelle N, Eychenne B, Denton D, et al. Prolonged intracerebroventricular infusion of neurosteroids affects cognitive performances in the mouse. *Brain Res* 2000;858:371–379.
73. Lambert J, Belelli D, Hill-Venning C, Peters J. Neurosteroids and $GABA_A$ receptor function. *Trends Pharmacol Sci* 1995;16:295–303.
74. Le Goascogne C, Robel P, Gouezou M, Sananes N, Baulieu E, Waterman M. Neurosteroids: Cytochrome P450scc in rat brain. *Science* 1987;237:1212–1215.
75. Le Goascogne C, Sananes N, Gouezou M, et al. Immunoreactive cytochrome P-450(17α) in rat and guinea-pig gonads, adrenal glands and brain. *J Reprod Fertil* 1991;93:609–622.
76. Le Goascogne C, Robel P, Gouezou M, Sananes N, Baulieu E, Waterman M. Neurosteroids: Cytochrome P450scc in rat brain. *Science* 1984;237:1212–1215.
77. Leblhuber F, Windhager E, Reisecker F, Steinparz F, Dienstl E. Dehydroepinadrosterone sulphate in Alzheimer's disease. *Lancet* 1990;336:449.
78. Legrain S, Berr C, Frenoy N, Gourlet V, Debuire B, Baulieu E. Dehydroepiandrosterone sulfate in a long-term care aged population. *Gerontology* 1995;41:343–51.
79. Legrain S, Massien C, Lahlou N, et al. Dehydroepiandrosterone replacement administration: Pharmacokinetic and pharmacodynamic studies in healthy elderly subjects. *J Clin Endocrinol Metab* 2000;85:3208–3217.
80. Li P, Rhodes M, Burke AM, Johnson D. Memory enhancement mediated by the steroid sulfatase inhibitor (p-O-sulfamoyl)-N-tetradecanoyl tyramine. *Life Sci* 1997;60: PL45–51.
81. Li X, Salido E, Gong Y, et al. Cloning of the rat steroid sulfatase gene (Sts), a non-pseudo-autosomal X-linked gene that undergoes X inactivation. *Mam Genome* 1996;7:420–424.
82. Liu C, Laughlin G, Fischer U, Yen S. Marked attenuation of ultradian and circadian rhythms of dehydroepiandrosterone in postmenopausal women: Evidence for a reduced 17,20-desmolase enzymatic activity. *J Clin Endocrinol Metab* 1990;71:900–906.
83. Lupien S, Lecours AR, Schwartz G, et al. Longitudinal study of basal cortisol levels in healthy elderly subjects: Evidence for subgroups. *Neurobiol Aging* 1996;17:95–105.
84. Majewska MD. Neurosteroids: Endogenous bimodal modulators of the $GABA_A$ receptor. Mechanism of action and physiological significance. *Prog Neurobiol* 1992;38:379–395.
85. Majewska M, Harrison NL, Schwartz RD, Barker JL, Paul S. M. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* 1986;232:1004–1007.
86. Martin F, Siddle DA, Gourley M, Taylor J, Dick R. P300 and traffic scenes: The effect of temazepam. *Biol Psychol* 1992;33:225–240.
87. Mathur C, Prasad V, Raju VS, Welch M, Lieberman S. Steroids and their conjugates in the mammalian brain. *Proc Natl Acad Sci USA* 1993;90:85–88.

88. Mathis C, Paul SM, Crawley JN. The neurosteroid pregnenolone sulfate blocks NMDA antagonist-induced deficits in a passive avoidance memory task. *Psychopharmacology (Berl)* 1994;116:201–6.
89. Matsuno K, Senda T, Kobayashi T, Mita S. Involvement of sigma 1 receptor in (+)-N-allylnormetazocine-stimulated hippocampal cholinergic functions in rats. *Brain Res* 1995;690:200–206.
90. Matsuno K, Senda T, Matsunaga K, Mita S. Ameliorating effects of sigma receptor ligands on the impairment of passive avoidance tasks in mice: Involvement in the central acetylcholinergic system. *Eur J Pharmacol* 1994;261:43–51.
91. Maurice T, Su TP, Privat A. Sigma1 (sigma 1) receptor agonists and neurosteroids attenuate B25–35-amyloid peptide-induced amnesia in mice through a common mechanism. *Neuroscience* 1998;83:413–428.
92. Maurice T, Lockhart BP. Neuroprotective and anti-amnesic potentials of sigma (sigma) receptor ligands. *Prog Neuropsychopharmacol Biol Psychiatry* 1997;21:69–102.
93. Maurice T, Roman F, Privat A. Modulation by neurosteroids of the *in vivo* (+)-[3H]SKF-10,047 binding to sigma 1 receptors in the mouse forebrain. *J Neurosci Res* 1996;46:734–743.
94. Maurice T, Roman F, Su T, Privat A. Beneficial effects of sigma agonists on the age-related learning impairment in the senescence-accelerated mouse. *Brain Res* 1996;733:219–230.
95. Mayo W, Dellu F, Robel P, et al. infusion of neurosteroids into the nucleus basalis magnocellularis affects cognitive processess in the rat. *Brain Res* 1993;607:324–328.
96. Melchior CL, Ritzmann RF. Neurosteroids block the memory-impairing effects of ethanol in mice. *Pharmacol Biochem Behav* 1996;53:51–56.
97. Meyer EM, Judkins JH, Momol AE, Hardwick EO. Effects of peroxidation and aging on rat neocortical ACh-release and protein kinase C. *Neurobiol Aging* 1994;15:63–67.
98. Mellon SH, Deschepper CF. Neurosteroid biosynthesis: Genes for adrenal steroidogenic enzymes are expressed in the brain. *Brain Res* 1993;629:283–292.
99. Mellon SH, Griffin LD. Neurosteroids: Biochemistry and clinical significance. *Trends Endocrinol Metab* 2002;13:35–43.
100. Mensah-Nyagan AG, Do-Rego JL, Beaujean D, Luu-The V, Pelletier G, Vaudry H. Neurosteroids: Expression of steroidogenic enzymes and regulation of steroid biosynthesis in the central nervous system. *Pharmacol Rev* 1999;51:63–81.
101. Mensah-Nyagan AG, Feuilloley M, Dupont E, et al. Immunocytochemical localization and biological activity of 3 beta-hydroxysteroid dehydrogenase in the central nervous system of the frog. *J Neurosci* 1994; 14:7306–7318.
102. Meziane H, Mathis C, Paul SM, Ungerer A. The neurosteroid pregnenolone sulfate reduces learning deficits induced by scopolamine and has promnestic effects in mice performing an appetitive learning task. *Psychopharmacology (Berl)* 1996;126:323–330.
103. Micheau J, Riedel G. Protein kinases: Which one is the memory molecule? *Cell Mol Life Sci* 1999;55:534–548.
104. Miller P, Taylor J, Rogerson S, et al. Cognitive and noncognitive symptoms in dementia patients: Relationship to cortisol and dehydroepiandrosterone. *Int Psychoger* 1998;10:85–96.
105. Mochly-Rosen D. Localization of protein kinases by anchoring proteins: A theme in signal transduction. *Science* 1995;268:247–251.
106. Mochly-Rosen D, Smith BL, Chen CH, Disatnik MH, Ron D. Interaction of protein kinase C with RACK-1, a receptor for activated C-kinase: A role in beta protein kinase C mediated signal transduction. *Biochem Soc Trans* 1995;23:596–600.
107. Moffat SD, Zonderman AB, Harman SM, Blackman MR, Kawas C, Resnick SM. The relationship between longitudinal declines in dehydroepiandrosterone sulfate concentrations and cognitive performance in older men. *Arch Intern Med* 2000;160:2193–2198.
108. Monnet FP, Mahe V, Robel P, Baulieu E. Neurosteroids, via sigma receptors, modulate the [^{3}H]norepinephrine release evoked by *N*-methyl-D-aspartate in the rat hippocampus. *Proc Natl Acad Sci USA* 1995;92: 3774–3778.
109. Morganti S, Freddi M, Rebecchi I, et al. Serum profile of dehydroepiandrosterone (DHEA) concentrations in DHEA-supplemented elderly subjects. *J Endocrinol Invest* 1999;22(10,Suppl):77–78.
110. Morrison MF, Redei E, TenHave T, et al. Dehydroepiandrosterone sulfate and psychiatric measures in a frail, elderly residential care population. *Biol Psychiatry* 2000;47:144–150.
111. Morrison MF, Katz IR, Parmelee P, Boyce A. A, TenHave T. Dehydroepiandrosterone sulfate (DHEA-S) and psychiatric and laboratory measures of frailty in a residential care. *Am J Geriatr Psychiatry* 1998;6: 277–284.
112. Nasman B, Olsson T, Backstrom T, et al. Serum dehydroepiandrosterone sulfate in Alzheimer's disease and in multi-infarct dementia. *Biol Psychiatry* 1991;30:684–90.
113. Nieschlag E, Loriaux D, Ruder H, Zucker I, Kirschner M, Lipsett M. The secretion of dehydroepiandrosterone and dehydroepindrosterone sulfate in man. *J Endocrinol* 1973;57:123–134.

114. Nishizuka Y. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science* 1992;258:607–14.
115. Orentreich N, Brind J, Vogelman J, Andres R, Baldwin H. Long-term longitudinal measurements of plasma dehydroepiandrosterone sulfate in normal men. *J Clin Endocrinol Metab* 1992;75:1002–1004.
116. Park IH, Han BK, Jo DH. Distribution and characterization of neurosteroid sulfatase from the bovine brain. *J Steroid Biochem Mol Biol* 1997;62:315–320.
117. Park-Chung M, Wu FS, Farb DH. 3 alpha-Hydroxy-5-beta-pregnan-20-one sulfate: A negative modulator of the NMDA-induced current in cultured neurons. *Mol Pharmacol* 1994;46:146–50.
118. Parker CR Jr, Mixon RL, Brissie RM, Grizzle WE. Aging alters zonation in the adrenal cortex of men. *J Clin Endocrinol Metab* 1997;82:3898–3901.
119. Pascale A, Fortino I, Govoni S, Trabucchi M, Wetsel WC, Battaini F. Functional impairment in protein kinase C by RACK-1 (receptor for activated C kinase 1) deficiency in aged rat brain cortex. *J Neurochem* 1996;67:2471–2477.
120. Pascale A, Govoni S, Battaini F. Age-related alteration of PKC, a key enzyme in memory processes: Physiological and pathological examples. *Mol Neurobiol* 1998;16:49–62.
121. Paul SM, Purdy RH. Neuroactive steroids. *FASEB J* 1992;6:2311–2322.
122. Plassart-Schiess E, Baulieu EE. Neurosteroids: Recent findings. *Brain Res Brain Res Rev* 2001;37: 133–140.
123. Racchi M, Govoni S, Solerte SB, Galli CL, Corsini E. Dehydroepiandrosterone and the relationship with aging and memory: A possible link with protein kinase C functional machinery. *Brain Res Rev* 2001;37: 287–293.
124. Rajkowski KM, Robel P, Baulieu EE. Hydroxysteroid sulfotransferase activity in the rat brain and liver as a function of age and sex. *Steroids* 1997;62:427–436.
125. Ravaglia G, Forti P, Maioli F, et al. Dehydroepiandrosterone sulphate and dementia. *Arch Gerontol Geriatr* 1998;Suppl 6:423–426.
126. Reddy DS, Kulkarni SK. The effects of neurosteroids on acquisition and retention of a modified passive-avoidance learning task in mice. *Brain Res* 1998;791:108–116.
127. Rison RA, Stanton PK. Long-term potentiation and *N*-methyl-D-aspartate receptors: foundations of memory and neurologic disease? *Neurosci Biobehav Rev* 1995;19:533–552.
128. Roberts E, Bologa L, Flood JF, Smith GE. Effects of dehydroepiandrosterone and its sulfate on brain tissue in culture and on memory in mice. *Brain Res* 1987;406:357–62.
129. Ron D, Chen CH, Caldwell J, Jamieson L, Orr E, Mochly-Rosen D. Cloning of an intracellular receptor for protein kinase C: A homolog of the beta subunit of G proteins. *Proc Natl Acad Sci USA* 1994;91:839–843.
130. Ron D, Jiang Z, Yao L, Vagts A, Diamond I, Gordon A. Coordinated movement of RACK-1 with activated beta II PKC. *J Biol Chem* 1999;274:27039–27046.
131. Rupprecht R, Holsboer F. Neuropsychopharmacological properties of neuroactive steroids. *Steroids* 1999; 64:83–91.
132. Salido EC, Li XM, Yen PH, Martin N, Mohandas TK, Shapiro LJ. Cloning and expression of the mouse pseudoautosomal steroid sulphatase gene (Sts). *Nat Genet* 1996;13:83–86.
133. Schumacher M, Akwa Y, Guennoun R, et al. Steroid synthesis and metabolism in the nervous system: Trophic and protective effects. *J Neurocytol* 2000;29:307–326.
134. Schumacher M, Guennoun R, Mercier G, et al. Progesterone synthesis and myelin formation in peripheral nerves. *Brain Res Brain Res Rev* 2001;37:343–59.
135. Seeman TE, Robbins RJ. Aging and hypothalamic-pituitary-adrenal response to challenge in humans. *Endocrinol Rev* 1994;15:233–260.
136. Senda T, Matsuno K, Okamoto K, Kobayashi T, Nakata K, Mita S. Ameliorating effect of SA4503, a novel sigma 1 receptor agonist, on memory impairments induced by cholinergic dysfunction in rats. *Eur J Pharmacol* 1996;315:1–10.
137. Schneider LS, Hinsey M, Lyness S. Plasma dehydroepiandrosterone sulfate in Alzheimer's disease. *Biol Psychiatry* 1992;31:205–208.
138. Stoffel-Wagner B. Neurosteroid metabolism in the human brain. *Eur J Endocrinol* 2001;145:669–679.
139. Stromstedt M, Waterman MR. Messenger RNAs encoding steroidogenic enzymes are expressed in rodent brain. *Brain Res Mol Brain Res* 1995;34:75–88.
140. Strott CA. Steroid sulfotransferases. *Endocrinol Rev* 1996;17:670–697.
141. Su TP. Delineating biochemical and functional properties of sigma receptors: Emerging concepts. *Crit Rev Neurobiol* 1993;7:187–203.
142. Su TP, Shukla K, Gund T. Steroid binding at sigma receptors: CNS and immunological implications. *Ciba Found Symp* 1990;153:107–113; discussion 113–116.
143. Su TP. Delineating biochemical and functional properties of sigma receptors: Emerging concepts. *Crit Rev Neurobiol* 1993;7:187–203.
144. Sunderland TS, Merril CR, Harrington MG, et al. Reduced plasma dehydroepiandrosterone concentrations in Alzheimer's disease. *Lancet* 1989;2:570.

145. Sui M, Yamazaki T, Kominami S, Tsutsui K. Avian neurosteroids. II. Localization of a cytochrome P450scc-like substance in the quail brain. *Brain Res* 1995;678:10–20.
146. Takase M, Ukena K, Yamazaki T, Kominami S, Tsutsui K. Pregnenolone, pregnenolone sulfate, and cytochrome P450 side-chain cleavage enzyme in the amphibian brain and their seasonal changes. *Endocrinology* 1999;140:1936–1944.
147. Tanaka C, Nishizuka Y. The protein kinase C family for neuronal signaling. *Annu Rev Neurosci* 1994;17: 551–567.
148. Tsutsui K, Yamazaki T. Avian neurosteroids. I. Pregnenolone biosynthesis in the quail brain. *Brain Res* 1995;678:1–9.
149. Ukena K, Usui M, Kohchi C, Tsutsui K. Cytochrome P450 side-chain cleavage enzyme in cerebellar Purkinje neuron and its neonatal change in rats. *Endocrinology* 1998;139:137–147.
150. Urani A, Privat A, Maurice T. The modulation by neurosteroids of the scopolamine-induced learning impairment in mice involves an interaction with sigma1 (sigma1) receptors. *Brain Res* 1998;799:64–77.
151. Vallee M, Mayo W, Darnaudery M, et al. Neurosteroids: Deficient cognitive performance in aged rats depends on low pregnenolone sulfate levels in the hippocampus. *Proc Natl Acad Sci USA* 1997;94: 14865–14870.
152. van Niekerk JK, Huppert FA, Herbert J. Salivary cortisol and DHEA: Association with measures of cognition and well-being in normal older men, and effects of three months of DHEA supplementation. *Psychoneuroendocrinology* 2001;26:591–612.
153. Valenti G, Banchini A, Denti L, Maggio M, Ceresini G, Ceda GP. Acute oral administration of dehydroepiandrosterone in male subjects: Effect of age on bioavailability, sulfoconjugation and bioconversion in other steroids. *J Endocrinol Invest* 1999;22(10,Suppl):24–28.
154. Vermeulen A. Dehydroepiandrosterone sulfate and aging. *Ann NY Acad Sci* 1995;774:121–127.
155. Walker JM, Bowen WD, Walker FO, Matsumoto RR, De Costa B, Rice KC. Sigma receptors: Biology and function. *Pharmacol Rev* 1990;42:355–402.
156. Watzka M, Bidlingmaier F, Schramm J, Klingmuller D, Stoffel-Wagner B. Sex- and age-specific differences in human brain CYP11A1 mRNA expression. *J Neuroendocrinol* 1999;11:901–5.
157. Webb EC. *Enzyme nomenclature*. New York: Academic Press, 1992:299–303.
158. Wichmann U, Wichmann G, Krause W. Serum levels of testosterone precursors, testosterone and estradiol in 10 animal species. *Exp Clin Endocrinol* 1984;83:283–290.
159. Wolf OT, Neumann O, Hellhammer DH, et al. Effects of a two-week physiological dehydroepiandrosterone substitution on cognitive performance and well-being in healthy elderly women and men. *Clin Endocrinol Metab* 1997;82:2363–2367.
160. Wolf OT, Koster B, Kirschbaum C, et al. A single administration of dehydroepiandrosterone does not enhance memory performance in young healthy adults, but immediately reduces cortisol levels. *Biol Psychiatry* 1997;42:845–848.
161. Wolf OT, Naumann E, Hellhammer DH, Kirschbaum C. Effects of dehydroepiandrosterone replacement in elderly men on event-related potentials, memory, and well-being. *J Gerontol A Biol Sci Med Sci* 1998;53: M385–M390.
162. Wolf OT, Kudielka BM, Hellhammer DH, Hellhammer J, Kirschbaum C. Opposing effects of DHEA replacement in elderly subjects on declarative memory and attention after exposure to a laboratory stressor. *Psychoneuroendocrinology* 1998;23:617–629.
163. Wolf OT, Kirschbaum C. Actions of dehydroepiandrosterone and its sulfate in the central nervous system: Effects on cognition and emotion in animals and humans. *Brain Res Brain Res Rev* 1999;30:264–288.
164. Wolkowitz OM, Reus VI, Roberts E, et al. Antidepressant and cognition-enhancing effects of DHEA in major depression. *Ann NY Acad Sci* 1995;774:337–339.
165. Wolkowitz OM, Reus VI, Roberts E, et al. Dehydroepiandrosterone (DHEA) treatment of depression. *Biol Psychiatry* 1997;41:311–318.
166. Wolkowitz OM, Reus VI, Keebler A, et al. Double-blind treatment of major depression with dehydroepiandrosterone. *Am J Psychiatry* 1999;156:646–649.
167. Yaffe K, Ettinger B, Pressman A, et al. Neuropsychiatric function and dehydroepiandrosterone sulfate in elderly women: A prospective study. *Biol Psychiatry* 1998;43:694–700.
168. Yanase T, Fukahori M, Taniguchi S, et al. Serum dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) in Alzheimer's disease and in cerebrovascular dementia. *Endocrinol J* 1996;43:119–123.
169. Zhu WJ, Vicini S. Neurosteroid prolongs $GABA_A$ channel deactivation by altering kinetics of desensitized states. *J Neurosci* 1997;17:4022–4031.
170. Zwain IH, Yen SS. Neurosteroidogenesis in astrocytes, oligodendrocytes, and neurons of cerebral cortex of rat brain. *Endocrinology* 1999;140:3843–3852.
171. Zwain IH, Yen SS. Dehydroepiandrosterone: Biosynthesis and metabolism in the brain. *Endocrinology* 1999;140:880–887.