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BIOSYNTHESIS AND REGULATION OF DEHYDROEPIANDROSTERONE (DHEA) IN BRAIN

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RESUM

Els esteroides sintetitzats en el sistema nerviós s'han anomenat *neuroesteroides* perquè es creu que estan dirigits a actuar exclusivament en les cèl·lules del cervell. Utilitzant com a model les vies esteroidogèniques definides en els teixits endocrins perifèrics, s'ha demostrat que les cèl·lules glials del cervell poden convertir el colesterol en pregnenolona, que és el precursor d'una sèrie d'esteroides moduladors de les funcions neuronals, com és la dihidroepiandrosterona (DHEA), un dels esteroides neuroactius principals sintetitzats en el cervell. Nogensmenys, les dades obtingudes fins avui no aporten elements clau de l'existència d'aquesta via en el cervell, tals com l'activitat 17α-hidroxilasa/17-20-liasa citocrom P450 (CYP17) responsable de la formació de DHEA en els teixits perifèrics. Això suggereix l'existència d'una via diferent, específica de cèl·lula cerebral, per a la síntesi de DHEA. El present manuscrit vol revisar els esforços per explorar i caracteritzar el mecanisme de síntesi i regulació del neuroesteroide DHEA. El nostre objectiu a llarg termini és comprendre les bases neuroquímiques de la biosíntesi esferoidal en el cervell. Durant els darrers anys, hem aportat evidència de l'existència d'una via alternativa de la biosíntesi de DHEA nova, específica de cervell i independent de CYP17. Tanmateix, hem demostrat que la formació de la DHEA en el cervell està regulada per l'estrès oxidatiu, i desencadenada pel ferro i el pèptid β-amiloide tant *in vitro* com *in vivo*. En conclusió, aquestes dades suggereixen l'existència d'una via específica cerebral per a la biosíntesi de DHEA.

Paraules clau: neuroesteroides, colesterol, andrògens, CYP17, neuropatologia.

SUMMARY

Steroids synthesized in the nervous system were termed *neurosteroids* because they are believed to be targeted exclusively to brain cells. Using as a model the steroidogenic pathway defined in peripheral endocrine tissues, it has been shown that brain glial cells can convert cholesterol to pregnenolone, precursor of a number of steroid modulators of neuronal func-

tions including dehydroepiandrosterone (DHEA), one of the main neuroactive steroids synthesized in brain. However, the data presented up to date did not find key elements of this pathway in brain, such as the 17α-hydroxylase/17,20-lyase cytochrome P450 (CYP17) activity responsible for DHEA formation in peripheral tissues, suggesting the presence of distinct, brain (cell)-specific, pathway for DHEA biosynthesis. The present manuscript reviews our efforts to explore and characterize the mechanism of synthesis and regulation of the neurosteroid DHEA. Our long-term goal is to understand the neurochemical basis of steroid biosynthesis in brain. During the past years we provided evidence for a novel, brain cell-specific CYP17-independent alternative pathway for DHEA biosynthesis. Moreover, we demonstrated that brain DHEA formation is regulated by oxidative stress, and triggered by iron and β-amyloid peptide both *in vitro* and*in vivo*. In conclusion, these data suggest that there is a brain-specific DHEA biosynthetic pathway.

Keywords: neurosteroids, cholesterol, androgens, CYP17, neuropathology.

It is now well established that steroid hormones act by regulating gene expression, inducing cellular processes such as growth and differentiation in steroid-sensitive tissues. The genomic effects of steroids are mediated through proteins members of the superfamily of steroid hormone receptors, a group of intracellular transcription factors (Beato, 1989). Steroids also have rapid, non-genomic effects, particularly in the brain, that were first observed in the 1940s (Selye, 1941; Selye, 1942). These actions initially involved anesthetic metabolites of progesterone, but have since been expanded to include a large number of steroid compounds. These non-genomic activities are characterized by extremely rapid effects, lasting from milliseconds to minutes, and do not require interaction with steroid hormone receptors (Orchinik *et al.*, 1993). In the CNS, these effects are thought to involve steroid modulation of membrane-bound neurotransmitter receptors (Majewska, 1987; Lambert *et al.*, 1995), including the $GABA_A$ receptor complex and the NMDA class of glutamate receptors (Mellon, 1994). Neuroactive steroids have been intensively studied in recent years, due to their great appeal as potential drugs for treatment of a number of neuropathological and clinical conditions. Because of their lipophilic structure, steroids can easily diffuse across the blood-brain barrier if given peripherally, thereby bypassing the issues of drug delivery from the circulation across the blood-brain barrier and into the brain. Furthermore, the amounts of steroid needed to induce changes in neural activity are extremely low, typically in the nanomolar range. The term *neurosteroids* was initially used by Baulieu and coworkers (Baulieu *et al.*, 1990) to designate the steroid hormone, DHEA and pregnenolone and their sulfated forms, which could be measured in the brain at concentrations independent of the known peripheral sources, such as the adrenal glands or the gonads (Corpechot *et al.*, 1981; Corpechot *et al.*, 1983).

DHEA is a naturally occurring steroid produced by the adrenal cortex in humans and is the most abundant steroid in the blood under normal conditions (Kroboth *et al.*, 1999). In adult humans, most DHEA secreted by the adrenal glands is released as the sulfated form (DHEA-S). Together, DHEA and DHEA-S are the precursors for ~50% of androgens produced in adult men, ~75% of estrogen in premenopausal women, and 100% of estrogen produced in postmenopausal women. Under normal conditions, DHEA is secreted with cortisol in response to ACTH stimulation of the adrenal cortex. There is some debate as to whether the function of DHEA is solely as

a precursor for androgen biosynthesis or if it has some biological activity of its own. Because there is no known intracellular steroid receptor for DHEA it seems likely that the majority of its actions are due to its conversion to estrogen and testosterone in target tissues. Importantly, DHEA production is age-dependent, with adrenal production peaking at about age 25 and declining to 15-20% of peak levels by age 80 (Baulieu *et al.*, 1998).

DHEA was first isolated in the brain in 1981 (Corpechot *et al.*, 1981), and shown to be present in the brain at higher levels than in the periphery (Corpechot *et al.*, 1981; Kroboth *et al.*, 1999). Furthermore, brain DHEA levels seem to be independent of peripheral steroid producing tissues (Corpechot *et al.*, 1981, 1993; Baulieu *et al.*, 1998), and will persist for up to two weeks following the removal of the adrenal glands and gonads in rats. This suggests either a sequestration of DHEA in the brain, or *de novo* synthesis of DHEA within the brain itself. In recent years, much interest has been directed towards DHEA in the brain and the role of DHEA in aging (Baulieu, 1996). DHEA is considered to be a neuroactive steroid, and as such, can modulate neuronal excitability through a number of different mechanisms (Baulieu *et al.*, 1998). DHEA has been shown to enhance memory in male mice (Roberts *et al.*, 1987; Flood *et al.*, 1992) and long-term potentiation in the hippocampus (Yoo *et al.*, 1996). DHEA can potentiate neuronal NMDA responses via sigma receptors (Bergeron *et al.*, 1996), which may account for some of its memory-enhancing effects. There is also some evidence that DHEA may be important in differentiation of glia and neurons during development (Roberts *et al.*, 1987) and in cortical organization (Compagnone *et al.*, 1998). There has also been a great deal of research trying to link DHEA with cognitive function associated with various pathologies, for a number of reasons. Age-related decrease in DHEA is one of the most striking changes in endocrine function over one's lifetime (Parker, 1999). DHEA, as well as other neurosteroids, may be involved in modulating learning and memory (Roberts *et al.*, 1987; Flood *et al.*, 1992; Yoo *et al.*, 1996) although there is not yet convincing evidence that DHEA is involved in human age-related cognitive decline or dementia (Guazzo *et al.*, 1996; Berr *et al.*, 1996; Wolf *et al.*, 1997*a*, 1997*b*). Ultimately, despite the huge public interest and use, the role of DHEA in the brain is, at this time, still unknown.

BIOSYNTHESIS OF DHEA IN THE PERIPHERY

All steroid hormones are derived from cholesterol. The initial step in steroid biosynthesis is the metabolism of cholesterol to pregnenolone, a process that occurs on the inner mitochondrial membrane, and is catalyzed by the cytochrome P450 side chain cleavage enzyme (CYP11A1). The rate-limiting step in steroid biosynthesis is the transport of cholesterol from the cytosol to the inner mitochondrial membrane, a process that is mediated by a complex of proteins on the cytoplasmic side of the outer mitochondrial membrane (Papadopoulos, 1993; Hall, 1994). DHEA is synthesized in the microsomal compartment of cells of adrenal cortex (as well as other steroidogenic cell types) by the metabolism of pregnenolone via the cytochrome P450 17α-hydroxylase/17,20-lyase (CYP17). The expression of the CYP17 enzyme mRNA is regulated by ACTH and LH and their second messenger cAMP (Whitlock, 1986). Further PCR analysis has demonstrated two products amplified from rat adrenal glands and brain, but only one product in testis (Sanne *et al.*, 1995), suggesting that alternative, tissue-specific, splice variants of the CYP17 enzyme exist. The enzyme activity can be also induced by pituitary hormones and cAMP (Whitlock, 1986) and although the enzyme is active on its own, high levels of

17,20-lyase activity require the presence of cytochrome P450 oxidoreductase as an electron donor (Sanne *et al.*, 1995). In the non-human primate adrenal gland, CYP17 is expressed in the zona fasciculata, where it is involved in the production of glucocorticoids, and in the zona reticularis, where it mediates androgen production (Mesiano *et al.*, 1993). In the human adrenal, DHEA is present in the zona fasciculate/reticularis (Miller *et al.*, 1997; Parker, 1999). The development of compounds affecting CYP17 activity allowed to better examine the role of the enzyme in *in vitro* and *in vivo* settings. CYP17 activity can be inhibited by SU 10603, a pyridine derivative (LaCagnin *et al.*, 1989) or the imidazole fungicide ketoconazole (Albertson *et al.*, 1988).

In the rat, where the majority of studies on DHEA synthesis and its neuroactive effects have been done, CYP17 activity and protein expression are limited to the theca cells of the ovary and the Leydig cells of the testis, both primary sources of estrogen and testosterone respectively (Berr *et al.*, 1996; LeGoascogne *et al.*, 1991). Although CYP17 mRNA is present in the rat adrenal (Stromstedt *et al.*, 1995) and embryonic rat central nervous system (Compagnone *et al.*, 1995) no CYP17 immunoreactivity and bioactivity were detected in either the adrenal cortex (Berr *et al.*, 1996) or the neonatal and adult rat brain (LeGoascogne *et al.*, 1987; Baulieu *et al.*, 1998; Cascio *et al.*, 2001). These data indicate that the presence of an mRNA transcript does not necessarily reflect the expression of a biologically active protein. This may explain many studies showing that rats do not make high levels of peripheral DHEA (Hall, 1985), and is intriguing in light of the high levels of DHEA present in brain (Baulieu *et al.*, 1998).

BIOSYNTHESIS OF DHEA IN THE CENTRAL NERVOUS SYSTEM

An issue in characterizing the physiological role of neurosteroids has been determining their source. Two possibilities have been considered: diffusion of steroids made in peripheral steroidogenic tissues, such as the gonads and adrenals, across the blood-brain barrier, or *de novo* steroid biosynthesis within the brain itself. Baulieu and colleagues demonstrated that the levels of neurosteroids in the rat brain are very high, and persist for up to two weeks after the removal of peripheral steroidogenic organs (Corpechot *et al.*, 1981; Corpechot *et al.*, 1983; Akwa *et al.*, 1991; Corpechot *et al.*, 1993; Baulieu *et al.*, 1998). In search of *de novo* synthesis of steroids in brain, the assumption was then made that steroidogenesis in brain will be the same as that described in classical peripheral steroidogenic tissues, such as adrenal and gonads.

In peripheral steroid biosynthesis, steroidogenesis begins with the transport of the precursor cholesterol from intracellular stores across the outer mitochondrial membrane to the inner mitochondrial membrane, where CYP11A1 is located (Hall, 1994; Jefcoate, 2002). This process is facilitated by two proteins, the peripheral-type benzodiazepine receptor (PBR; Papadopoulos, 1993; Papadopoulos *et al.*, 1997) and the steroidogenic acute regulatory protein (StAR; Stocco *et al.*, 1996). PBR was found to be abundant in the glial cell of the CNS (Papadopoulos, 1993; Papadopoulos *et al.*, 2005) and PBR drug ligands were shown to stimulate pregnenolone formation in vitro and in vivo (Papadopoulos *et al.*, 1992; Costa *et al.*, 1994; Papadopoulos *et al.*, 2005). StAR was also found to be present in the CNS, although in limited amounts (King *et al.*, 2002); its function in neurosteroidogenesis is under investigation.

As noted above, cholesterol in mitochondria is metabolized to pregnenolone by CYP11A1. The localization of CYP11A1 to the white matter of the rat brain was established by immunocytochemistry (LeGoascogne *et al.*, 1987), suggesting that oligodendrocytes are a source of neurosteroids in the brain (LeGoas-

cogne *et al.*, 1987; Robel *et al.*, 1995). Primary cultures of mixed glia (Hu *et al.*, 1987, 1989; Jung-Testas *et al.*, 1989; Robel *et al.*, 1995) and gliomas (Guarneri *et al.*, 1992; Papadopoulos *et al.*, 1992) were then shown to metabolize cholesterol to pregnenolone and progesterone. Furthermore, mRNA and protein for the CYP11A1 and other steroid metabolizing enzymes have been found in specific brain areas and cell types (Jung-Testas *et al.*, 1989; Mellon *et al.*, 1992; Stromstedt *et al.*, 1995; Kohchi *et al.*, 1998). In peripheral tissues, pregnenolone is subsequently metabolized to various steroid products. Indeed, the conversion of pregnenolone to progesterone in rat glial cultures (Hu *et al.*, 1987; Hu *et al.*, 1989; Jung-Testas *et al.*, 1989; Robel *et al.*, 1995) and the production of progesterone and 5α-reduced metabolites of progesterone by rat brain (Jung-Testas *et al.*, 1989; Melcangi *et al.*, 1994) have been demonstrated. Interestingly, these metabolites were shown to be positive allosteric modulators of the $GABA_A$ receptor complex (Majewska, 1987; Lambert *et al.*, 1995).

Despite these observations indicating that steroids in brain may indeed be formed via the same enzymatic pathways as those described in adrenals and gonads, there is controversy about the mechanism of DHEA synthesis. DHEA, the first neurosteroid described is a major neuroactive steroid, and constitutes a main portion of the neurosteroids found in the rat brain (Corpechot *et al.*, 1981; Baulieu *et al.*, 1998). However, convincing evidence for CYP17 expression and activity in the brain has been more difficult to find. First, conflicting reports exist on the presence of CYP17 mRNA transcripts in the CNS. Although CYP17 mRNA has been found by *in situ* hybridization to be present during rat embryonic development (Compagnone *et al.*, 1995), there is no solid evidence for CYP17 mRNA in the adult (Mellon *et al.*, 1993; Stromstedt *et al.*, 1995; Kohchi *et al.*, 1998). A similar contradiction has been shown in the rat adrenal, a tissue devoid of CYP17 activity (Hall, 1994) where the CYP17 mRNA is present (Stromstedt *et al.*, 1995). Moreover, there has been no demonstration on the presence of either CYP17 protein expression (LeGoascogne *et al.*, 1991; Cascio *et al.*, 1999, 2001) or enzyme activity (Akwa *et al.*, 1991; Baulieu *et al.*, 1998; Cascio *et al.*, 1999, 2001) in adult rat brain. Detailed studies using incubations of the precursor pregnenolone with brain slices, homogenates, and microsomes, with primary cultures of mixed glia cells, or with astrocytes and neurons of rat and mouse embryos failed to demonstrate any CYP17 activity (Baulieu *et al.*, 1998; Cascio *et al.*, 2001). Interestingly, in other species such as in the songbird zebra finch, CYP17 activity is also absent from brain (Schlinger *et al.*, 1999).

In contrast to these findings, Hojo (Hojo *et al.*, 2004) recently reported that the adult male rat hippocampus synthesizes estradiol from pregnenolone by CYP17 and cytochrome P450 aromatase localized in neurons. CYP17 immunoreactivity was found in pyramidal neurons of CA1-CA3 regions of hippocampus and in the granule cells in the dentate gyrus. CYP17 expression was confirmed by western blot and RT-PCR. Moreover, the authors reported that hippocampal cubic slices converted radiolabeled pregnenolone to estradiol through DHEA and testosterone (Hojo *et al.*, 2004). It is evident that such findings stirred an enormous interest since other laboratories have been unable to demonstrate such data in adult brain slices, cells or microsomes (Compagnone *et al.*, 1995; Baulieu *et al.*, 1998; Cascio *et al.*, 1999, 2001). The most critical finding was the demonstration for the first time of CYP17 activity. However, a closer analysis of the data presented demonstrated that considering the expression level of CYP17 in hippocampus, which is at least 50-100-fold less than in testis, and the 1 to 10,000 transformation rate of radiolabeled pregnenolone achieved in the experiments presented by the authors, the reported activity could not account for the formation of

0.42 ng DHEA per gram brain tissue (Corpechot *et al.*, 1981).

Taken together these findings suggest that DHEA is formed in brain via a CYP17-independent pathway. This possibility is also supported by clinical findings from patients with CYP17 defects (Yanase, 1995; Zachmann, 1995). Deficiency in CYP17 in humans is not lethal (Yanase, 1995; Zachmann, 1995). Because of the importance of CYP17 in testosterone and estrogen formation (Corpechot *et al.*, 1993), male pseudohermaphroditism or absence of pubertal development has been described for the CYP17-deficient humans (Yanase, 1995; Zachmann, 1995). However, no patient has shown neurological or mental problems. Considering that DHEA can modulate neuronal excitability (Baulieu *et al*., 1998), enhance memory in male mice (Roberts *et al.*, 1987; Flood *et al.*, 1992), enhance long-term potentiation in the hippocampus (Yoo *et al.*, 1996), potentiate neuronal NMDA responses (Bergeron *et al.*, 1996) and foremost being important in differentiation of glia and neurons during development (Roberts *et al.*, 1987) and in cortical organization (Compagnone *et al.*, 1998) one wonders how the brain could function without DHEA. The only logical explanation would be that in humans deficient in CYP17 brain DHEA is formed via a CYP17-independent mechanism.

These data leave us with a crucial question: how the brain is able to synthesize high levels of DHEA if the CYP17 enzyme is absent, present are extremely low levels or inactive? Many concepts in science are based on dogmas, such as that of CYP17 being indispensable for DHEA synthesis. Thus, to answer the question one has to go against that dogma. This dogma is based exclusively on *in vitro* data, because there is not any *in vivo* data available since there are no specific/exclusive inhibitors for CYP17 that do not inhibit other cytochrome P450s and CYP17 knock-out animal models. This dogma was first challenged openly by Dr. S. Lieberman in a review (Lieberman *et al.*, 2001), where he even questioned the role of CYP17 in the biosynthesis of gonadal steroids. In 1994, Prasad *et al.* reported the formation of DHEA in organic extracts of rat brain treated with different oxidizing and reducing agents (Prasad *et al.*, 1994). This paper proposed the existence of alternative precursors for neurosteroids in the brain that could be metabolized under appropriate oxidative conditions. This was the first indication that there could be a novel CYP17-independent mechanism for steroid biosynthesis in the brain. Since the Prasad et al. study examined this alternative pathway in an artificial and non-physiological system, we have looked for it in living cells. Studies in our laboratory using Fe^{++} ions as a redox tool demonstrated the presence of an alternative pathway for DHEA synthesis in rat C6-2B glioma cells, which lack both the CYP17 mRNA and protein (Cascio *et al.*, 1998, 1999).

Adding $FeSO_4$ to C6 cells increased the synthesis of both pregnenolone and DHEA. Even in the presence of aminoglutethimide, an inhibitor of CYP11A1, $FeSO_4$ increased the synthesis of both steroids, indicating that the Fe^{2+}-sensitive process does not involve CYP11A1. Likewise the $FeSO_4$-induced formation of DHEA was not blocked by the CYP17 inhibitor, SU-10603. These results suggest that the $FeSO_4$-induced synthesis of DHEA as well as of pregnenolone in C6 cells may be due to the fragmentation of *in situ*-formed tertiary hydroperoxides. It is likely, however, that the effect of the Fe^{2+} is not limited to this reaction. When exogenous pregnenolone was added to C6 microsomes, along with $FeSO_4$, the amount of DHEA formed was greater than control values, indicating that Fe^{2+} facilitated the conversion of pregnenolone to DHEA. Unlike the constituents that are converted by Fe^{2+} to pregnenolone, the precursor of DHEA in C6 cells is not soluble in a 1:1 mixture of ether and ethylacetate. In search of the mechanism of DHEA formation, we examined the nature of its pu-

tative precursor using successive treatments with reducing and oxidizing agents. C6 cells were treated with KI (to reduce peroxy compounds to their corresponding alcohols), then with $NaBH_4$ (to reduce ketones, including preexisting DHEA, to alcohols) and finally with $NaIO_4$ (to oxidize glycols to carbonyl products). Treatment of C6 cells with KI, $NaBH_4$ or HIO_4 resulted in an increase in DHEA synthesis in comparison to that of untreated cells, suggesting that a C17,C20-glycol was the proximal precursor of DHEA. The most obvious steroid glycol is pregn-5-ene-3β,17,20-triol, in which case the initial peroxy precursor is 17-hydroperoxide of pregnenolone, which can be converted to DHEA through a process of ketone formation by β-fragmentation (Cascio *et al.*, 1998). However, the evidence presented does not exclude other peroxy precursors, particularly one derived from a sterol, like cholesterol, such as the 17,20-dihydroxycholesterol. In control dishes DHEA was reduced to the diol by $NaBH_4$. From this it seems clear that a precursor of the DHEA produced in C6 cells is a steroid where both C-17 and C-20 are oxygenated (Cascio *et al.*, 1998).

This alternative pathway was subsequently found in microsomes isolated from neonatal rat brain cortex and primary cultures of rat glial cells (Cascio *et al.*, 2001), but not in other tissues examined. In contrast to the C6-2B glioma cells, CYP17 mRNA and protein were found in immature rat oligodendrocyte precursors and mature oligodendrocytes. In the presence of substrate (pregnenolone), these cells made DHEA. However, addition of Fe^{++} increased DHEA formation in these cells. DHEA formation in the presence of pregnenolone and Fe^{++} was not inhibited by the CYP17 inhibitors SU-10603 and ketoconazole, indicating that the formation of DHEA in oligodendrocytes occurs independently of the CYP17 protein present in the cells (Cascio *et al.*, 1999; Cascio *et al.*, 2001). These data could also explain the data reported by Hojo *et al.*, (2004) because it shows that pregnenolone can be metabolized to DHEA, in a CYP17-independent manner, but in the presence of prooxidants. It is likely that during incubation of brain slices and cells such conditions might occur if oxygen levels are not carefully controlled. Isolated type I astrocytes do not express CYP17 mRNA or protein, but will respond to Fe^{++} by producing DHEA. Thus, DHEA production in both types of cells occurred independently of active CYP17. Furthermore, these results suggest that in differentiating oligodendrocytes and astrocytes, DHEA is formed via an oxidative stress-dependent alternative pathway (Cascio *et al.*, 1999). Another study also demonstrated DHEA production when cultures of isolated rat glial cells, but not brain tissue, are incubated with pregnenolone as a precursor (Zwain *et al.*, 1999). However, whether this reaction was mediated by CYP17 and the identity of the steroids formed remain to be established.

We subsequently investigated the presence of the alternative pathway in human glial cells by treating them with $FeSO_4$ and looking at DHEA production. We found that both human oligodendrocytes and astrocytes produce DHEA in response to $FeSO_4$, but not human neurons (Brown *et al.*, 2000). This pathway does not appear to be an artifact of cell culture, because treating homogenates of human brain with $FeSO_4$ also resulted in DHEA production (Brown *et al.*, 2003). Moreover, we demonstrated that DHEA formation by human cells is regulated by the levels of intracellular free radicals and can be blocked by antioxidants such as Vitamin E (Brown *et al.*, 2000). The mechanism by which we detect the alternative pathway activity, treatment with $FeSO_4$, is a harsh and non-physiologic environment, with high levels of oxidative stress. This brought up the question of whether or not this pathway is relevant *in vivo*. We addressed this question, in the case of Alzheimer's disease (AD), by treating cells in culture with β-

amyloid (Aβ) and examining samples of human AD brain for evidence of the alternative pathway. Recent data suggest that increased oxidative stress and inflammation may play a large role in the pathogenesis of AD (Markesbery, 1997). Aβ, a peptide important in the pathogenesis of AD, has been shown to cause increases in free radicals in neurons (Subbarao *et al.*, 1990; Mecocci *et al.*, 1994; Nunomura *et al.*, 1999) and glia (Brown *et al.*, 2000), directly producing hydrogen peroxide through metal ion reduction (Huang *et al.*, 1999). This suggests that Aβ may play a direct role in increasing oxidative stress in the AD brain. We examined the ability of Aβ to activate the alternative pathway in human cells in culture. We found that Aβ increases the levels of cellular reactive oxygen species and DHEA after 24 hours of treatment. Both of these increases could be blocked by co-treatment with Vitamin E, an anti-oxidant (Brown *et al.*, 2000). These data suggested that the alternative pathway for DHEA synthesis could be important in pathological conditions involving increased oxidative stress. Furthermore, in tissue samples isolated from the hippocampus, hypothalamus and frontal cortex of severe AD patients (containing high levels of Aβ), the levels of DHEA were significantly increased as compared to age-matched controls. We believe this to be representative of the increased Aβ-induced oxidative stress thought to be the hallmark of the pathogenesis of AD (Markesbery, 1997). Interestingly, CYP17 was not found in the human hippocampus by immunohistochemistry or RT-PCR followed by Southern blot analysis (Brown *et al.*, 2003). Treatment of hippocampus and hypothalamus with $FeSO_4$ caused an increase in DHEA levels in control but not in AD patients (Brown *et al.*, 2003), suggesting that the levels of the DHEA precursor in control are higher than in AD specimens. These results indicate that DHEA is formed in the AD brain by the oxidative stress-mediated metabolism of an unknown steroid precursor (probably due to increased levels of Aβ).

To test the hypothesis that there is a CYP17-indepednent pathway of DHEA formation we attempted to generate mice with a targeted deletion of *CYP17* (Liu *et al.*, 2005). If CYP17 is indeed not involved in brain DHEA formation, then we could focus our efforts on the characterization of the biochemical pathway responsible for DHEA formation in brain. Although in chimeric mice, Leydig cell CYP17 mRNA and intratesticular and circulating testosterone levels were dramatically reduced, the remaining testosterone was sufficient to support spermatogenesis as evidenced by the generation of phenotypical black C57BL/6 mice. However, male chimeras consistently failed to generate heterozygous *CYP17* mice and after five matings, chimeric mice stopped mating indicating a change in sexual behavior. These results suggested that *CYP17* deletion caused a primary phenotype (infertility) probably not due to the anticipated androgen imbalance and a secondary phenotype (change in sexual behavior) due to the androgen imbalance. Surprisingly, CYP17 mRNA was found in mature sperm, and serial analysis of gene expression identified CYP17 mRNA in other testicular germ cells. CYP17 mRNA levels were directly related to percent chimerism. Moreover, more than 50% of the sperm from high percentage chimeric mice were morphologically abnormal and half of them failed the swim test. Furthermore, 60% of swimming abnormal sperm was devoid of *CYP17*. These results suggest that YP17, in addition to its role in steroidogenesis and androgen formation, is present in germ cells where it is essential for sperm function and deletion of one allele prevents genetic transmission of mutant and wild-type alleles causing infertility followed by change in sexual behavior due to androgen imbalance (Liu *et al.*, 2005). The next step will be to examine the levels of DHEA in the brain of wild-type

and chimeric mice carrying the *CYP17* gene deletion.

In conclusion, the generated data provides evidence that CYP17 is not involved in brain DHEA formation. Considering our findings that brain DHEA formation is regulated by oxidative stress, and triggered by iron and Aβ both *in vitro* and *in vivo*, and the recent confirmation of these findings by an independent group (Maayan *et al.*, 2005), it is evident that the next steps in our studies would be to provide the final evidence for the role of CYP17 in brain DHEA synthesis by examining the levels of DHEA in the brain of CYP17 chimeric mice and identify the mechanism(s) responsible for brain DHEA synthesis and regulation.

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REFERENCES

Akwa, Y.; Young, J.; Kabbadj, K.; Sancho, M. J.; Zucman, D.; Vourc'h, C.; Jung-Testas, I.; Hu, Z. Y.; LeGoascogne, C.; Jo, D. H.; Corpechot, C.; Simon, P.; Baulieu, E. E.; Robel, P. (1991). «Neurosteroids: Biosynthesis, metabolism and function of pregnenolone and dehydroepiandrosterone in the brain». *J. Steroid Biochem. Mol. Biol.*, 40: 71-81.

Albertson, B. D.; Frederick, K. L.; Maronian, N. C.; Feuillan, P.; Schorer, S.; Dunn, J. F.; Loriaux, D. L. (1988). «The effect of ketoconazole on steroidogenesis: Leydig cell enzyme activity in vitro». *Res. Commun. Chem. Pathol. Pharmacol.*, 61: 17-26.

Baulieu, E. E. (1996). «Dehydroepiandrosterone (DHEA): A fountain of youth?». *J. Clin. Endo. Metab.*, 81: 3147-3151.

Baulieu, E. E.; Robel, P. (1990). «Neurosteroids: a new brain function?». *J. Steroid Biochem. Mol. Biol*, 37: 395-403.

— (1998). «Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) as neuroactive neurosteroids». *Proc. Natl. Acad. Sci. USA*, 95: 4089-4091.

Beato, M. (1989). «Gene regulation by steroid hormones». *Cell*, 56: 335-344.

Bergeron, R.; Montigny, C. D.; Debonnel, G. (1996). «Potentiation of neuronal NMDA response induced by dehydroepiandrosterone and its suppression by progesterone: effects mediated by sigma receptors». *J. Neurosci.*, 16: 1193-1202.

Berr, C.; Lafont, S.; Debuire, B.; Dartigues, J. F.; Baulieu, E. E. (1996). «Relationships of dehydroepiandrosterone sulfate in the elderly with functional, psychological, and mental status, and short-term mortality: A French community-based study». *Proc. Natl. Acad. Sci. USA*, 93: 13410-13415.

Brown, R. C.; Cascio, C.; Papadopoulos, V. (2000). «Pathways of neurosteroid biosynthesis in cell lines from human brain: regulation of dehydroepiandrosterone formation by oxidative stress and beta-amyloid peptide». *J. Neurochem.*, 74: 847-859.

Brown, R. C.; Han, Z.; Cascio, C.; Papadopoulos, V. (2003). «Oxidative stress mediated dehydroepiandrosterone formation in Alzheimer's disease pathology». *Neurobiol. Aging*, 24: 57-65.

Cascio, C.; Brown, R. C.; Hales, D. B.; Papadopoulos, V. (2001). «Pathways of dehydroepiandrosterone formation in rat brain glia». *J. Steroid Biochem. Mol. Biol.*, 75: 77-186.

Cascio, C.; Guarneri, P.; Li, H.; Brown, R. C.; Amri, H.; Boujrad, N.; Kotoula, M.; Vidic, B.; Drieu, K.; Papadopoulos, V. (1999). «Peripheral-type benzodiazepine receptor. Role in the regulation of steroid and neurosteroid biosynthesis». In: Baulieu, E. E. [ed.]. *Neurosteroids:* A *new regulatory function in the central nervous system. Contemporary endocrinology*. The Humana Press Inc, p. 75-96.

Cascio, C.; Prasad, V. V. K.; Lin, Y. Y.; Lieberman, S.; Papadopoulos, V. (1998). «Detection of P450c17-independent pathways for dehydroepiandrosterone (DHEA) biosynthesis in brain glial tumor cells». *Proc. Natl. Acad. Sci. USA*, 95: 2862-2867.

Compagnone, N. A.; Bulfone, A.; Rubenstein, J. L. R.; Mellon, S. H. (1995). «Steroidogenic enzyme P450c17 is expressed in the embryonic central nervous system». *Endocrinology*, 136: 5212-5223.

Compagnone, N. A.; Mellon, S. H. (1998). «Dehydroepiandrosterone: A potential signaling molecule for neocortical organization during development». *Proc. Natl. Acad. Sci. USA*, 95: 4678-4683.

Corpechot, C.; Robel, P.; Axelson, M.; Sjovall, J.; Baulieu, E. E. (1981). «Characterization and measurement of dehydroepiandrosterone sulfate in rat brain». *Proc. Natl. Acad. Sci. USA*, 78: 4704-4707.

Corpechot, C.; Synguelakis, M.; Talha, S.; Axelson, M.; Sjovall, J.; Vihko, R.; Baulieu, E. E.; Robel, P. (1983). «Pregnenolone and its sulfate ester in the rat brain». *Brain Res.*, 270: 119-125.

Corpechot, C.; Young, J.; Calvel, M.; Wehrey, C.; Veltz, J. N.; Touyer, G.; Mouren, M.; Prasad, V. V. K.; Banner, C.; Sjovall, J.; Baulieu, E. E.; Robel, P. (1993). «Neurosteroids: 3alpha-hydroxy-5beta-pregnan-20-one and its precursors in the brain, plasma,

and steroidogenic glands of male and female rats». *Endocrinology*, 133: 1003-1009.

Costa, E.; Cheney, D. L.; Grayson, D. R.; Korneyev, A.; Longone, P.; Pani, L.; Romeo, E.; Zivkovich, E.; Guidotti, A. (1994). «Pharmacology of neurosteroid biosynthesis». *Ann. NY Acad. Sci.*, 746: 223-242.

Flood, J. F.; Morley, J. E.; Roberts, E. (1992). «Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it». *Proc. Natl. Acad. Sci. USA*, 89: 1567-1571.

Guarneri, P.; Papadopoulos, V.; Pan, B.; Costa, E. (1992). «Regulation of pregnenolone synthesis in C6-2B glioma cells by 4'-chlorodiazepam». *Proc. Natl. Acad. Sci. USA*, 89: 5118-5122.

Guazzo, E. P.; Kirkpatrick, P. J.; Goodyer, I. M.; Shiers, H. M.; Herbert, J. (1996). «Cortisol, dehydroepiandrosterone (DHEA) and DHEA sulfate in the cerebrospinal fluid of man: relation to blood levels and the effects of age». *J. Clin. Endo. Metab.*, 81: 3951-3959.

Hall, P. F. (1985). «Role of Cytochrome P-450 in the biosynthesis of steroid hormones». *Vitamin. Horm.*, 42: 315-368.

— (1994). «Cellular organization of steroidogenesis». *Int. Rev. Cytol.*, 86: 53-95.

Hojo, Y.; Hattori, T. A.; Enami, T.; Furukawa, A.; Suzuki, K.; Ishii, H. T.; Mukai, H.; Morrison, J. H.; Janssen, W. G.; Kominami, S.; Harada, N.; Kimoto, T.; Kawato, S. (2004). «Adult male rat hippocampus synthesizes estradiol from pregnenolone by cytochromes P45017alpha and P450 aromatase localized in neurons». *Proc. Natl. Acad. Sci. USA*, 101: 865-870.

Hu, Z. Y.; Bourreau, E.; Jung-Testas, I.; Robel, P.; Baulieu, E. E. (1987). «Neurosteroids: oligodendrocyte mitochondria convert cholesterol to pregnenolone». *Proc. Natl. Acad. Sci. USA*, 84: 8215-8219.

Hu, Z. Y.; Jung-Testas, I.; Robel, P.; Baulieu, E. E. (1989). «Neurosteroids: Steroidogenesis in primary cultures of rat glial cells after release of aminoglutethimide blockade». *Biochem. Biophys. Res. Comm.*, 161: 917-922.

Huang, X.; Atwood, C.; Hartshorn, M.; Multhaup, G.; Goldstein, L.; Scarpa, R.; Cuajungco, M.; Gray, D.; Lim, J.; Moir, R.; Tanzi, R.; Bush, A. (1999). «The beta-amyloid peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction». *Biochemistry*, 38: 7609-7616.

Jefcoate, C. R. (2002). «High-flux mitochondrial cholesterol trafficking, a specialized function of the adrenal cortex». *J. Clin. Invest.*, 110: 881-890.

Jung-Testas, I.; Hu, Z. Y.; Baulieu, E. E.; Robel, P. (1989). «Neurosteroids: biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells». *Endocrinology*, 125: 2083-2091.

King, S. R.; Manna, P. R.; Ishii, T.; Syapin, P. J.; Ginsberg, S. D.; Wilson, K.; Walsh, L. P.; Parker, K. L.; Stocco, D. M.; Smith, R. G.; Lamb, D. J. (2002). «An essential component in steroid synthesis, the steroidogenic acute regulatory protein, is expressed in discrete regions of the brain». *J. Neurosci.*, 22: 10613-10620.

Kohchi, C.; Ukena, K.; Tsutsui, K. (1998). «Age- and region-specific expressions of the messenger RNAs encoding for steroidogenic enzymes P450scc, P450c17 and 3β-HSD in the postnatal rat brain». *Brain Res.*, 801: 233-238.

Kroboth, P.; Salek, F.; Pittenger, A.; Fabian, T.; Frye, R. (1999). «DHEA and DHEA-S: A review». *J. Clin. Pharm.*, 39: 327-348.

LaCagnin, L.; Levitt, M.; Bergstrom, J.; Colby, H. (1989). «Inhibition of adrenocortical, mitochondrial and microsomal monooxygenases by SU-10'603, a steroid 17alpha-hydroxylase inhibitor». *J. Steroid Biochem.*, 33: 599-604.

Lambert, J. J.; Belelli, D.; Hill-Venning, C.; Peters, J. A. (1995). «Neurosteroids and $GABA_A$ receptor function». *Trends Pharm. Sci.*, 16: 295-303.

LeGoascogne, C.; Robel, P.; Gouezou, M.; Sananes, N.; Baulieu, E. E.; Waterman, M. (1987). «Neurosteroids: cytochrome P-450scc in rat brain». *Science*, 237: 1212-1215.

LeGoascogne, C.; Sananes, N.; Gouezou, M.; Takemori, S.; Kominami, S.; Baulieu, E. E.; Robel, P. (1991). «Immunoreactive cytochrome P-450(17 alpha) in rat and guinea pig gonads, adrenal glands and brain». *J. Reprod. Fertil.*, 93: 609-622.

Lieberman, S.; Warne, P. A. (2001). «17-hydroxylase: an evaluation of the present view of its catalytic role in steroidogenesis». *J. Steroid Biochem. Mol. Biol.*, 78: 299-312.

Liu, Y.; Yao, Z. X.; Bendavid, C.; Borgmeyer, C.; Han, Z.; Cavalli, L. R.; Chan, W. Y.; Folmer, J.; Zirkin, B. R.; Haddad, B. R.; Gallicano, G. I.; Papadopoulos, V. (2005). «Haploinsufficiency of cytochrome P450 17α-hydroxylase/17,20 lyase (CYP17) causes infertility in male mice». *Mol. Endocrinol.*, 19: 2380-2389.

Maayan, R.; Touati-Werner, D.; Ram, E.; Galdor, M.; Weizman, A. (2005). «Is brain dehydroepiandrosterone synthesis modulated by free radicals in mice?» *Neurosci. Lett.*, 377: 130-135.

Majewska, M. D. (1987). «Steroids and brain activity». *Biochem. Pharmaco.*, 36: 3781-3788.

Markesbery, W. R. (1997). «Oxidative stress hypothesis in Alzheimer's disease». *Free Rad. Biol. Med.*, 23: 134-147.

Mecocci, P.; MacGarvey, U.; Beal, M. (1994). «Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease». *Ann. Neurol.*, 36: 747-751.

Melcangi, R. C.; Celotti, F.; Martini, L. (1994). «Progesterone 5alpha reduction in neuronal and in different types of glial cell cultures: Type 1 and 2 astrocytes and oligodendrocytes». *Brain Res.*, 639: 202-206.

Mellon, S. H. (1994). «Neurosteroids: biochemistry, modes of action and clinical relevance». *J. Clin. Endo. Metab.*, 78: 1003-1008.

Mellon, S. H.; Deschepper, C. F. (1993). «Neurosteroid biosynthesis: genes for adrenal steroidogenic enzymes are expressed in the brain». *Brain Res.*, 629: 283-292.

Mesiano, S.; Coulter, C. L.; Jaffe, R. B. (1993). «Lo-

calization of cytochrome P450 cholesterol side-chain cleavage, cytochrome P450 17alpha-hydroxylase/17,20-lyase, and 3beta-hydroxysteroid dehydrogenase isomerase steroidogenic enzymes in human and rhesus monkey fetal adrenal glands: Reappraisal of functional zonation». *J. Clin. Endo. Metab.*, 77: 1184-1189.

Miller, W. L.; Auchus, R. J.; Geller, D. H. (1997). «The regulation of 17,20 lyase activity». *Steroids*, 62: 133-142.

Nunomura, A.; Perry, G.; Pappolla, M.; Wade, R.; Hirai, K.; Chiba, S.; Smith, M. (1999). «RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease». *J. Neurosci.*, 19: 1959-1964.

Orchinik, M.; McEwan, B. (1993). «Novel and classical actions of neuroactive steroids». *Neurotransmissions*, 9: 1-6.

Papadopoulos, V. (1993). «Peripheral-type benzodiazepine/diazepam binding inhibitor receptor: biological role in steroidogenic cell function». *Endocr. Rev.*, 14: 222-240.

Papadopoulos, V.; Amri, H.; Boujrad, N.; Cascio, C.; Culty ,M.; Garnier, M.; Hardwick, M.; Li, H.; Vidic, B.; Brown, A. S.; Reversat, J. L.; Bernassau, J. M.; Drieu, K. (1997). «Peripheral benzodiazepine receptor in cholesterol transport and steroidogenesis». *Steroids*, 62: 21-28.

Papadopoulos, V.; Guarneri, P.; Krueger, K. E.; Guidotti, A.; Costa, E. (1992). «Pregnenolone biosynthesis in C6 glioma cell mitochondria: regulation by a diazepam binding inhibitor mitochondrial receptor». *Proc. Natl. Acad. Sci. USA*, 89: 5113-5117.

Papadopoulos, V.; Lecanu, L.; Brown, R. C.; Han, Z.; Yao, Z. X. (2005). «Peripheral-type benzodiazepine receptor in neurosteroid biosynthesis, neuropathology and neurological disorders». *Neuroscience*. [In press]

Parker, C. J. (1999). «Dehydroepiandrosterone and dehydroepiandrosterone sulfate production in the human adrenal during development and aging». *Steroids*, 64: 640-647.

Prasad, V. V. K.; Vegesna, S. R.; Welch, M.; Lieberman, S. (1994). «Precursors of the neurosteroids». *Proc. Natl. Acad. Sci. USA*, 91: 3220-3223.

Robel, P.; Young, J.; Corpechot, C.; Mayo, W.; Perche, F.; Haug, M.; Simon, H.; Baulieu, E. E. (1995). «Biosynthesis and assay of neurosteroids in rats and mice: functional correlates». *J. Steroid Biochem. Mol. Biol.*, 53: 355-360.

Roberts, E.; Bologa, L.; Flood, J. F.; Smith, G. E. (1987). «Effects of dehydroepiandrosterone and its sulfate on brain tissue in culture and on memory in mice». *Brain Res.*, 406: 357-362.

Sanne, J. L.; Krueger, K. (1995). «Aberrant splicing of rat steroid 17α-hydroxylase transcripts». *Gene*, 165: 327-328.

Schlinger, B. A.; Lane, N. I.; Grisham, W.; Thompson, L. (1999). «Androgen synthesis in a songbird: a study of Cyp17 (17α-hydroxylase/C17,20-lyase) activity in the zebra finch». *Gen. Comp. Endocrinol.*, 113: 46-58.

Selye, H. (1941). «Anesthetic effects of steroid hormones». *Proc. Soc. Exp. Biol.*, 46: 116-121.

— (1942). «Correlations between the chemical structure and the pharmacological actions of the steroids». *Endocrinology*, 30: 437-453.

Stocco, D. M.; Clarkm B. J. (1996). «Regulation of the acute production of steroids in steroidogenic cells». *Endocr. Rev.*, 17: 221-244.

Stromstedt, M.; Waterman, M. R. (1995). «Messenger RNAs encoding steroidogenic enzymes are expressed in rodent brain». *Mol. Brain. Res.*, 34: 75-88.

Subbarao, K.; Richardson, J.; Ang, L. (1990). «Autopsy samples of Alzheimer's cortex show increased peroxidation in vitro». *J. Neurochem.*, 55: 205-228.

Whitlock, J. P. (1986). «The regulation of cytochrome P-450 gene expression». *Annu. Rev. Pharmacol. Toxicol.*, 26: 333-369.

Wolf, O. T.; Koster, B.; Kirschbaum, C.; Pietrowsky, R.; Kern, W.; Hellhammer, D. H.; Born, J.; Fehm, H. L. (1997*a*). «A single administration of dehydroepiandrosterone does not enhance memory performance in young healthy adults, but immediately reduces cortisol levels». *Biol. Psychiatry*, 42: 845-848.

Wolf, O. T.; Neumann, O.; Hellhammer, D. H.; Geiben, A. C.; Strasburger, C. J.; Dressendorger, R. A.; Pirke, K. M.; Kirschbaum, C. (1997*b*). «Effects of a two-week physiological dehydroepiandrosterone substitution on cognitive performance and well being in healthy elderly women and men». *J. Clin. Endo. Metab.*, 82: 2363-2367.

Yanase, T. (1995). «17alpha-hydroxylase/17,20-lyase defects». *J. Steroid Biochem. Mol. Biol.*, 53: 153-157.

Yoo, A.; Harris, J.; Dubrovsky, B. (1996). «Dose-response study of dehydroepiandrosterone sulfate on dentate gyrus long term potentiation». *Exp. Neurol.*, 137: 151-156.

Zachmann, M. (1995). «Defects in steroidogenic enzymes. Discrepancies between clinical steroid research and molecular biology results». *J. Steroid Biochem. Mol. Biol.*, 53: 159-164.

Zwain, I.; Yen, S. (1999). «Dehydroepiandrosterone: biosynthesis and metabolism in the brain». *Endocrinology*, 140: 880-887.