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NEUROSTEROIDS - A POTENTIAL THERAPEUTIC AGENT IN ALZHEIMER'S DISEASE.

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline. This review examines the potential neuroprotective roles of neurosteroids like allopregnanolone (AlloP) and 24(S)-hydroxycholesterol (24(S)-HC) in AD. Multiple preclinical studies in rodent models demonstrate the ability of AlloP to promote neurogenesis, myelination, and anti-inflammatory effects via GABA receptor modulation. Enhanced AlloP levels reduced amyloid-beta pathology in an AD mouse model. The enzyme CYP46A1 converts cholesterol to 24(S)-HC which can exit the brain. CYP46A1 upregulation appears neuroprotective in some AD models through improved cholesterol homeostasis. Analysis of human AD patient brain tissues reveals reduced AlloP levels correlating with disease progression. Overall, these neurosteroids show promise as novel AD therapeutics by targeting pathogenic mechanisms like neurodegeneration, neuro inflammation and amyloid-beta aggregation. Further research especially large-scale clinical trials are warranted to strengthen the preclinical evidence. In conclusion, neurosteroids augmentation could provide a new avenue for prevention and symptomatic management in AD. In summary, this draft abstract highlights the key findings from the research review on neuroprotective roles of allopregnanolone and 24(S)-hydroxycholesterol in Alzheimer's disease models and patients. It summarizes the major outcomes demonstrating their abilities to reduce neurodegeneration, inflammation and amyloid-beta pathology. The abstract concludes that these neurosteroids represent promising novel therapeutic candidates for Alzheimer's disease worthy of further clinical investigation. Please let me know if you would like me to modify or expand the abstract in any way.

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INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory loss and cognitive impairment. With increasing life expectancy, AD presents a major global public health challenge (statistics). However, current therapeutic options provide only symptomatic relief without stopping disease progression. The failure of numerous clinical trials targeting amyloid-beta highlights the need to explore alternative pathogenic mechanisms as treatment targets.

Neurosteroids like allopregnanolone (AlloP) and 24(S)-hydroxycholesterol (24(S)-HC) have emerged as promising neuroprotective candidates. These steroids are synthesized in the brain from cholesterol and regulate diverse processes from neurogenesis to inflammation. Modulating their levels may confer protection against the neuronal damage and cognitive decline in AD.

This review aims to critically evaluate the current preclinical and clinical evidence on the neuroprotective potential of AlloP and 24(S)-HC in AD. The key objectives are to:

1) Summarize the mechanisms of AlloP and 24(S)-HC production and their regulatory roles in the central nervous system
2) Analyze results from animal models and human studies on their ability to reduce AD pathology and symptoms
3) Identify remaining knowledge gaps and future research directions
4) Clarifying the therapeutic promise of these neurosteroids will provide crucial insights into novel pathogenic pathways and treatment opportunities for AD.

75 years of age or older make up % of the population. 10.7% of adults aged 65 or older or 1 in 9 have Alzheimer's disease. Deaths from dementia and Alzheimer's increased by 17% in 2020 as a result of COVID-19.

Emil Kraepelin (1856–1926), a German physician, made the distinction between senile and presenile dementia in 1910. In honour of his student Alois Alzheimer (1866-1915), who recognised the pathological features of presenile dementia, he was the one who gave the illness the name "Alzheimer's disease." In the brain tissue of a woman who died in 1906 from a rare mental illness, Dr. Alzheimer discovered changes. Her warning signals were unpredictability in her behaviour, memory loss, and speech issues. Once she had passed away, he examined her brain and found what are now known as amyloid plaques, which are assemblages of aberrant folds and bundles of twisted fibres (now called neurofibrillary tangles, or tau.**[1]**

NEUROPATHOLOGY OF ALZHEIMER'S DISEASE.

Positive lesions (due to accumulation), which are characterised by the accumulation of neurofibrillary tangles, amyloid plaques, dystrophic neurites, neuropil threads, and other deposits found in the brains of AD patients, are one of two types of neuropathological changes in AD that provide evidence about the disease's progression and symptoms. Moreover, there are two negative lesions (caused by losses), which are characterised by significant atrophy brought on by neuronal, neuropil, and synaptic loss. In addition, other variables like neuroinflammation, oxidative stress, and damage to cholinergic neurons might result in neurodegeneration.**[2]**

SENILE PLAQUES(SP)

The senile plaques, also known as neuritic, diffuse, dense-cored, classic, and compact type plaques, are extracellular aggregates of β-amyloid protein (Aβ).

The transmembrane amyloid precursor protein (APP) is used as the building block for the production of A deposits by proteolytic cleavage enzymes like α-secretase andγ-secretase. These enzymes break down APP into a number of amino acid fragments, resulting in the end forms Aβ40 and Aβ42, which are amino acids 43, 45, 46, 48, 49, and 51. Large, soluble amyloid fibrils that can build up to create amyloid plaques and soluble oligomers that can spread throughout the brain are two different types of Aβ monomers. Accumulation of denser plaques in the hippocampus, amygdala, and cerebral cortex can lead to stimulation of astrocytes and microglia, harm to axons, dendrites, and loss of synapses, in addition to cognitive impairments because Aβ plays a significant part in neurotoxicity and neural function.**[2]**

NEUROFIBRILLARY TANGLES(NFT)

The abnormal tau protein filaments known as NFT can sometimes form paired helical filaments (PHF) and accumulate in neuralperikaryal cytoplasm, axons, and dendrites. This results in a loss of cytoskeletal microtubules and proteins associated with tubulin. In the brains of AD patients, hyperphosphorylated tau protein makes up the majority of NFTs, and its evolution can be interpreted in terms of the NFTs' morphological stages, which include: (1) the pre-tangle phase, one type of NFT, in which phosphorylated tau proteins accumulate in the somatodendritic compartment without the formation of PHF; (2) mature NFTs, which are characterised by tau protein filament aggregation and the displacement of the nucleus to periphery part of the soma; (3) the extracellular tangles, also known as the "ghost NFTs stage," that develop after neuronal loss as a consequence of an abundance of filamentous tau protein that is only partially proteolyzable.**[2]**

CAUSES AND RISK FACTOR OF ALZHEIMER'S DISEASE.

CHOLINERGIC HYPOTHESIS.

Ach plays a role in a number of bodily processes including learning, memory, attention and other crucial processes. It has been discovered that AD is characterized by cholinergic neuron degeneration, which alters brain function and impairs memory. Cholinergic neurotransmission is thought to be affected by β-amyloid, which also causes a reduction in choline absorption and release of Ach.The neurotoxicity of Aβ oligomers and the interactions between AChE and Aβ peptide have been linked to cholinergic synaptic loss and the development of amyloid fibrils, according to studies. The loss of nicotinic and muscarinic (M2) Ach receptors, which are found on presynaptic cholinergic terminals, and the deficit in excitatory amino acid (EAA) neurotransmission, where glutamate concentration and D-aspartate uptake are significantly reduced in many cortical areas in AD brains, are additional factors that contribute to the progression of AD. Additionally, cholinergic receptor antagonists like scopolamine, which was discovered to cause amnesia, are used. Utilizing substances that stimulate acetylcholine formation will reverse this effect.[3,4]

AMYLOID HYPOTHESIS

According to the amyloid hypothesis, aging or pathological conditions impair α and γ-secretase's ability to break down Aβ, which results in the accumulation of Aβ peptides (Aβ-40 and Aβ-42). A amyloid fibril formation is induced by a rise in the Aβ-42/Aβ-40 ratio.

Resulting in neurotoxicity and the induction of tau pathology, which in turn causes neuronal cell death and neurodegeneration. A catabolism and anabolism were found to be affected by AD risk factors and mutations of several genes, including APP, PSEN1, and PSEN2, which quickly lead to an accumulation of Aβ and a rapid progression of neurodegeneration.**[5,6]**

GENETIC FACTORS

AMYLOID PRECURSOR PROTEIN(APP)

Thirty mutations in the APP gene have been identified, of which 25 are linked to AD and cause an accumulation of Aβ with high levels. Mutations like T714I, V715A, V715M, V717I, V717L, L723P, K724N, and I716V increase the AB42/AB40 ratio, whereas mutations like E693G, E693K, D694N, and A692G affect the γ-secretase cleavage site and cause polymorphic aggregates that can affect bilayer stability.**[7,8]**

PSEN-1 & PSEN-2

PSEN1 is a core protein that plays a crucial part in the synthesis of Aβ from APP by activating the γ-secretase complex. PSEN1 plays a crucial role in preserving memory and neurons, as shown by PSEN1 knockout studies that resulted in synaptic dysfunction and memory impairment in mice. Simple PSEN1 mutations involve a single amino acid substitution, and severe mutations can occur when two amino acids are substituted . By lowering the levels of Aβ-40, mutations in the PSEN1 gene increase the ratio of Aβ-42 to Aβ-40.

In contrast, PSEN-2 mutations are rare and play a minor role in Aβ production. any mutation PSEN-2 severely affects the Aβ-42/40 ratio and may cause familial AD in common disease PSEN-1 allele. Some of her PSEN-2 mutations cause a significant increase in γ-secretase activity With increasing Aβ-42 and Aβ42/40 ratios such as N141I, T122P, M239V, M239I, Others are rare polymorphisms and do not affect Aβ-42 and Aβ 42/40 ratio levels not considered a pathogenic variant.**[9,10]**

ATP BINDING CASSETE TRANSPORTER A1(ABCA1)

Adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1) is part of the large ABC. Apolipoprotein-AI (ApoAI).

And like ApoE it enters the brain. Furthermore, ABCA1 maintains apoE lipid stability and As a mediator of high-density lipoprotein (HDL) formation, reflecting its role in atherosclerosis and cardiovascular disease. Studies in AD mouse models showed increased ABCA1 deficiency Clears amyloid plaques and eliminates ApoE lipidation . Humans have mutations in ABCA1 causes Tangier disease characterized by low levels of high-density lipoprotein (HDL) and apoAI in Plasma, tissue cholesterol accumulation and pathogenesis of AD.[11,12]

CLUSTERIN GENE & BRIDGING INTEGRATOR 1 (CLU & BIN 1 GENE)

CLU may have a protective role by interacting with Aβ and promoting its clearance, and a neurotoxic role by decreasing Aβ clearance. The value of the Aβ ratio determines whether the role of CLU is neuroprotective or neurotoxic. (BAR) Adapter proteins involved in the generation of endocytic membrane bending and other cellular functions. BIN1 has multiple isoforms. Some are in the brain that interact with various proteins such as clathrin, synaptojanin and amphiphysin 1, while others regulate synaptic vesicle endocytosis. BIN1 was recently identified as the second most important risk factor for LOAD, after ApoE, and plays a role in Aβ production and as a modulator of tau and her NFT pathology.**[13,14]**

NEUROSTEROIDS AND ALZHEIMER'S DISEASE.

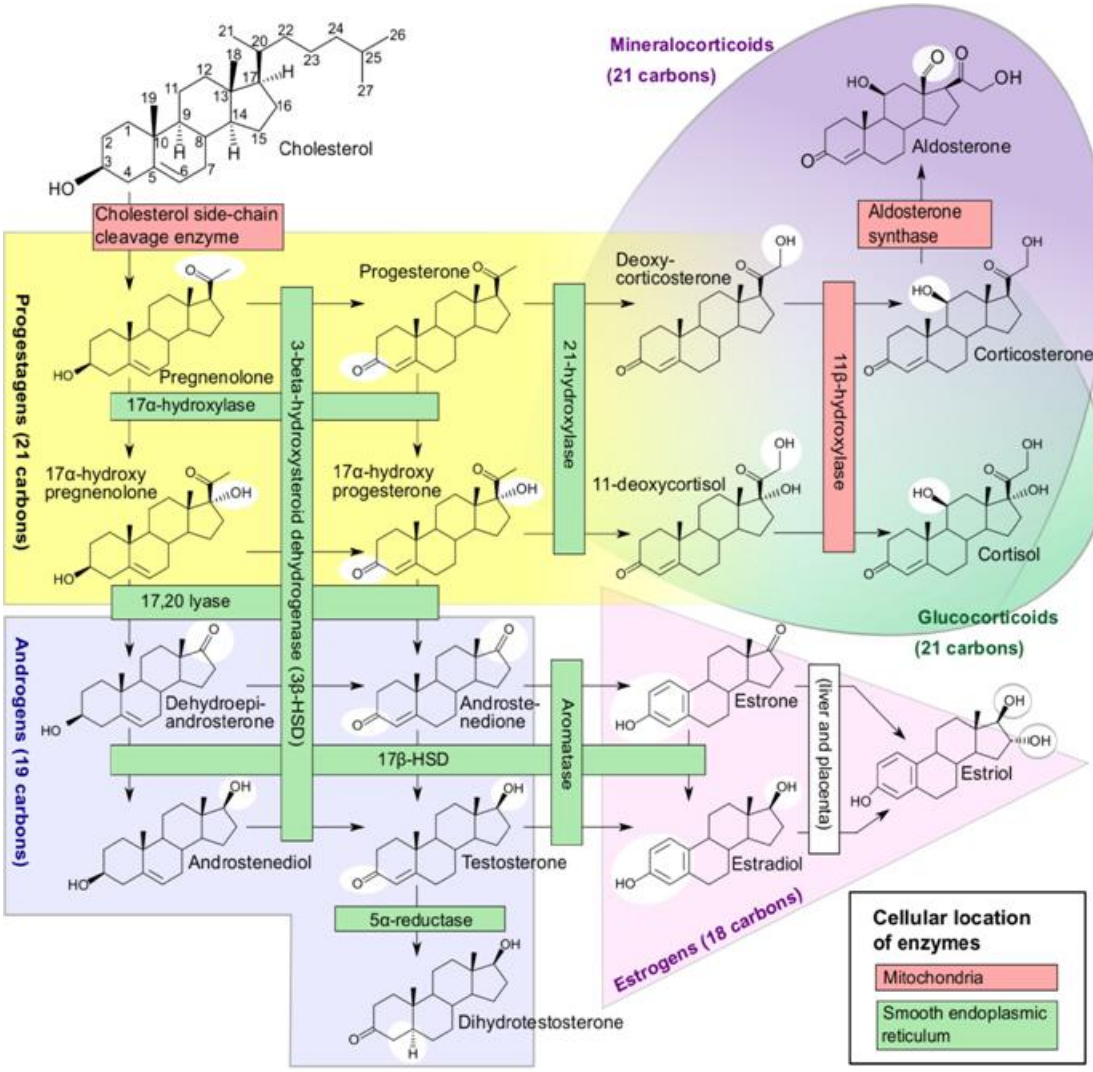

FIG-1: BIOSYNTHESIS OF STEROIDS FROM CHOLESTROL.

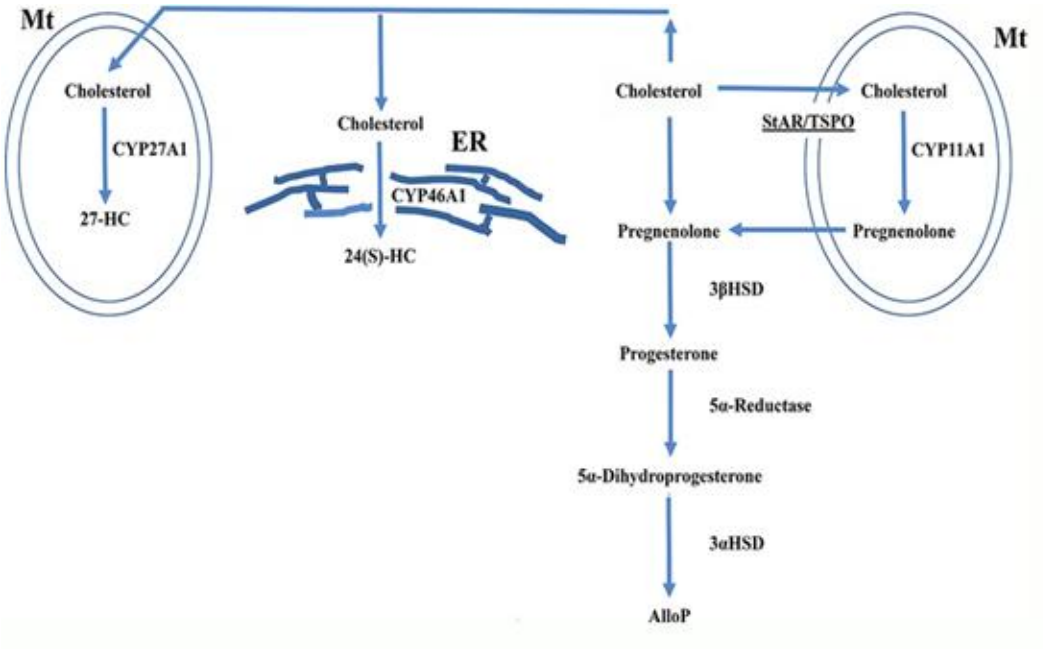

FIG-2:Key stages and enzymes involved in the production of AlloP, 24(S)-HC, and 27-HC from cholesterol are shown. The enzymes CYP46A1 and CYP27A1 catalyse the formation of 24(S)-HC and 27-HC, respectively. Both CYP46A1 and CYP27A1 are found in the endoplasmic reticulum (ER) and the mitochondria (Mt). The enzymes 3β-hydroxysteroid dehydrogenase (3βHSD), 5α-reductase (5αRD), and 3β-hydroxysteroid dehydrogenase (3βHSD) work together to produce alloP from cholesterol. The factors that govern the synthesis of 24(S)-HC, 27-HC, and AlloP from cholesterol in the retina and brain are poorly known.

OXYSTEROLS

Oxysterols are divided into two classes: Sterol ring oxidized oxysterols such as 7-Ketocholesterol or 7α/β-hydroxycholesterol, and side-chain oxidized oxysterols, such as 24(S)-HC, 25-hydroxycholesterol (25-HC) or 27-hydroxycholesterol (27-HC). general, The former class is produced by reactive oxygen species (ROS), the latter enzymatically produced from cholesterol.

25-HC appears to be a weak partial agonist of NMDARs and has antagonistic effects against 24(S)-HC of NMDARs . 25-HC has been reported to induce neuronal apoptosis at high concentrations. Cholesterol 25-hydroxylase is the enzyme that catalyzes the formation of this oxysterol. In Alzheimer's disease, 25-HC promotes the insertion of amyloid-β peptides into cell membranes (penetration of peptides by 25-HC into membranes has been confirmed in experiments using artificial model membranes and affects give leads to apoptosis mediated by mitochondria or endosomes and subsequently by oxidative stress. Recently, it was reported that 25-HC induces neuronal cell death in amyotrophic lateral cords sclerosis (ALS), another serious neurodegenerative disease.

In addition, 25-HC also induced neuronal apoptosis by activating it.

Glycogen synthase kinase 3 beta (GSK-3β)/liver X receptor (LXR) pathway in vitro ALS model.

27-HC is a metabolite of cholesterol that is formed primarily in the periphery and is capable of entering in the brain. Potent inhibitor of amyloid-β, in contrast to 24S-HC enhances Peptide formation and thus increases amyloid-β peptide and oxidative stress in vitro. Using LC/MS, Abdel-Khalik et al., (2017) measured non-esterified cholesterol and its metabolites in serum cholesterol and CSF from ALS patients in comparison to a group of healthy controls. When cholesterol metabolites from CSF and serum were compared, it was hypothesised that CYP27A1 impairment could result in the CNS's inability to eliminate excess cholesterol, which could be toxic to neuronal cells. This hypothesis was supported by a decrease in the neuroprotective LXR ligand 3β,7α-dihydroxycholest-5-enoic acid. Mateos et al. proved that 27-HC lowers NMDAR expression as well as activity-regulated cytoskeleton-associated protein (Arc) levels in rat primary hippocampal neurons. Arc is believed to contribute to the molecular processes underpinning memory and learning.

Enzymatic conversion of cholesterol into 24(S)-HC, which diffuses through the blood-brain barrier, removes the majority of it from the brain. Hence, it is suggested that 24(S)-HC be used as a measure of brain cholesterol metabolism. The brain and neuron-specific enzyme CYP46A1 (cholesterol 24-hydroxylase), which is encoded by the CYP46A1 gene, produces 24(S)-HC from cholesterol. Although while the participation of 24(S)-HC in the genesis of Alzheimer's disease has also been researched , the relationship between 24(S)-HC and Alzheimer's disease is still unclear. Several studies have shown that 24(S)-HC levels in CSF and plasma of Alzheimer's disease patients are higher or lower than in controls , however some of these differences may be related to the stage of the disease. On the other hand, in animal models of Huntington's disease, another serious neurodegenerative disease, overexpression of CYP46A1 is also discovered to be neuroprotective . In order to provide cholesterol to neurons during synaptogenesis or neuritic remodelling, astrocytes may receive signals from 24(S)-HC produced by neurons that instruct them to produce more lipidated ApoE particles .

Moreover, changes in 24(S)-HC's transcriptional control of ApoE-mediated cholesterol export may have an impact on the development of neurological disorders, including Alzheimer's disease. CYP46A1 and 24(S)-HC may exert neuroprotection in neurodegenerative disorders, while this is still up for debate.**[31-35]**

ALLOPREGNANOLONE

A powerful and efficient positive modulator of the main inhibitory neurotransmitter, GABA, AlloP is an endogenous neurosteroid produced in the Brain from cholesterol. According to earlier research, AlloP was produced under pathological circumstances by astrocytes or other glial cells . Current research has shown that primary excitatory neurons produce GABAergic neurosteroids . The rate-limiting processes in the manufacture of endogenous AlloP are the translocation of cholesterol to the mitochondrial inner membrane by translocator protein 18 kD (TSPO) and the catalytic reaction by 5-reductase (5aRD) . In both in vitro cell culture and in vivo animal models , AlloP shows neuroprotective characteristics via potentiating the activation of $GABA_A$receptors . Moreover, AlloP boosts neurogenesis , decreases inflammation , and lowers apoptosis . It also promotes myelination . Lack of AlloP may result in excitotoxicity, neurodegeneration, disruption of myelination, neurogenesis, apoptosis, and inflammation , all of which may contribute to the pathogenesis of Alzheimer's disease. According to reports, a decrease in AlloP in the temporal brain of people with Alzheimer's disease is unfavourably related to the illness's development.

Reduced amounts of AlloP are linked to the existence of the APOE 4 allele, a risk factor for getting Alzheimer's disease. Patients homozygous or heterozygous for the APOE 4 allele (2.86 ng/g, n=36) have substantially lower AlloP median levels in the temporal cortex than patients without the APOE 4 gene (5.23 ng/g, n=44) (Mann Whitney test: 0.04).**[33-36]**

DHEAS(DIHYROEPIANDROSTANE) AND IT'S SULFATE ESTERS AS NEUROPROTECTIVE AGENT.

The most prevalent steroids in people are dehydroepiandrosterone (DHEA) and its sulphate derivative, DHEAS. They are primarily made in the human adrenal cortex's zona reticularis. DHEA(S) release from the adrenal gland rises during the adrenarche. Circulating DHEA(S) levels peak between the ages of 20 and 30; after that, they begin to sharply decline1-4, peaking in people over the age of 70.Additionally, different parts of the central and peripheral nervous systems (CNS and PNS), respectively, of humans and other animals can synthesise these hormones from scratch**.**

Because these steroids shield CNS neurons from noxious substances, it is most likely that the decrease in neurosteroid levels that occurs with ageing is linked with neuronal malfunction and degeneration. In fact, allopregnanolone (Allo) lessens NMDA-induced excitotoxicity in human neurons while DHEA shields rodent hippocampal neurons from the damage caused by NMDA. In fact, new research examining the physiopathological implications of neurosteroids in Alzheimer's disease (AD) has revealed a substantial decrease in neurosteroid concentrations in specific brain areas of AD patients compared to elderly nondemented controls. In the striatum and cerebellum, pregnenolone sulphate (PREGS) and DHEAS were considerably lower, and DHEAS was also significantly decreased in the hypothalamus in these individuals.**[15,16]**

PREVENT SYMPATHOADRENAL CELL APOPTOSIS BY INTRODUCING ANTIAPOPTOTIC BCL-2 PROTEINS.

Indeed, the antiapoptotic proteins Bcl-2 and Bcl-xL are expressed in response to DHEA(S) and Allo. Since the protective impact of neuroactive steroids was almost completely eliminated when their synthesis was inhibited by antisense oligonucleotides (directed against the translation initiation site of the Bcl-2 transcript), it appears that these proteins play an important function. The cAMP-response element (CRE) and the NF-κB sensitive motif are present in the promoter regions of the anti-apoptotic Bcl-2 and Bcl-xL genes. Transcription factors CREB and NF-κB have also been implicated in the neuroprotective and survival mechanisms of central and peripheral neurons as positive regulators of Bcl-2 and Bcl-xL gene expression.

Confocal laser scanning microscopy localization of p65 NF-κB reveals that NF-κB is almost entirely localised within the nucleus in PC12 cells cultured in serum-supplemented media, whereas NF-κB is located in the cytoplasm in cells kept in serum-free media. Similar to serum-supplemented cells, serum-depleted cells subjected to DHEA or Allo exhibit NF-κB labelling primarily in the nucleus. The phosphorylation and stimulation of the CREB protein is also impacted by these neurosteroids.

In fact, Western blot analysis using cell extracts from serum-depleted PC12 cells treated for 1 hour with DHEA, DHEAS, and Allo, as well as antibodies specific for the phosphorylated and total forms of CREB, demonstrates that serum depletion causes a sharp decrease in phosphorylated CREB within 1 hour when compared to serum-supplemented cells. However, levels of phosphorylated CREB are almost entirely returned to those seen with serum addition in serum-depleted cells exposed to neuroactive steroids. Bcl-2 must be phosphorylated at serine 70 in order to perform its antiapoptotic role.**[17,18]**

DIRECTLY STIMULATE NEUROPROTECTIVE CATECHOLAMINE BIOSYNTHESIS AND SECRETION AND NEUROPROTECTIVE EFFECT BY BINDING ON G-PROTEIN COUPLE RECEPTOR BINDING DOMAIN.

In models of AD, noradrenergic pathways appear to be crucial in regulating basalocortical cholinergic system activity and its response to damage, as well as in altering cognitive processes including memory and attention. By influencing neuroplasticity, neurotrophic factors (BDNF), neurogenesis, inflammation, cellular energy metabolism, excitotoxicity, and oxidative stress, catecholamines aid in the repair of neurological damage.

Recent research reveals that neuroactive hormones including DHEA, DHEAS, and Allo indirectly modulate the turnover of catecholamines in the brain. In fact, DHEAS has been demonstrated to enhance NMDA-evoked norepinephrine release in rat hippocampus cells. Meanwhile, in mice, DHEA shields striatal neurons from MPTP-induced dopamine depletion. It now appears that neurosteroids might control neuroprotective catecholamines directly to exert some of their neuroprotective effects. In fact, DHEA, DHEAS, and Allo may boost dopamine and norepinephrine release from PC12 sympathoadrenal cells quickly (within 10 min).

Neurosteroids not only influence catecholamine secretion but also catecholamine synthesis directly. By promoting the production of tyrosine hydroxylase (TH), the rate-limiting enzyme of catecholamine biosynthesis, DHEAS and Allo do in fact have a long-lasting impact on catecholamines in vitro. Tyrosine hydroxylase (TH) mRNA and protein levels are strongly four-fold induced by DHEAS and Allo within 6 h and 8 h, respectively, according to results from RTPCR, real-time PCR, and Western blot tests, indicating a direct transcriptional influence on TH expression. AMPT and NSD-1015, inhibitors of TH and L-aromatic amino acid decarboxylase, respectively, totally prevent the effects of DHEAS and Allo, further supporting the idea that their action involves catecholamine production.

Since their protective impact was eliminated in the presence of 10^{-6}M pertussis toxin, experimental evidence points to the involvement of Gi protein in the DHEAand DHEA-BSA-induced protection of PC12 cells against serum deprivation-induced apoptosis. (PTX). PTX also entirely eliminated DHEA and DHEA-BSA's capacity to defend against serum deprivation-induced reduction of the antiapoptotic and prosurvival Bcl-2/Bcl-xL proteins.DHEA dose-dependently boosted the specific binding of [35S]-GTPS to PC12 cell membrane preparations.

Furthermore, it was proposed that DHEA stimulates Src kinase via a Gi-dependent mechanism in light of recent results revealing that Src tyrosine kinase is directly activated by Gi via phosphorylation. Interestingly, the phosphorylation of Src was quickly enhanced by both DHEA and DHEA-BSA. (within 5 min of exposure). It should be mentioned that activation of the Src-PKC pathway promotes PC12 cell viability by increasing NF-κB activity.

All of these findings point to the possibility that DHEA exerts its protective effects by first activating G-protein-associated membrane binding sites, followed by the activation of prosurvival Src-PKC kinases, which in turn triggers the release of transcription factor NF-κB and the production of anti-apoptotic Bcl-2 proteins.**[19-21]**

VARIOUS STUDIES ON EFFECT OF NEUROSTEROIDS ON THE NERVOUS SYSTEM USING ANIMAL MODELS.

In 2002 a study was conducted by the Iñigo Azcoitia and his research colleagues on the reduction of myelin fiber loss and myelin fiber abnormalities in the sciatic nerve of rats by progesterone and it's derivatives (Dihydroprogesterone and Tetrahydroprogesterone)

Male Sprague-Dawley rats, Crl:CD BR, Charles River, Calco, Italy, aged (22-24 months) and adult (3 months) were used. The temperature and humidity in the department's livestock accommodations were kept under control for the creatures. The rodents were beheaded to death 24 hours after the last dose. The sciatic nerves were quickly removed, cut into tiny pieces (1-2 millimetres long), and fixated by immersion in 1% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate solution, pH 7.4, for 24 hours at 4 °C.

The total cross-sectional area of the nerve, comprising the endo- and perineurium, was measured from camera lucida drawings at a final magnification of 150×. For the morphometric analysis of myelinated fibers and unmyelinated axons, areas of the semi-thin sections, covering at least 25% of the total cross-sectional profile of the nerve, were chosen by systematic random sampling of squares. At least five rats were studied for each experimental group.

Microscopic studies shown the effect of ageing on the sciatic nerves of the rats which include- Loss of myelinated and unmyelinated fibers, disorganization of the myelin sheath, Changes in myelin compaction like unusually broad incisions and aberrant lamellar detachment, presence of myelin infoldings in the axoplasm and Abnormal change in the size and shape of myelinated fibers with aging.**[22]**

EFFECTS OF NEUROSTEROIDS

Progesterone and its derivative DHP, and THP had one of the most notable impacts on small-caliber myelinated fibres. The amount of the tiny myelinated fibres greatly increased after treatment with these steroids. Furthermore, P, DHP, and THP markedly improved the g ratio of tiny myelinated fibres.The rise in myelinated fibres could be due to the elderly sciatic nerves' greater remyelination of small fibres. This theory is supported by the fact that these fibres' increased g ratio values following P, DHP, and THP therapies. Additionally, after being treated with P or its compounds, the number of myelinated fibres increased while the number of big unmyelinated axons decreased by a similar amount. This indicates that this axonal subpopulation's remyelination may have been aided by P and its compounds. P has also been demonstrated to promote the remyelination of nerve fibres in sciatic nerve that has undergone cryolesion.

Therefore, the increased number of tiny myelinated fibres in the sciatic nerves of ageing rats following P, DHP, and THP therapies is most likely the result of remyelination.

The decrease in the number of axons with myelin anomalies was another notable result of the P, DHP, and THP therapies. P also decreased the percentage of irregularly shaped fibres. As previously stated, abnormal myelin and erratic fibre patterns are usual indicators of ageing in peripheral nerves.

Indeed, older rats' sciatic nerves exhibit substantially less production of myelin proteins like Po, peripheral myelin protein 22 (PMP22), and myelin basic protein (MBP). The shape of myelin sheaths depends on each of these myelin proteins. Specifically, Po, which makes up more than half of all peripheral myelin proteins is believed to have a crucial physiological role in the preservation of the PNS myelin's multilamellar structure , acting as a bifunctional structural component connecting neighbouring lamellae and stabilising the myelin's assembly. THP appears to be able to particularly stimulate PMP22 expression while P, DHP, and THP can all boost Po expression. The expression of Po in the sciatic nerve is markedly increased when elderly adult rats are treated with DHP.On the other hand, Po expression in elderly rats was not substantially impacted by P or THP. Additionally, P, DHP, and THP have no impact on the levels of PMP22 mRNA in the sciatic nerve of old adult rats. Additionally, THP's impact on the expression of this myelin protein is unclear, whereas P and DHP are unable to alter MBP expression in the peripheral nerves of elderly animals.**[23-26]**

In 2005 a study was conducted by Jun Ming Wang, Roberta Diaz Brinton and their research colleagues on the effect of allopregnanolone on the promotion of rodent and human neural progenitor cells.

Male Sprague-Dawley rats was selected for the studies . These rodents were kept in a 24°C room with a 14/10 h light/dark cycle, served standard rat food, and given access to tap water. Allopregnanediol (5-pregnan-3, 20-diol), allopregnanetriol (5-pregnan-3, 17, 20-triol), 5-pregnan-3-ol, and pregnenolone sulphate (5-pregnan-3-ol-20-one sulphate) are some of the steroids that were used for the studies. Cultures of hippocampal neurons and the human neural stem cells were prepared and immunocytochemical staining, BrdU incorporation(By detecting the absorption of BrdU in the S phase of the cell cycle, cell proliferation was first assessed). Thymidine uptake(Thymidine uptake was used to identify the selectivity of AP and its stereoisomers, as well as its parent neurosteroid progesterone, on DNA replication.),

Murine leukemia virus– enhanced green fluorescent protein viral particle preparation and cell labeling (The number of retrovirus-enhanced green fluorescent protein (eGFP)-labeled dividing rNPCs was quantitatively measured using a fluorescence-activated cell sorting (FACS) assay to confirm that AP-induced DNA amplification was indicative of mitosis and not DNA repair and that AP caused a complete mitosis.), FACS analysis and morphological observation of MuLV–GFP-positive cells, gene array assay and Real time revere transcriptase PCR (RTPCR) and Western blot analyses for CDC2 and PCNA protein expression were carried out.**[29]**

EFFECTS OF NEUROSTEROIDS

It is well known that AP increases chloride inflow by acting as an allosteric modulator of the GABAAR, hyperpolarizing the neuronal membrane potential, and reducing neuron excitability. In stark contrast to this response in adult neurons, activation of GABAAR by GABA or AP results in a chloride efflux in immature neurons. The elevated intracellular chloride concentration in embryonic cells flips the chloride concentration gradient, causing chloride efflux to depolarize the membrane and open the VGLCC**.** Blockade of AP-induced neurogenesis by an inhibitor of VGLCCs is consistent with our finding of an AP-induced rise in intracellular calcium via activation of VGLCCs. The discovery of an AP-induced increase in intracellular calcium via activation of VGLCCs is consistent with the blocking of APα-induced neurogenesis by a VGLCC inhibitor. By activating VGLCCs via $GABA_AR$ in cultured hippocampal neurons, studies showed that APα causes a quick and developmentally controlled calcium influx that may stimulate neurogenesis. Therefore, discovery suggest that AP stimulation of neurogenesis in both rat neural progenitors and human neural stem cells is significantly influenced by $GABA_AR$-activated VGLCCs and following calcium influx.

We looked into the neurogenic properties of APα in human neural stem cells generated from the human brain cortex to see if our results in rodent neural progenitor cells were applicable to the proliferation of human neural stem cells. These studies' findings show that APα significantly increased the absorption of BrdU.

The results from gene-array and real-time RT-PCR support APα neurogenic impact. APα increased the expression of genes like CDC2, cyclin-B, and PNCA that are involved in cell cycle progression and growth. In line with this, APα reduced the expression of genes involved in the inhibition and degradation of CDKs and cyclins, including the ubiquitin-activating enzyme E1 and the CDK4 and CDK6 inhibitors p16 and p18. These enzymes are necessary for ubiquitinating mitotic cyclins and promoting cell cycle exit. In these study, AP not only controlled DNA amplification and cell-cycle protein expression, but it also caused the rNPCs to undergo a full mitosis.

Two well-known cell-proliferating markers, CDC2 and PNCA, were further examined by Western blot on the basis of the gene-array analysis findings to ascertain whether increases in mRNA for mitotic cell-cycle genes were indicative of increases in protein. Western blot was used to check for CDC2 and PNCA in whole-cell lysates from hippocampal neurons treated with 500nm APα and normal neurons. APα increased CDC2 and PNCA protein levels by 1.5 or 2 times.

Neurogenic effect of APα is stereospecific, Thymidine incorporation was used as a stand-in measure of DNA synthesis and mitosis in order to ascertain the steroid selectivity for inducing neurogenesis. According to the findings of these studies, 250nm APα significantly increased thymidine accumulation by (150 ± 21%) compared to the control. A (126 ±12%) rise was brought on by progesterone ($p < 0.05$ vs. control). Despite the fact that progesterone's % average was smaller than APα.

In primary cultured rat hippocampal progenitor cells, the steroid specificity analysis demonstrates both the specificity of APα-induced mitogenesis and supportive evidence that factors that support morphological differentiation, such as pregnenolone sulfate, have an effect opposite to that of APα.**[27-30]**

CONCLUSION

In conclusion, the neurosteroids allopregnanolone (AlloP) and 24(S)-hydroxycholesterol (24(S)-HC) exhibit significant neuroprotective properties that could be leveraged as novel Alzheimer's disease therapeutics. The bottom line from the accumulated preclinical evidence is that these steroids reduce Alzheimer's pathology and symptoms in animal models by targeting neurodegeneration, inflammation, and amyloid-beta aggregation. Enhancing AlloP and 24(S)-HC levels appears to restore neuronal function and regenerative capacity. Findings from human AD brain studies also support their deficiency in disease states.

However, large-scale clinical trials are critically needed to translate these preliminary results into viable treatment strategies. Future research should focus on:

1. Determining optimal dosage and timing of AlloP and 24(S)-HC administration in AD patients
2. Evaluating efficacy and safety through randomized controlled trials with cognitive outcomes
3. Identifying responsive patient subgroups through biomarker profiling
4. Developing selective steroid modulators with improved drug-like properties
5. Elucidating interactions between neurosteroid therapy and current acetylcholinesterase inhibitors

In summary, allopregnanolone and 24(S)-hydroxycholesterol are promising neuroprotective candidates against Alzheimer's pathogenesis. Advancing these neurosteroids from preclinical models to clinical applications could provide much-needed therapeutic innovations for managing this devastating disease.

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