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NEUROSTEROIDS IN OXIDATIVE STRESS-MEDIATED INJURY IN ALZHEIMER DISEASE

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8.1 INTRODUCTION

The brain is considered to be especially vulnerable to oxidative stress due to high levels of prooxidant factors and relatively low antioxidant defence. Putative prooxidant factors consist of a high metabolic rate, high levels of unsaturated fatty acids that readily undergo lipid peroxidation reactions, and relatively high levels of iron in some brain regions that facilitate hydroxyl radical formation from Fenton reactions [1]. Furthermore, neuronal activity results in high levels of intracellular calcium ions after depolarization that are linked to activation of phospholipase A_2, release of arachidonic acid, and subsequent formation of reactive oxygen species (ROS) from cyclooxygenase and lipoxygenase reactions (Fig. 8.1). Calcium ions also facilitate mitochondrial depolarization with release of mitochondrial factors that promote ROS formation. Furthermore, calcium ions are required for nitric oxide synthesis via endothelial and neuronal nitric oxide synthases (eNOS and nNOS). The brain contains relatively high levels of nitric oxide that can give rise to formation of highly reactive peroxynitrite. Also, catecholamine metabolism involves increased ROS formation: Superoxide can be generated from semiquinone formation, and hydrogen peroxide is released as a by-product of catecholamine synthesis by tyrosine hydroxylase and degradation by monoamine oxidases.

Despite these prooxidant factors, the brain possesses only relatively low levels of antioxidant defenses. Catalase activity is extremely low in brain tissue, and glutathione peroxidase as well as superoxide dismutase show low activity compared with other organs such as liver, heart, and kidney [2]. As a consequence, increased levels of ROS can be especially detrimental to brain tissue. Oxidative stress has accordingly been suggested to be a primary factor in the pathogenesis of several chronic neurodegenerative disorders, most prominently Alzheimer disease (AD), Parkinson disease (PD), and Huntington disease (HD).

Several studies, mainly in animals, suggest neurosteroid involvement in neuroprotection [3]. However in humans, the role of neurosteroidogenesis in the regulation of degenerative mechanisms is unknown. Since the process of neurosteroid biosynthesis is a pivotal mechanism intervening in the protection or viability of nerve cells, it might be regulated or significantly affected under oxidative stress conditions. However, the key factors interacting with neurosteroid biosynthesis under pathophysiological conditions are poorly understood. New findings demonstrate an amino acid sequence-dependent action of amyloid-β (Aβ) on neurosteroidogenic pathways [4]. The data also indicate that, unlike progesterone neosynthesis, regulation of endogenous estradiol formation by pathogenic factors may be a deciding process controlling cell death mechanisms. Targeting estradiol

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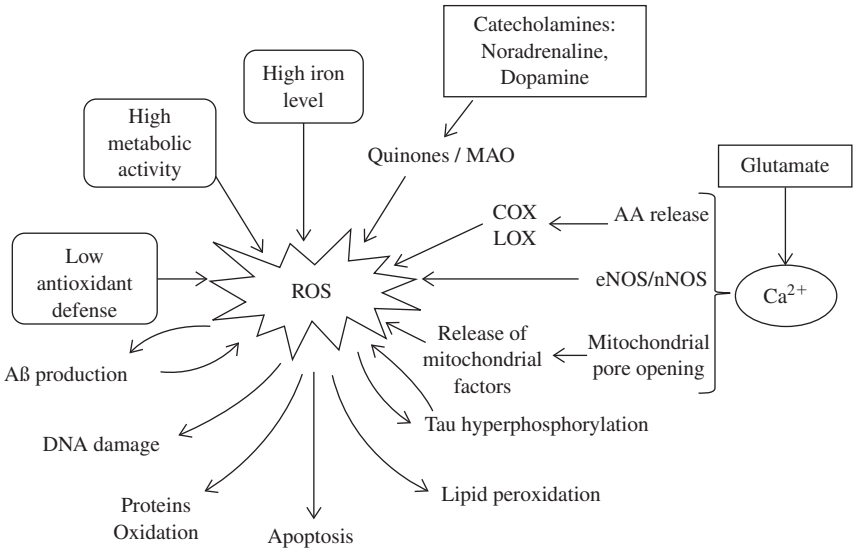

Fig. 8.1 Factors contributing to the accumulation of reactive oxygen species (ROS) in brain tissue and their implications in Alzheimer disease. See text for details. MAO, monoamine oxidase; COX, cyclooxygenase; LOX, lipoxygenase; AA, arachidonic acid; eNOS/nNOS, endothelial/neuronal nitric oxide synthase; Aβ, amyloid-β peptide.

biosynthetic pathways in nerve cells may therefore be interesting in development of neuroprotective strategies.

8.2 EVIDENCE FOR A PATHOLOGICAL ROLE FOR OXIDATIVE STRESS IN AD

AD is a neurodegenerative brain disease and the most common form of dementia among the elderly. It is characterized by clinical symptoms of severe and progressive loss of memory, language skills, as well as spatial and temporal orientation. The pathology of AD is characterized by extracellular senile plaques, composed of aggregated amyloid-β peptide, and intracellular neurofibrillary tangles, consisting of aggregates of abnormally hyperphosphorylated Tau protein, and is accompanied by mitochondrial dysfunction, but the mechanisms underlying AD-related dysfunction and neurodegeneration are poorly understood.

Identification of factors that contribute to the pathology of AD comes from epidemiological as well as genetic studies: AD can be classified into two different forms, rare familial forms (FAD), in which the disease onset is at an age younger than 60 years, and the vast majority of sporadic AD cases, in which onset occurs at an age over 60. Both forms of AD show the same clinical symptoms and neuropathology. Genetic studies in FAD patients have identified mutations in the genes encoding for the amyloid precursor protein (APP) and for the presenilins PS1 and PS2 that cause an autosomal inherited form of AD with 100% penetrance. These FAD mutations consistently lead to increased production of Aβ from its precursor protein APP, which prompted Hardy and Higgins [5] to suggest a direct and pathological role for Aβ accumulation in the development of AD. In the sporadic form of the disease, several risk factors have been found that increase the risk to develop the disease but—unlike FAD mutations—do not necessarily lead to development of AD. Aging is by far the most important risk factor for AD, but the apolipoprotein E4 allele and female sex also predispose to the development of AD.

Immunohistochemical studies of postmortem AD brains have established that neurons undergo apoptotic cell death. Since ROS can elicit apoptotic signaling, the hypothesis that oxidative stress is involved in the pathogenic steps that lead to the development of AD was proposed in the 1990s by several groups [6—9]. There is a large body of evidence in support of this hypothesis: Oxidative stress has been repeatedly shown to be associated with Aβ toxicity and with risk factors for sporadic AD—mostly aging and the apolipoprotein E4 genotype.

8.2.1 Evidence for a Role for Oxidative Stress in Sporadic AD

Oxidative stress has been associated with the risk factors for sporadic AD, most prominently with aging [10], suggesting that an age-associated rise in accumulation of ROS can render the brain more vulnerable to the development of AD. Consequently, increased markers of oxidative stress have been found in AD patients: Several studies have reported elevated levels of lipid peroxidation products, oxidatively modified proteins, and oxidized DNA and RNA bases in brains and cerebrospinal

fluid from AD patients compared to age-matched nondemented control subjects [11–13]. Furthermore, tissue samples from AD brains display a higher susceptibility to in vitro oxidation [14], suggesting an impairment of antioxidant defense in AD patients. Reports on antioxidant parameters in AD brains have, however, been contradictory so far. Several antioxidant enzymes have been studied in AD brains with inconsistent results, but the majority of reports found elevations in antioxidant enzymes, suggesting an upregulation of antioxidant defense in response to increased ROS levels [15]. Interestingly, upregulation of antioxidant defence was more pronounced in female patients, and levels of 4-hydroxynonenal (HNE), a neurotoxic aldehyde derived from lipid peroxidation reactions, were elevated in female compared to male patients. These findings suggest that brains from female AD patients are under higher oxidative pressure [15], consistent with epidemiological findings that AD is more frequent in postmenopausal women compared to age-matched men. This observation can possibly be linked to the lack of sexual hormones, especially estrogens, that can modulate cognitive function and nonreproductive behaviours in humans and other mammalian species [16]. Potential sources of ROS in AD brains include ROS derived from impaired mitochondrial function [17, 18] and secondary ROS formation due to inflammatory reactions. Furthermore, increased monoamine oxidase B activity and increased levels of potentially pro-oxidative heavy metals like iron have been identified in AD brains [19, 20] and in patients with mild cognitive impairment (MCI), the "clinical precursor of AD," suggesting that oxidative stress is an early event of the disease [21].

Apart from aging, the apolipoprotein E4 allele is the second most important risk factor for the development of AD. Apolipoprotein E seems to play a role in brain lipid metabolism and neuronal and glial development. It can exist in three different alleles, E2, E3, and E4, which differ in only two amino acids: The E2 isoform contains two and the E3 isoform one cysteine residue, while the E4 isoform contains none. Carriers of the apolipoprotein E4 are at increased risk to develop sporadic AD, especially when they are homozygous carriers. The apolipoprotein E4 allele has been associated with increased oxidative damage in AD brains, with the greatest impact in homozygotic carriers [22] and an increased susceptibility to cell death in lymphocytes from carriers bearing at least one E4 allele [23]. In vitro studies have evidenced that apolipoprotein E4 is less efficient in binding HNE, a cytotoxic lipid peroxidation product. These findings suggest that the Apo E4 isoform increases susceptibility to oxidative damage, thereby possibly predisposing to the development of AD.

8.2.2 Oxidative Stress and Toxicity of Mutant APP, Presenilins, and Tau

Since the proposal of the amyloid hypothesis of AD, toxic mechanisms caused by mutant APP and presenilins related to an increased production of Aβ have been extensively studied. Cells exposed to Aβ undergo apoptotic cell death, and the toxicity of Aβ has been shown to be related to the production of ROS [24, 25]. Furthermore, toxicity of Aβ depends on its aggregation state, which can be influenced by oxidation. Thus oxidative stress can cause formation of toxic Aβ species, which in turn can further exacerbate accumulation of ROS in a vicious cycle (Fig. 8.1). This could also explain why the prevalence of AD increases with advancing age—due to rising oxidative stress levels with aging favoring Aβ toxicity.

Toxicity of Aβ is also evident in cell cultures overexpressing APP/Aβ. PC12 cells transfected with mutant APP Swedish showed higher sensitivity to ROS-induced cell death and increased mitochondrial impairment after challenge with hydrogen peroxide [26]. Similar observations were obtained in human neuroblastoma cells (SH-SY5Y) overexpressing human wild-type APP (wtAPP) [27]. The study demonstrated that chronic exposure to Aβ protein resulted in activity changes of complexes III and IV of the oxidative phosphorylation system (OXPHOS) in mitochondria coupled with a drop of ATP levels and an increase of ROS production, which may finally instigate loss of synapses and neuronal cell death in AD. Furthermore, treatments of untransfected SH-SY5H cells with Aβ or human amylin aggregates induced an increase of ROS production and had a negative impact on mitochondrial respiration by their action on OXPHOS system [25].

Toxicity of Aβ has also been evidenced in animal models of the disease. Mice transgenic for mutant APP have high levels of Aβ in their brains and show an age-dependent formation of Aβ plaques similar to the plaques found in AD patients. Increased markers of oxidative stress have been detected in brains of transgenic mice bearing mutant APP, accompanied by markers for mitochondrial damage [28]. Furthermore, mutant APP transgenic mice show reduced levels of the antioxidant enzyme copper/zinc superoxide dismutase [29]. In agreement, increased markers of oxidative stress and reduced antioxidant defense by catalase as well as a trend toward reduced activity of SOD were found in brains from FAD patients [30]. The results provide an important link of studies on toxicity of mutant APP in cell culture and animal models mimicking the pathogenesis of the disease in FAD patients, all of them bearing mutations finally causing an increased generation of Aβ.

Mutations in the presenilins PS1 and PS2 account for the majority of FAD cases and have similarly been linked with oxidative stress. Oxidative toxicity of mutant

presenilins can be either (i) due to increased formation of toxic Aβ, especially the $A\beta_{1-42}$ isoform, or (ii) due to direct toxic effects of mutant presenilins. Several mutations in the presenilins have been found that consistently lead to increased production of the long $A\beta_{1-42}$ from its precursor protein APP [31], resulting in increased Aβ levels and toxicity via the above-mentioned mechanisms. Expression of mutant presenilins in cell culture and transgenic mice sensitizes cells to apoptotic stimuli by increasing ROS production and mitochondrial damage [32, 33]. Furthermore, brains from PS1 mutant transgenic mice display reduced activities of antioxidant enzymes [34], and lymphocytes from these mice display increased sensitivity to apoptosis accompanied by high intracellular ROS and calcium levels [35]. Interestingly, increased ROS accumulation, disturbed calcium homeostasis, and diminished levels of antioxidants have also been identified in peripheral cells from FAD patients bearing APP or PS mutations as well as in cells from sporadic AD patients [36]. These results suggest that the oxidative toxicity observed in transgenic animal models of the disease can indeed play an important role to the pathogenesis of sporadic as well as familial AD in humans.

The second main hallmark lesion of AD is intracellular neurofibrillary tangles (NFTs) built up of hyperphosphorylated Tau. This protein may block the transport of mitochondria, leading to energy deprivation and oxidative stress at the synapse as well as to neurodegeneration [37]. Functional analysis showed mitochondrial dysfunction in transgenic mice (pR5 mice) expressing P301L mutation of Tau, with a reduced complex I activity and, with age, impaired mitochondrial respiration and ATP synthesis. Mitochondrial dysfunction was associated with higher levels of ROS in aged pR5 mice. Increased Tau pathology as in aged homozygous pR5 mice revealed modified lipid peroxidation levels and upregulation of antioxidant enzymes in response to oxidative stress. These findings demonstrated for the first time that not only the Aβ but also the Tau pathology acts on the enzyme metabolism of the brain and the oxidative conditions in AD. However, more recently, the successful development of double, and even triple, transgenic mouse models has facilitated the investigation of pathogenic mechanisms in AD and assisted in an understanding of the interplay of Aβ and Tau on bioenergetics processes in vivo [37]. These findings support the idea that Aβ and Tau act synergistically in amplifying mitochondrial respiratory deficits, mainly of complex I and IV activities [18].

8.2.3 Is Oxidative Stress an Early Event in the Pathogenesis of AD?

From the above evidence it can be concluded that oxidative stress is a feature of sporadic as well as familial forms of AD. However, it remains to be elucidated whether oxidative stress is a primary factor in the pathogenesis of the disease or only a secondary contributing mechanism. The fact that oxidative damage and mitochondrial dysfunction can be detected at early stages in animal models [28]—even before the presence of Aβ plaques [38]—and that oxidative stress parameters have been detected at highest levels in early stages of the disease in AD patients [39] suggest that oxidative stress is a primary event in the course of the disease. This is supported by studies that reported a reduced risk of AD in users of antioxidant vitamin supplements [40]. Although further clinical trials are needed, antioxidant therapeutic approaches seem to be most effective at very early stages of AD and are even better utilized to modulate disease risk.

8.3 NEUROSTEROIDS

Steroid hormones are now well-defined molecules that are mainly produced by endocrine glands, such as adrenal gland, gonads, and placenta. They are involved in the control of a lot of physiological processes, from reproductive behavior to stress responsiveness. With their ability to cross cellular membranes, and thus the blood-brain barrier, steroid hormones have also an important role in the development, maturation, and differentiation of the central and peripheral nervous systems.

Three decades ago, Baulieu and co-workers were the first to show a steroid production within the nervous system itself. They discovered that some steroids, such as pregnenolone (PREG) and dehydroepiandrosterone (DHEA), were more concentrated in the brain than in the plasma [41]. In addition, they could show that the level of these steroids remained elevated in the brain even after adrenalectomy and castration. These molecules are now called "neurosteroids" and are defined as neuroactive steroids that are synthesized within the nervous system, independently of peripheral endocrine glands. Enzymatic activities of proteins involved in steroidogenesis have been shown in many regions of the central and peripheral nervous systems, in neurons as well as in glial cells [42]. Pharmacological and behavioral studies showed that neurosteroids were implicated in several physiological mechanisms, for example, cognition, anxiety, depression, neuroprotection, and even nociception [43]. Thus the conservation of the ability to produce neurosteroids during vertebrates' evolution suggests that this category of molecules is important for living beings.

8.3.1 Biosynthesis of Neurosteroids

Neurosteroids derive from cholesterol and other blood-borne steroidal precursors. The first step of neurosteroidogenesis is the transfer of molecules of cholesterol from

the outer to the inner mitochondrial membrane. Free cholesterol accumulates outside of mitochondria and binds to the steroidogenic acute regulatory protein (StAR), a hormone-induced mitochondria-targeted protein that initiates cholesterol transfer into mitochondria. Then, molecules are transported inside mitochondria by a protein complex including translocator protein (TSPO), a cholesterol-binding mitochondrial protein also known under the name of peripheral-type benzodiazepine receptor (PTBR), which permits cholesterol transfer into mitochondria and subsequent steroid formation [44]. This translocation from the outer membrane to the inner membrane of mitochondria is the rate-limiting step in the production of neurosteroids. In fact, the ability of cholesterol to enter into mitochondria to be available to cytochrome *P*450 cholesterol side chain cleavage enzyme (P450scc), located in the inner side of the mitochondrial membrane and responsible for the conversion of cholesterol to PREG, will determine the efficiency of steroidogenesis.

PREG, precursor of all steroid hormones, is then transported to the endoplasmic reticulum, where it is metabolized to form neuroactive steroids (Fig. 8.2). The next enzymatic step in neurosteroidogenesis is the conversion of PREG into DHEA by the cytochrome *P*450c17 enzyme (P450c17), also called 17α-hydroxylase/17,20 lyase. This enzyme catalyzes the 17α-hydroxylation of PREG in a two-step reaction that gives first 17-hydroxyPREG (17OH-PREG) and then the final product, DHEA. Each step requires the molecules NADPH and O_2.

PREG can also be catalyzed by another enzyme called 3β-hydroxysteroid dehydrogenase (3β-HSD) into progesterone (PROG). In general, 3β-HSD uses NAD^+ as a cofactor to oxidize hydroxysteroids, such as PREG, 17OH-PREG, and DHEA, into their respective ketosteroids, PROG, 17OH-PROG, and androstenedione. Then, neurosteroidogenesis follows two main pathways with PROG as precursor: the androgen/estrogen pathway and the corticoid pathway.

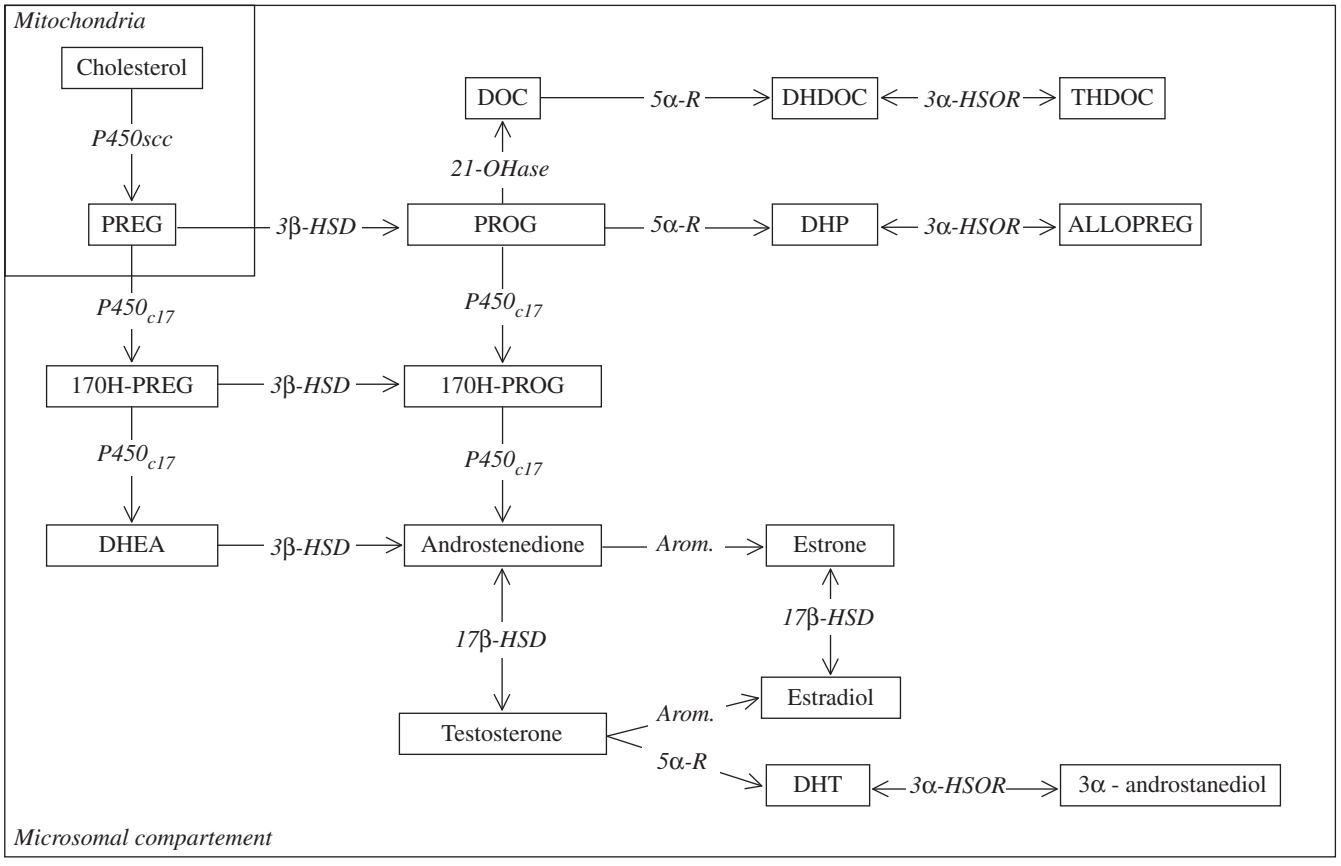

Fig. 8.2 Main biochemical pathways for neurosteroid biosynthesis and metabolism in the vertebrate brain. 17OH-PREG, 17-hydroxypregnenolone; 17OH-PROG, 17-hydroxyprogesterone; DHEA, dehydroepiandrosterone; DOC, deoxycorticosterone; DHDOC, dihydroxydeoxycorticosterone; THDOC, tetrahydroxydeoxycorticosterone; DHP, dihydroprogesterone; ALLOPREG, allopregnenolone; DHT, dihydrotestosterone; P450scc, cytochrome *P*450 cholesterol side chain cleavage; $P450_{c17}$, cytochrome P450c17; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 5α-R, 5α-reductase; Arom., aromatase; 21-OHase, 21-hydroxylase; 3α-HSOR, 3α-hydroxysteroid oxydoreductase;17β-HSD, 17β-hydroxysteroid dehydrogenase.

In the first pathway, PROG is metabolized by the same enzyme as PREG, the cytochrome *P*450c17, which converts PROG into androstenedione with the 17-hydroxyPROG as an intermediate product of reaction. Androstenedione is then converted in a reversible manner into testosterone by another hydroxysteroid dehydrogenase called 17β-HSD. Of note, this enzyme possesses several isoforms, and one of them, 17β-HSD-10, also called ABAD (Aβ binding alcohol dehydrogenase) or ERAB (endoplasmic reticulum-associated amyloid β-peptide binding protein), is in mitochondrial matrix. This isoform was recently linked to AD because of its ability to bind Aβ peptide, thus inducing mitochondrial dysfunction [45]. 17β-HSD is also responsible for the reversible conversion of estrone, an estrogen stemming from aromatization of androstenedione by the enzyme aromatase into estradiol. The second way to synthesize estrogens is via testosterone molecules, which can, in turn, be metabolized into estradiol by aromatase or continued metabolism via the androgen pathway. The 5α-reductase enzyme (5α-R), a microsomal NADPH-dependent protein, intervenes at this level and catalyzes the transfer of two atoms of hydrogen from NADH to form the 5α-reduced metabolite of testosterone, dihydrotestosterone (DHT) [42]. Finally, the enzyme 3α-hydroxysteroid oxido-reductase (3α-HSOR), also called 3α-hydroxysteroid dehydrogenase, catalyzes the reversible conversion of DHT into the neuroactive steroid 3α-androstanediol.

The latter enzymes also intervene at another level, in the second main steroidogenic pathway which starts with PROG. In fact, PROG is successively metabolized by the 5α-R and the 3α-HSOR to form dihydroprogesterone (DHP) and 3α/5α-tetrahydroprogesterone (3α/5α-THP), also known under the name allopregnenolone, another neuroactive steroid.

To finish by the corticoid pathway, molecules of deoxycorticosterone (DOC), stemming from the transformation of PROG by the enzyme 21-hydroxylase (21-OHase), are in turn successively converted into dihydroxydeoxycorticosterone (DHDOC) and tetrahydroxydeoxycorticosterone (THDOC) by the 5α-R and the 3α-HSOR, respectively.

8.3.2 Mechanisms of Action of Neurosteroids

The main role of steroid hormones produced by gonads or adrenal glands is now well defined and consists of a feedback loop on the hypothalamus-pituitary axis, to inhibit or activate their own synthesis. Thus they act at a distance from their glands of origin in an endocrine way. In contrast, neurosteroids are synthesized by the nervous system and act on the nervous system in an autocrine/paracrine configuration [46]. The ability of neurosteroids to cross cellular membranes allows them to act on nuclear receptors and to have a genomic action by regulating gene transcription. This action seems to be important during neonatal life, when it has been shown that neurosteroids, such as PROG or estradiol, are able to promote dendritic growth, spinogenesis, synaptogenesis, and cell survival, particularly in the cerebellum [47]. The most studied steroid nuclear receptors are the estrogen receptors α and β, which are expressed in metabolic tissue such as adipose tissue, skeletal muscle, liver, and pancreas, as well as in the central nervous system. Some studies have demonstrated that these receptors play a role in the regulation of glucose homeostasis and lipid metabolism [48], whereas other studies showed that they were also implicated in neuroprotection [49].

Neurosteroids can also act via membrane receptors and play a role in general as allosteric modulators of neurotransmitter receptors. For example, sulfate esters of DHEA and PREG are known to be excitatory neurosteroids and can inhibit the effect of GABA, an inhibitor neurotransmitter, at physiological concentration by acting via the $GABA_A$ receptor [46]. On the contrary, allopregnenolone is a positive allosteric modulator of $GABA_A$ receptors, strengthening the effects of GABA. PREG sulfate can also potentiate the effect of the main excitatory neurotransmitter glutamate by binding to *N*-methyl-D-aspartate (NMDA) receptors. On the other hand, it is well known that neurosteroids modulate neurotransmitter binding sites or receptors including calcium channels and P2X receptors in the brain, spinal cord, as well as dorsal root ganglia (DRG) [50].

Furthermore, recent clinical and pharmaceutical studies showed that estrogens can interact with several neurotransmitter systems, such as the cholinergic and serotoninergic systems, to influence cognitive performance in animals and humans [51]. Thus neurosteroids seem to play an important role in the nervous system during development as well as in adult brain, by regulating gene transcription and different neurotransmitter systems. Their implication was already demonstrated in several pathologies, such as AD or neuropathic pain [42, 52]. Thus it can be speculated that they might be an important therapeutic target to develop in the next years.

8.4 NEUROSTEROIDS AND OXIDATIVE STRESS

During recent years, a growing body of evidence has shown that neurosteroids, in particular estrogens, are implicated in the regulation of oxidative stress by acting on mitochondria [53]. However, on one hand, depending

on the level of oxidative stress within cells estrogens can have a protective effect or, on the contrary, show a negative action on cell survival. On the other hand, oxidative stress itself can have an effect on neurosteroid production within nerve cells.

8.4.1 Regulation of Neurosteroidogenesis by Oxidative Stress and Aβ Peptide

It is established that steroids can be synthesized by nonglandular tissue within the nervous system. But the regulation of their biosynthesis is still poorly understood. Recent findings showed that several glial cells, in particular oligodendrocytes, upregulated their production of DHEA under oxidative stress conditions induced by treatment with Aβ peptide or Fe^{2+} [54]. Modulation of neurosteroid production was also observed in neuroblastoma (SH-SY5Y) cells overexpressing the key AD proteins, APP/Aβ or Tau (Fig. 8.3) [52]. Indeed, overexpression of human wild-type Tau (hTau 40) protein induced an increase in production of progesterone, 3α-androstanediol, and 17-hydroxyprogesterone, in contrast to overexpression of the abnormally hyperphosphorylated Tau bearing the P301L mutation and leading to a decrease in the production of these neurosteroids. In parallel, a decrease of progesterone and 17-hydroxyprogesterone production was observed in cells expressing human wild-type APP (wtAPP), whereas

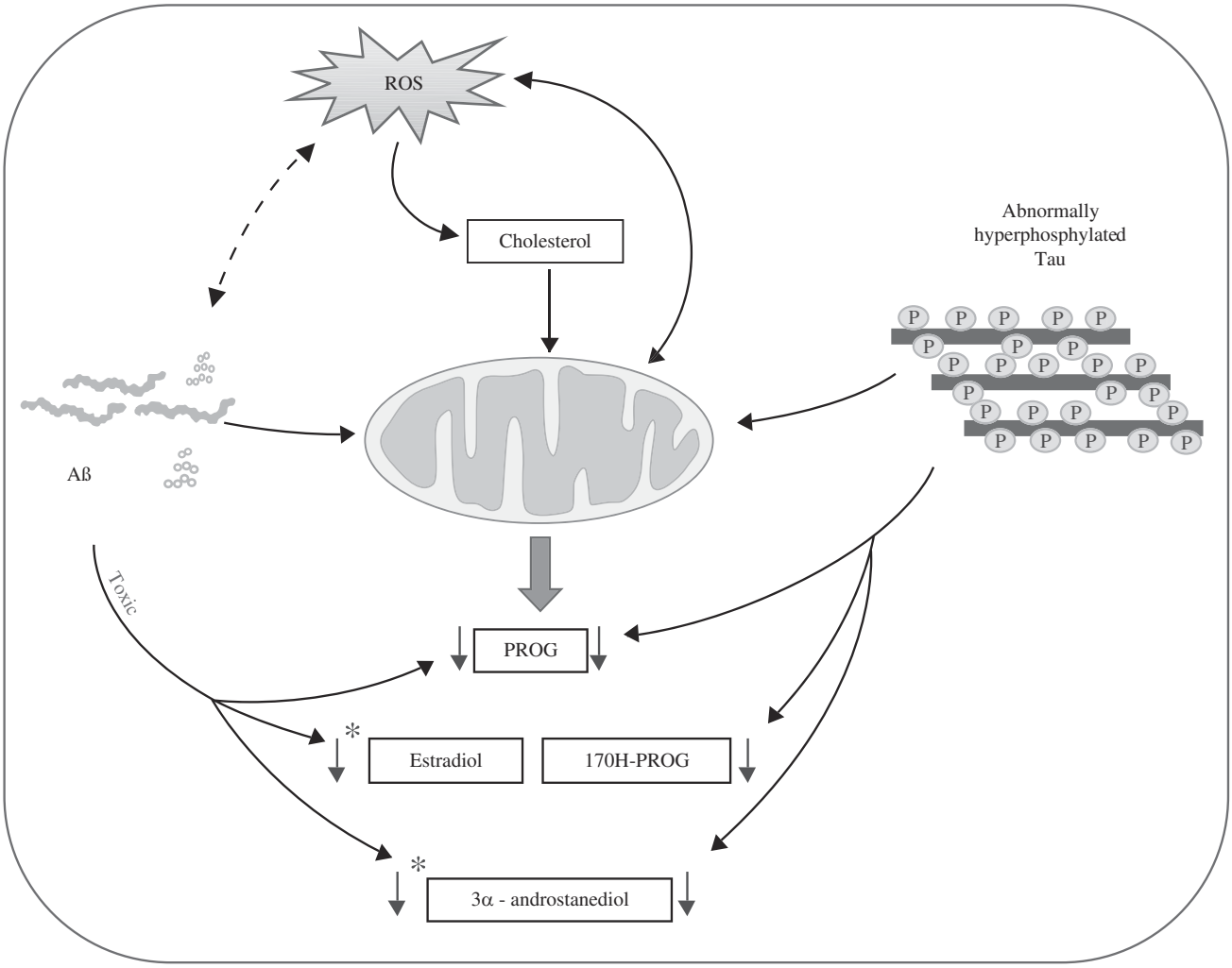

Fig. 8.3 Effect of toxic concentrations of Aβ peptides and abnormally hyperphosphorylated Tau protein on neurosteroid biosynthesis. Aβ induced a drop of the level of progesterone (PROG), estradiol, and 3α-androstanediol by acting on reactive oxygen species (ROS) formation and mitochondrial function and/or directly on steroidogenesis. The presence of abnormally hyperphosphorylated Tau protein had the same effect by inducing a decrease of progesterone, 17-hydroxyprogesterone (17OH-PROG), and 3α-androstanediol. On the other hand, it has been shown that nontoxic concentrations of Aβ induced an increase in estradiol and 3α-androstanediol levels (this pathway is marked by *). *(See color insert.)*

3α-androstanediol and estradiol level were increased. The latter finding was additionally confirmed with in vitro treatment experiments [4]. APPwt SH-SY5Y cells secrete Aβ levels within the nanomolar concentration range. Consistently, treatment of native SH-SY5Y cells with "nontoxic," that is, non-cell death-inducing, $A\beta_{1\text{-}42}$ concentrations in vitro revealed an increase in estradiol production, whereas toxic $A\beta_{1\text{-}42}$ concentrations within the micromolar range strongly reduced estradiol levels revealing the exact opposite effect. Of note, oxidative stress was able to modify neogenesis of neurosteroids in a similar pattern [55]. In fact, treatment with H_2O_2 for 24 h or 48 h induced a decrease of estradiol synthesis that was correlated to a downregulation of the aromatase, the enzyme responsible for estradiol formation from testosterone. Furthermore, an increase of cell death was observed in the presence of letrozole, an inhibitor of aromatase. This suggests that endogenous estradiol formation is very important for human neuroblastoma cells and plays a critical role in cell survival. Interestingly, when cells were pretreated with estradiol, it was possible to rescue neuroblastoma cells from H_2O_2 as well as from letrozole-evoked death. In agreement, similar results were also found in stress condition experiments using heavy metals, such as cobalt and mercury, and once again estradiol was able to reverse their deleterious effect by reducing oxidative stress and β-amyloid secretion [56].

8.4.2 Estrogens and Neuroprotection

Neuroprotective effects of estrogens against a variety of brain injuries have been described for many years. Treatment with 17β-estradiol was able to protect the brain against excitotoxicity, Aβ peptide-induced toxicity, free radical generators, and ischemia in animal studies [53], but the basis of these effects is still poorly understood. It was recognized from former studies that estrogen depletion in postmenopausal women represents a significant risk factor for the development of AD and that an estrogen replacement therapy may decrease this risk and even delay disease progression [57, 58].

However, results from the "Woman's Health Initiative Memory Study" (WHIMS) including 4532 postmenopausal woman aged over 68 years indicated a twofold increase in dementia after 4.2 years of treatment (p.o. treatment with premaxin plus medroxyprogesterone). In addition, it indicated potential risks for breast cancer, pulmonary embolism, and stroke [59, 60]. Besides warrantable criticism with regard to the synthetic hormones used in the WHIMS trial, the outcome results were unexpected and disappointing. One can ask the question, "How could it be that so many scientific studies before the WHIMS trial were wrong?" Thus the currently prevailing view points about the "critical window hypothesis" [16] are asking about the critical period in which one might expect a neuroprotective effect to occur. The results of the WHIMS study also initiated a discussion about a two-edged effect of estradiol. Thus estradiol can possibly also exhibit a "prooxidant effect" in the presence of ongoing oxidative stress [53]. Thereby, estradiol can be hydroxylated to give catecholestrogens that can enter a redox cycle generating superoxide radical. In an oxidative environment, this redox cycling can lead to a continuous formation of ROS that amplifies even more oxidative stress and increases neuronal loss.

On the contrary, animal studies, especially in rodents and transgenic mice models for AD, seem to confirm positive effects of estrogen treatment on the pathophysiology of the disease. It has been shown that treatment with estrogen in mice expressing mutations in human APP (Swedish and Indiana) had an impact on APP processing, decreasing levels of Aβ and so its aggregation into plaques [61]. In triple transgenic AD mice, depletion of sex steroid hormones induced by ovariectomy in adult females significantly increased Aβ accumulation and had a negative impact on cognitive performance [62]. Treatment of these ovariectomized mice with estrogens was able to prevent these effects. Of note, when PROG was administered in combination with estrogens, the beneficial effects on Aβ accumulation were blocked but not effects on cognitive performance. Furthermore, PROG reduced Tau hyperphosphorylation when administered alone. This suggests that estrogen and PROG can interact to regulate APP processing but can also act independently on different AD pathways.

At the cellular level, estrogen was able to activate antioxidant defense systems by reducing ROS production, limiting mitochondrial protein and DNA damage, and improving the activity of the electron chain transport during oxidative phosphorylation [53]. Thus estrogen can have direct antioxidant effects by increasing reduced glutathione (GSH) levels and decreasing oxidative DNA damage in mitochondria of ovariectomized female rats [63]. This is correlated with an upregulation of the expression of two enzymes: manganese superoxide dismutase (Mn-SOD) and glutathione peroxidase, both of them implicated in the antioxidant defense system. Of note, estrogen can modulate the redox state of cells by intervening in several signaling pathways, such as MAPK, G protein-regulated signaling, NF-κB, c-fos, CREB, phosphatidylinositol-3-kinase, PKC, and Ca^{2+} influx [3, 64]. On the basis of this complex mode of action, estrogen seems to be able to decrease oxidative stress markers, including lipid peroxidation, protein oxidation, and DNA damage.

Recently, it has been proposed that estrogens exert their beneficial effects by acting directly on mitochondria

via estrogen receptor β (ERβ) [65]. In fact, incubation of isolated mitochondria from rat brain with estradiol leads to a decrease of H_2O_2 production by this organelle coupled with an increase of the mitochondrial membrane potential. Moreover, estradiol seems to prevent the release of cytochrome *c* by mitochondria (a mechanism known to induce apoptosis of cells by activating the caspase cascade in the cytoplasm), which increases the efficiency of the respiratory chain. In addition, estrogens are able to bind to nuclear receptors, such as estrogen receptor α and β (ER α/β), and to act as transcription factors. Thus estrogens enhanced the expression of the antiapoptotic proteins, Bcl-2 and Bcl-xL, preventing the initialization of the cell death program by mitochondria [3]. They were also able to increase the expression of F1 subunits of ATP synthase and glucose transporter subunits and regulate enzymes involved in the tricarboxylic acid (TCA) cycle, which has the effect of improving glucose utilization by cells.

As described recently, estrogens can have an effect on the transcription of mitochondrial genes, especially on the electron transport chain components [66]. Treatment of ovariectomized female rats with estradiol induced an increase of mitochondrial respiratory function translated into an enhancement of O_2 consumption and coupled to an increased expression and activity of cytochrome *c* oxidase (electron transport chain complex IV).

Finally, another means for estrogens to avoid negative effects of oxidative stress is to regulate calcium homeostasis by inducing mitochondrial sequestration of cytosolic calcium [53]. In fact, an imbalance of calcium handling can lead to an increase of ROS production by activating the enzyme nitric oxide synthase, which can sensitize neural cells to oxidative damage. It has been shown that estradiol treatment of primary hippocampal neurons was able to potentiate glutamatergic response via NMDA receptor, which resulted in an increased influx of calcium in cells. This effect was coupled with an induction of mitochondrial sequestration of cytosolic calcium and an increase of the mitochondrial calcium load tolerability, to avoid calcium-induced excitotoxicity and to promote cell survival.

8.5 CONCLUSION

In summary, it is now clear that oxidative stress is an important actor involved in AD pathophysiology and intervenes already at an early disease stage. Furthermore, good evidence is provided that neurosteroids, such as estrogens, are able to limit oxidative damage by reducing lipid peroxidation, protein oxidation, Ca^{2+} overload in cytosol, and DNA damage in mitochondria as well as in the nucleus. These effects are mediated by several mechanisms, from transcription of genes coding for antioxidant enzymes to the regulation of antiapoptotic pathways, by way of improvement of mitochondrial respiratory chain efficiency and glucose metabolism. Thus, with their abilities to counter excess oxidative stress, estrogens seem to be able to prevent AD-related toxic mechanisms, such as Aβ peptide aggregation, Tau hyperphosphorylation, and neuronal loss. Better human studies taking into account the critical window hypothesis are essential before drawing a final conclusion on efficacy of neurosteroids in prevention of AD.

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