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Molecular Mechanisms Underlying Heme Action in Promoting the Pathogenesis of Alzheimer's Disease

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ABSTRACT

Heme is a central molecule for mitochondrial respiration and for all processes involved in oxygen utilization. Heme serves as a prosthetic group in many oxidative phosphorylation enzymes and oxygen-utilizing hemoproteins. Heme also directly regulates the synthesis, translocation, and assembly of these enzyme complexes. Most, if not all, human cells can synthesize heme de novo and uptake heme from circulation. Cells that have increased demand for cellular energy generation via oxidative phosphorylation, such as neuronal cells and many cancer cells, are known to intensify heme biosynthesis and/or uptake. Hence, it is not surprising that recent studies have heavily implicated heme in the pathogenesis of Alzheimer's disease (AD). Heme readily complexes with amyloid beta (Aβ) peptides. Notably, three amino acid residues—Arg5, Tyr10, and His13—in the Aβ peptide that are unique to at-risk humans but not risk-free rodents play essential roles in heme binding, supporting the idea that heme binding is important for AD pathogenesis. The heme-Aβ complex possesses demonstrable capacity to produce reactive oxygen species (ROS)

and to provide peroxidase activity. Hence, this complex can cause increased oxidative stress and the generation of ROS and radicals in the brain. Increased oxidative stress ultimately leads to the oxidation of various neurotransmitters and synaptic proteins, as well as increased Aβ peptide aggregation and elevated levels of nitrated proteins. These events lead to neuronal dysfunction and the accumulation of neurotoxic compounds, thereby causing extensive neurotoxicity and AD pathogenesis. This chapter reviews recent biochemical studies illuminating the mechanism by which heme interacts with the Aβ peptide and causes neurotoxicity. It also summarizes non-heme factors that may reduce or increase AD neurotoxicity and pathogenesis.

Keywords: Heme; Alzheimer's disease**;** Amyloid beta peptide; Peroxidase; ROS

ABBREVIATIONS

AD-Alzheimer's Disease**;** ALAD: 5-Aminolevulenic Acid Dehydratase; ALAS-Aminolevulinic Acid Synthase; Aβ- Amyloid Beta; CPO-Coproporphyrinogen Oxidase; FLVCR1-Feline Leukemia Virus Subgroup C Receptor 1; HCP1-Heme Carrier Protein 1; HMB-Hydroxymethylbilane; HO-Heme Oxygenase; HRG1-Heme-Responsive Gene 1; PBG-Porphobilinogen; PBGD-Porphobilinogen Deaminase; PPO-Protoporphyrinogen Oxidase; PROS-Partially Reduced ROS; ROS-Reactive Oxygen Species; TCA-Tricarboxylic Acid; UROD-Uroporphyrinogen Decarboxylase; UROS-Uroporphyrinogen III Synthase

INTRODUCTION

Heme, also known as iron protoporphyrin IX, is an essential molecule for human survival [1]. Heme is a central molecule for mitochondrial function and for all processes involved in oxygen utilization [2,3]. Heme can coordinate two axial ligands (Figure 1). One ligand is often an amino acid residue from proteins, which allows heme to bind to proteins. Another ligand can be an amino acid residue from proteins or a gaseous molecule, which enables hemoproteins to bind gaseous molecules. This property of heme enables it to perform diverse biological functions [1]. For example, heme serves as a prosthetic group in several oxidative phosphorylation enzymes and other oxygen-utilizing hemoproteins. Heme also directly regulates the synthesis, translocation, and assembly of these enzyme complexes [4]. Most, if not all, human cells can synthesize heme de novo and uptake heme from the circulation [3-6]. It is also worth noting that a plethora of epidemiological studies have shown that high heme intake is associated with increased risk of several cancers, type 2 diabetes, and myocardial infarction [[3] and references therein]. However, heme deficiency can also cause serious disorders, such as anemia and porphyrias [7]. Recent experimental evidence increasingly suggests that functional heme deficiency is a major factor contributing to the pathogenesis of AD disease [8,9]. In this review, we discuss and summarize the latest research investigating the molecular mechanisms by which heme impacts the pathogenesis of AD.

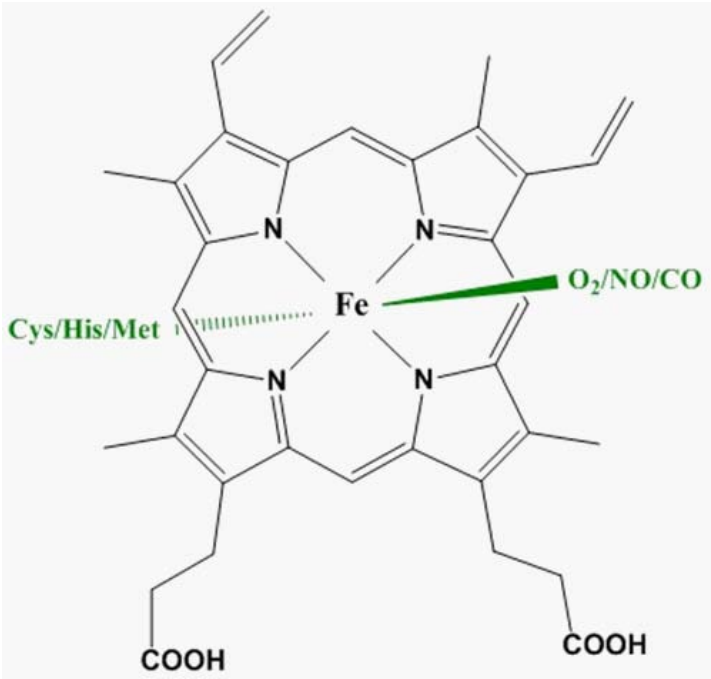

Figure 1: The structure of heme. Heme is composed of a macrocycle of four pyrrole rings, with four methyl groups, two vinyl groups and two propionate groups attached. The four nitrogen atoms of pyrrole rings coordinate the heme iron ion. Iron ion can coordinate two axial ligands, which may be Cys, His or Met residue in proteins or small molecules, including oxygen, nitric oxide and carbon monoxide.

HEME CAN BE ACQUIRED BY THE HUMAN BY INTAKE AND DE NOVO SYNTHESIS

Ubiquitous among organisms, heme is acquired through diet, synthesized, or both [1,3]. Dietary heme is present in a wide array of sources. Heme is most concentrated in meat, especially red meat. Heme is our primary source of iron, which is the most abundant metal in the human body. Heme is not well-absorbed in the stomach due to low gastric pH, instead being absorbed by enterocytes via vesicular transport facilitated by HCP1 and HRG-1 as it enters the small intestine and the acidic environment is neutralized (Figure 2). From there, a heme exporter known as FLVCR1 releases heme from the vesicles into the blood [10]. From there, heme is often used to synthesize hemoproteins or degraded to free iron by heme oxygenase, or HO. This heme degradation determines the rate of heme absorption.

Most, if not all, human cells can synthesize heme. This process is enabled by 8 enzymes, which are part of either a housekeeping or erythroid-specific set [1,7]. In erythroid cells, heme biosynthesis is controlled by the erythroid-specific set of enzymes (Figure 3). Outside of

erythroid cells, the housekeeping enzymes control synthesis. Both succinyl-CoA and glycine act as a substrate for heme synthesis. The first step of heme biosynthesis condenses succinyl CoA, which is produced by the TCA cycle, and glycine, which is an abundant amino acid present in the cell, to form 5-aminolevulinic acid, or 5-ALA. This step is assisted by the enzyme aminolevulinic acid synthase, or ALAS (Figure 3). ALAS1 is the housekeeping analogue for the erythroid-specific Period after ALAS2 The process takes place within the mitochondria [7].

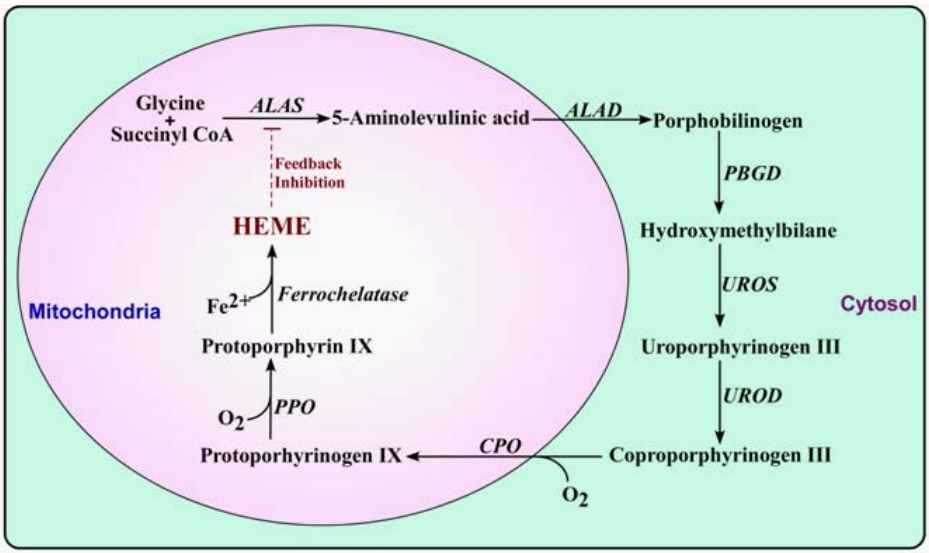

Figure 2: The heme biosynthetic pathway. In mammals, the first and the last three steps of heme biosynthesis occur in the mitochondria, and the other four steps takes place in the cytosol. The citric acid cycle (also known as the TCA or Krebs cycle) provides the succinyl CoA needed for heme biosynthesis, and the amino acid glycine is found readily in the cell. The first step in heme biosynthesis is a condensation reaction between glycine and succinyl CoA, resulting in 5-aminolevulinic acid (ALA), and is catalyzed by 5-aminolevulinic acid synthase (ALAS). ALAS1 is a housekeeping enzyme, while ALAS2 is an erythroid-specific enzyme. The second step is carried out by ALA dehydratase (ALAD), which catalyzes the condensation of two molecules of ALA to form porphobilinogen (PBG). Porphobilinogen deaminase (PBGD) catalyzes the condensation of four molecules of PBG to generate hydroxymethylbilane (HMB), a linear tetrapyrrole. Uroporphyrinogen III synthase (UROS) catalyzes the intramolecular rearrangement and ring closure of HMB to form uroporphyrinogen III, a cyclic tetrapyrrole. It is then converted to coproporhyrinogen III by uroporphyrinogen decarboxylase (UROD) by decarboxylation and formation of methyl groups. The next step is the decarboxylation and oxidation of coproporphyrinogen III, to form protoporphyrinogen IX, which is catalyzed by coproporphyrinogen oxidase (CPO). Protoporphyrinogen oxidase (PPO) catalyzes the formation of methene bridges in protoporphyrin IX. In the last step of heme biosynthesis, ferrochelatase inserts ferrous iron (Fe^{2+}) into the center of protoporphyrin IX, and heme is formed. Heme exerts a feedback inhibition on ALAS.

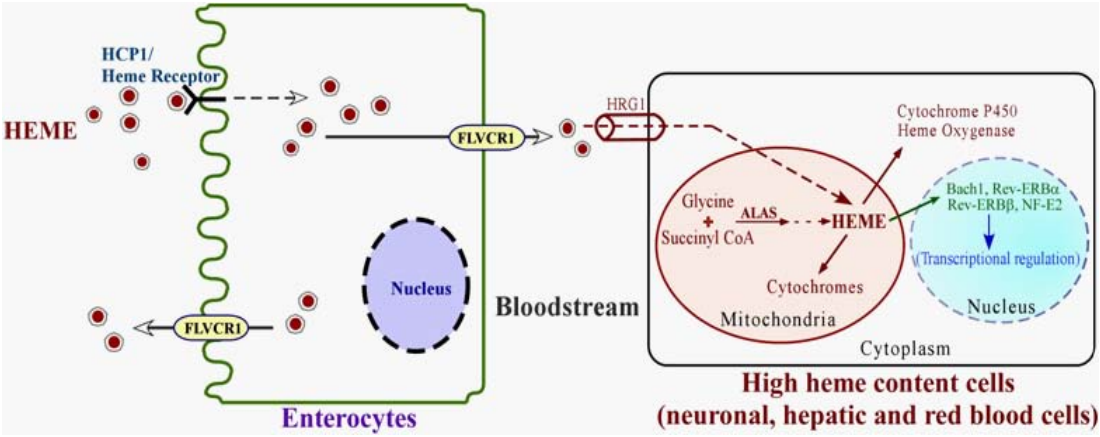

Figure 3: Human cells can acquire heme via de novo heme biosynthesis and uptake from the circulation. Heme uptake to enterocytes is facilitated by the heme transporter or heme receptor (HCP1). Then, a fraction of intact heme is released directly into the blood stream via heme transporter FLVCR1. FLVCR1 exports cytoplasmic heme, and it can export heme into the lumen during elevated cellular heme content to protect from heme toxicity. Heme also can be taken up from the blood stream directly by neuronal, hepatic and red blood cells via HRG1. Heme serves as a prosthetic group in numerous enzymes and proteins that transport, store, or use oxygen, such as mitochondrial cytochromes and cytochrome P450. Heme also regulates the activity of cellular signaling and regulatory molecules, such as Bach1, Rev-ERBα, Rev-ERBβ. HCP1, heme carrier protein 1; HRG-1, heme responsive gene-1; FLVCR1, cell surface receptor for feline leukemia virus, subgroup C, cellular receptor 1.

The second step of heme biosynthesis converts 5-ALA to porphobilinogen, or PBG. Two molecules of 5-ALA condense to form one molecule of PBG, and this reaction, which takes place within the cytosol, is allowed by 5-aminolevulenic acid dehydratase, or ALAD. Both versions of ALAD are coded by a single gene, but each form is unique because of alternative gene splicing [7]. The third step of heme biosynthesis occurs in the cytosol when the enzyme porphobilinogen deaminase, or PBGD, combines four PBG molecules to create hydroxymethylbilane, or HMB. HMB contains the four pyrrole groups of heme, albeit in a linear fashion as opposed to a cyclic one. Alternative splicing of mRNA leads to the formation of two separate versions of PBGD for the erythroid and housekeeping sets of enzymes. The fourth step of heme biosynthesis occurs in the cytosol as well, and it is catalyzed by uroporphyrinogen III synthase, or UROS. In this step, the PGBD rearranges and forms a ring. The end product is uroporphyrinogen III. Again, alternative splicing contributes to the difference between erythroid and housekeeping versions of UROS [1].

Next, uroporphyrinogen decarboxylase, or UROD, forms coproporphyrinogen III from uroporphyrinogen III. This enzyme decarboxylates and forms methyl groups. This takes place in the cytosol. The gene responsible for this enzyme has only one promoter [7]. The sixth step of

heme biosynthesis includes the decarboxylation and oxidation of coproporphyrinogen III. This forms protoporphyrinogen IX, and the reaction is enabled by coproporphyrinogen oxidase, or CPO. This step, and all successive steps, take place in the mitochondria. The seventh step of heme biosynthesis allows the formation of methene bridges in protoporphyrinogen IX via the enzyme protoporphyrinogen oxidase, or PPO. This forms protoporphyrin IX. Finally, iron is added to the center of the protoporphyrin IX by ferrochelatase. The product of this reaction is heme.

HEME DEFICIENCY CAN CAUSE SERIOUS DISEASES IN HUMANS, INCLUDING AD

Each of these enzymes can malfunction, and each malfunction has a specific physiological consequence. Genetic malfunctions of heme pathway enzymes are known as porphyrias [7]. Porphyrias can have very severe symptoms, ranging from neurological decline to photosensitivity of the skin. In fact, porphyrias are believed by some to be the origin of werewolf and vampire mythologies [1]. Notably, recent experimental evidence increasingly suggests that lack of functional heme underlies at least part of AD pathogenesis [9,11]. Altered heme metabolism in the brain is part of the AD pathology. Heme deficiency causes molecular and biochemical consequences similar to that of AD, including mitochondrial dysfunction and mitochondrial complex IV loss, energy hypometabolism, loss of iron homeostasis, dimerization of Aβ precursor protein, and oxidative stress [11-13]. The most commonly noted AD pathologies are the extracellular amyloid plaques and intracellular neurofibrillary tangles [14,15]. The AD brain also has abnormally high levels of certain metal ions, such as aluminum ions [16]. In addition, it has very low levels of cytochrome c function, and very low levels of ALAS1 mRNA and PBGD mRNA enzyme functions [17]. ALAS1 mRNA has been shown to be reduced 90%, and PBGD mRNA has been shown to be reduced 60% [17]. These are the primary and secondary rate-limiting enzymes in the heme biosynthetic pathway, respectively [17]. Heme-a, on the other hand, is thought to be drastically increased in the cerebrum. Compared to controls, it has been shown to be increased two-fold in the AD brain [17]. From this information, it has been speculated that AD pathogenesis is caused by consequential cell metabolism dysfunction or iron homeostasis dysregulation. The Aβ peptide is heavily implicated in AD pathology [11,15]. Aβ is a primary contributor to amyloid plaques. The lipophilic site (L site, Figure 4), containing amino acid residues 17-42, are identical among all species with or without the development of AD pathology [12]. All species which exhibit AD pathologies share the same sequence Aβ [13] (Figure 4). Rodents, which do not exhibit AD pathologies, have a differing sequence (amino acid residues 1-16) which varies by three amino acids, Arg5, Tyr10, and His13 [12,13,18,19]. Transgenic mice expressing Aβ with the human sequence can display AD pathologies [13]. These three amino acids are thought to be crucial to the understanding of AD pathogenesis [12,13].

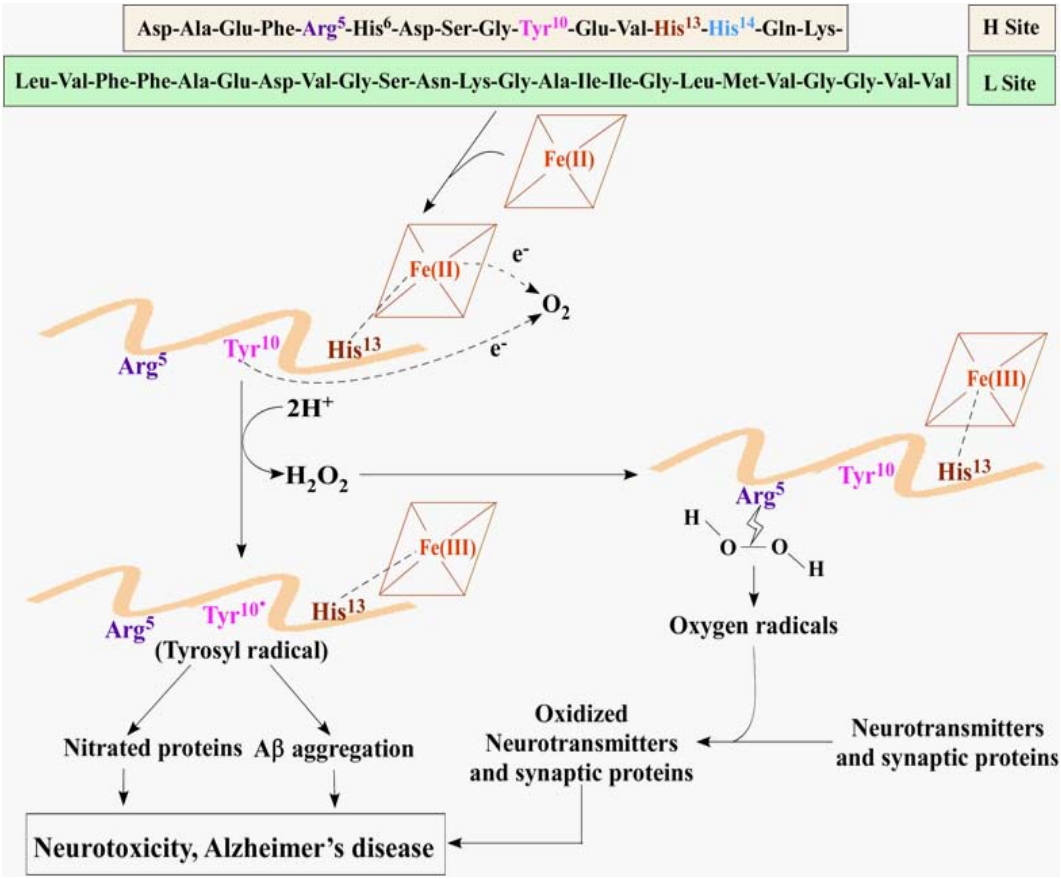

Figure 4: Molecular events underlying neurotoxicity of the heme-Aβ complex. When heme (pictured above as a ring with an iron center) binds to Aβ (pictured above as a peach-colored ribbon) at the His13 residue, free heme diminishes, causing functional heme deficiency, and forming ROS. The iron center in heme and the Tyr10 residue donate one electron each. These electrons reduce O_2, which disproportionates to H_2O_2. The Arg5 residue splits the H_2O_2 at the O-O bond, generating oxygen radicals. These oxygen radicals are responsible for the nitration of proteins, which can affect conformation and therefore function. The ROS can also oxidize neurotransmitters, diminishing neurotransmitter levels and creating neurotoxic products.

HEME-Aβ COMPLEX FORMATION PLAYS A CRITICAL ROLE IN INCREASING OXIDATIVE STRESS IN THE BRAIN

Many lines of experimental data have shown that heme interacts with Aβ [8,12,18,20-22]. Human Aβ Arg5, Tyr10, and His13 amino acids are thought to be important because they distinguish the immune rodent Aβ from the at-risk human Aβ [12,13,18,19]. Aβ has two binding sites, the hydrophilic heme binding motif, known as site-H, and the lipophilic region, also known

as site-L [21,22] (Figure 4). Heme first binds to site-H, which displaces the iron out of the plane of the tetrapyrole ring. Binding to site-H is hypothesized to expose site-L. There is no difference between site-L in rodents and in humans. Human Aβ binds to heme much more efficiently than rodent Aβ. The K_d of Aβ to site-H is 140+/-60 nM. The K_d of Aβ to binding site-L is 210 +/- 80 nM [13].

Heme binds to Aβ via His13 [8,21]. Once bound, the iron center of heme-Aβ complexes can offer one electron to O_2, and the Tyr10 residue of the Aβ peptide likely offers another electron [8]. Consequently, H_2O_2 is formed, and the Tyr10 residue of the Aβ peptide becomes a radical (Figure 4). The formation of reactive oxygen species (ROS) is decreased by roughly half when Tyr10 is omitted. The tyrosyl radical then can cause Aβ aggregation and permits the nitration of the Aβ peptide [18,23]. Dityrosine cross-linking and tyrosine nitration accelerate the aggregation process. Both of these processes occur when the Tyr10 residue forms a tyrosyl radical [8,19,23]. Additionally, the oxygen-oxygen bond of H_2O_2 can be attacked by the Arg5 residues, forming oxygen radicals, which can oxidize neurotransmitters and synaptic proteins [21,24,25] (Figure 4).

In addition to simple heme-Aβ interactions, recent literature suggests that copper ions are also important in promoting the generation of ROS and oxidative stress in neurons [14,26,27]. Heme-Aβ-Cu complexes stimulate Aβ aggregation. Although it was once believed that copper could bind to three histidine residues simultaneously, it has since been demonstrated that one of the histidines binds instead to heme [18,22,27]. Aβ peptides can be complexed with heme or copper ions to produce peroxidase activity, but maximum neurotoxicity results from the maximum quantity of partially reduced ROS created. The culmination of all three substrates into one toxic complex appears to have the most damaging effect [26-28].

PEROXIDASE ACTIVITY OF THE HEME-Aβ COMPLEX CAUSES NEUROTRANSMITTER OXIDATION AND PROTEIN NITRATION

Neurotransmitters impact an array of physiological processes including memory, mood, appetite, and movement, all of which are afflicted by AD [15]. Peroxide produced by complexes of Aβ attack several forms of neurotransmitters [29,30]. Serotonin is a well-studied neurotransmitter affected by AD [30]. Serotonin is crucial for memory, mood, appetite, sleep, movement, and more. Heme-Aβ complexes have the ability to catalyze oxidative degradation of serotonin, which alone would have major complications in the human body. Further, the products formed by this critical reaction, dihydroxybitryptamine and tryptamine-4,5-dione, are neurotoxic, causing yet more damage in the AD brain [30]. The oxidative degradation of serotonin and likely other neurotransmitters is first order relative to both heme-Aβ complexes and peroxide, and is optimal at physiological pH. This is due to Arg5, a residue which is present in the H-site of human Aβ but absent in rodent amyloid beta. Arg5 has been demonstrated to function as a second-sphere residue catalyzing O-O bond cleavage (Figure 4) [21]. When Arg5 is mutated, serotonin oxidation is diminished. Tyr10 is also implicated in serotonin oxidation [30].

Many proteins are significantly nitrated in AD [19,23]. Aβ has been shown to increase the levels of NO. Under conditions of oxidative stress, NO can form NO_2^-. Due to the demonstrably high level of peroxidase activity present in the heme-Aβ complex, it has been hypothesized that abnormally high levels of protein nitration characteristic of AD brain may be generated through the Aβ–heme peroxidase–NO_2^-–H_2O_2 pathway [19]. Upon nitration, the conformation of the protein may change, which therefore affects the activity of the protein. Furthermore, significant oxidative damage may be inflicted upon these proteins, further inhibiting or altering protein function. According to Yuan [19], protein nitration and oxidation caused by Aβ has a demonstrable correlation with tau fibrillation, cholinergic dysfunction, mitochondrial dysfunction, oxidative stress, and importantly cognitive impairment.

As β has been demonstrated to bind tightly with heme, producing a complex with high peroxidase activity, two phenomena may be expected. Firstly, regulatory heme is diminished, leading to heme deficiency [9,31]. This is known to decrease levels of mitochondrial complex IV, a known symptom of AD [11,32]. Secondly and consequently, Aβ-heme complexes can generate ROS or partially reduced ROS (PROS). It has been proposed that mitochondrial complex IV deficiency yields ROS. Thirdly, high levels of Aβ is associated with protein nitration [19,23].

One major protein which is nitrated by this pathway is enolase [19,23]. Enolase catalyzes the second to last step in glycolysis, therefore it is crucial for energy metabolism. Its dysfunction may cause a wide array of symptoms in AD. In the presence of a heme-Aβ complex, enolase nitration increases over three-fold [19,23]. Likewise, synaptosomes also exhibit greatly increased nitration in the presence of an Aβ complex. $Aβ_{1-42}$ increases nitration 1.65-fold, and $Aβ_{1-40}$ increases nitration 3.6-fold [19]. Mechanistically, Yuan [19] proposed that heme-Aβ complex binds non-covalently to enolase at varying sites, and the nitration of proximal tyrosine residues is enabled. Heme alone binds preferentially to amino acid Tyr12, whereas either heme-$Aβ_{1-40}$ complex or heme-$Aβ_{1-42}$ binds preferentially to Tyr191 and Tyr426, signifying a shift in nitration site selectivity after either peptide is added to heme. This property probably extends beyond interactions with enolase alone.

Heme-$Aβ_{1-42}$ is less effective at nitration of proteins, and has lower peroxidase activity than heme-$Aβ_{1–40}$ [26,33]. This appears to be due to heme's high affinity for the larger peptide sequence, which is evidenced by both the additional binding site for $Aβ_{1–42}$ present in heme as well as the increased rate of fibrillation characteristic of heme-$Aβ_{1–42}$. This larger complex forms aggregates, which certainly exacerbate heme deficiency in AD patients. Alternatively, heme-$Aβ_{1–40}$ has higher peroxidase activity, which leads to other symptoms characteristic of AD, such as degradation of neurotransmitters—and therefore formation of neurotoxic products—and nitration—and therefore dysfunction— of proteins. Consequently, the elevation of both complexes is thought to be critical for AD pathogenesis [33].

HEME OXYGENASE CAN ALSO IMPACT AD PATHOGENESIS

Considering that oxidative stress is a major factor in AD pathogenesis, it should come as no surprise that heme oxygenase, an antioxidant adaptive responder to stress, has been implicated in AD [34-38]. Heme oxygenase plays a vital role in heme degradation. It catalyzes the reaction of heme into free iron. Humans have both heme oxygenase 1, which is approximately 32 kDa, and heme oxygenase 2, which is about 36 kDa [39,40]. These are coded for by HMOX1 and HMOX2, respectively. Biliverdin reductase-A helps reduce biliverdin to bilirubin by dissociating heme oxygenase from biliverdin. Heme oxygenase levels increase in neocortical and vascular tissues of the AD brain [41].

If heme oxygenase 1 and biliverdin reductase-A become impaired, their neuroprotective properties are also impaired. To help reestablish the function of heme oxygenase-1, phosphorylation may occur. However, if phosphorylation continues for extended periods of time, increasing oxidative stress in the hippocampus [36]. The overwhelmed heme oxygenase/biliverdin reductase-A system, therefore, does more harm than good [42]. Both heme oxygenase 1 and 2 are overexpressed in the hippocampi, and heme oxygenase 2 is also overexpressed in the astrocytes. Heme oxygenase 2 is more strongly implicated than heme oxygenase 1 in early AD pathogenesis. It was suggested that heme oxygenase 1 overexpression may cause neurofibrillary tangles to form [34].

A VARIETY OF COMPOUNDS ARE KNOWN TO AMELIORATE SYMPTOMS OF AD

Hemin helps regulate protein folding, which guards peptides like Aβ against aggregation [43]. Hemin has been shown to inhibit $A\beta_{1-42}$ from forming beta sheet structures. Beta sheet formation is strongly correlated with amyloid plaque formation. Therefore, hemin may prevent amyloid plaques from forming. Furthermore, hemin may also reduce cytotoxicity of existing amyloid plaques. Partially formed fibrils are disabled by hemin, and amorphous aggregation is also inhibited by hemin [43]. Additionally, methylene blue and 8-hydroxyquinoline both help dismantle small copper- Aβ aggregates; however larger fibrils are not as strongly affected, thus increasing their toxicity [26]. Methylene blue also raises levels of mitochondrial complex IV approximately 30%, escalates levels of heme synthesis, increases oxygen consumption rates by approximately 37-70%, and helps to undo damage done by both cadmium and hydrogen peroxide. Methylene blue is thought to inhibit senescence by inhibiting reactive oxygen species formation. Importantly, methylene blue can easily be reduced from methylene blue to leucomethylene blue. This ability to cycle between reduced and oxidized states enables methylene blue's neuroprotective abilities [44].

Nitric oxide synthase is down-regulated in AD patients, which suggests that perhaps the lack of nitric oxide has deleterious effects on AD patients [15]. Nitric oxide is a vital signaling molecule which aids in memory formation, among other things. Aggregated Aβ hinders the nitric oxide

signaling pathway and disables nitric oxide's neuroprotective abilities [37]. However, some research suggests that nitric oxide also disrupts the bond between Aβ and heme, effectively breaking the complex into its component pieces and reducing toxicity [25]. Nitric oxide oxidizes copper site, His14, which would otherwise donate an electron to produce reactive oxygen species (Figure 4). Together, these effects suggest that nitric oxide may lessen the effects of heme deficiency and decrease the levels of reactive oxygen species, and thereby protect important neurotransmitters [25].

Cytochrome *c* has neuroprotective qualities. It has demonstrated an ability to suppress the formation of reactive oxygen species, which prevents the oxidation of important chemicals, such as neurotransmitters [45]. Cytochrome *c* is found in the intermembrane space of mitochondria. It signals apoptosis and aids in cellular metabolism. Much like nitric oxide is hypothesized to accept an electron from copper bound to amyloid beta, cytochrome *c* is hypothesized to oxidize the iron center in heme, thereby inhibiting the formation of reactive oxygen species [45].

HEAVY METALS EXACERBATE AD PATHOGENESIS

As has been discussed earlier in this chapter, copper can bind to the His14 residue of the H-site of the Aβ peptide. It donates an electron to oxygen, just as heme and the Tyr10 residue do [8]. Copper can reduce oxygen in the presence of heme or in the absence of heme. When the copper Aβ complex is compared to the heme Aβ complex at physiological potentials, the copper Aβ complex produces more reactive oxygen species per mole of O_2 [14]. Other metals, including aluminum, iron, calcium, and magnesium have been shown to reduce the V_{max} of the active site for cytochrome *c* oxidase [28,32,46,47]. AD is characterized by both low cytochrome *c* oxidase activity and high levels of metal ions [15]. The metal ions are thought to increase ROS levels, which then attack mitochondria and cytochrome *c* oxidase. This would cause lowered levels of adenosine triphosphate synthesis and increased heme levels, which in turn causes further ROS increase. These circumstances are all thought to promote neurodegeneration.

In summary, recent experimental evidence suggests a model for how heme may promote AD pathogenesis. Initially, in the AD brain, heme binds to Aβ causing functional heme deficiency. Additionally, the heme-Aβ complex enables ROS generation and provides peroxidase activity. This in turn causes the oxidation of neurotransmitters, nitration of proteins, and mitochondrial dysfunction. Consequently, neuronal functions are disrupted, and neurotoxic products accumulate, leading to AD pathogenesis.

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