Experimental Research on Nitric Oxide and the Therapy of Alzheimer Disease: A Challenging Bridge

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by severe cognitive impairment due to neuronal death. Although the lost of cognitive function is the main problem for AD subjects, death occurs due to secondary issues such as concomitant infections, respiratory complications or multi-organ failure. Current drugs used in AD are acetylcholinesterase inhibitors and antagonists of the N-methyl-D-aspartate receptor. These drugs may only slightly improve cognitive functions but have only very limited impact on the clinical course of the disease. Over the last 5 years, new targets were identified and innovative drugs against AD have been designed and developed. Worthy of mention are β-secretase inhibitors, monoclonal antibodies against amyloid-β-peptide and tau inhibitors. However, although promising beneficial effects were highlighted in the data from preclinical studies, only few of these new drugs improved cognitive functions for a significant time frame in AD subjects. Controversial is the therapeutic effect on AD obtained through the manipulation of the nitric oxide synthase/nitric oxide system since the potential toxic effects on brain function could overcome the beneficial effects. The aim of this review is to analyze from a pharmacologic point of view both old and new drugs developed for the treatment of AD. In addition, the risk/benefit ratio related to the modulation of the nitric oxide synthase/nitric oxide system in AD brain will be analyzed.

Keywords: Acetylcholinesterase inhibitors, Alzheimer disease, amyloid-β-peptide, memantine, nitric oxide, secretases, tau protein.

INTRODUCTION

Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by cognitive and memory decline, speech loss, and changes in personality. It is one of the major causes of admission to nursing homes [1, 2]. Alzheimer disease affects approximately 35 million people worldwide including 5.5 million people in the United States [2]. Epidemiological data show that the incidence of AD increases with age and doubles every 5 years after 65 years of age with 1275 new cases/100000 persons/year [2]. The prevalence of AD was estimated to be about 1% in persons aged 60-64 but increases up to 33% in people aged 85 or older in the Western hemisphere [3]. However, the annual incidence worldwide ranges from 1% to 7% at the ages of 70 and 85, respectively [4]. Sporadic AD is the more common form of the disease, accounting for 90% of all cases, whereas only 1% accounts for the familial form [1]. Although it was not definitely proven, most of the sporadic AD is associated with the ε4 allele of the apolipoprotein E (APOE), a plasma protein implicated in the transport of cholesterol, which binds amyloid-β-peptide (Aβ) [1, 5]. On the other hand, familial AD is an autosomal dominant disorder, whose early onset was associated with mutations in specific genes such as amyloid-β precursor protein (APP) located on chromosome 21, presenilin 1 on chromosome 14, and presenilin 2 on chromosome 1 [1]. Despite the huge amount of data derived from preclinical and clinical studies about the pathogenesis of AD, a limited number of drugs were developed over the last years.

The aim of this paper is to provide a focused pharmacological point of view on the commercially available drugs used to treat AD as well as some new drugs still under development.

PROPOSED PATHOGENESIS OF AD

Amyloid-β-Peptide, Tau Protein and Neuronal Damage

Post-mortem analysis of human AD brains reveals a variable degree of cortical atrophy with narrowed gyri and widened sulci most apparent in the frontal, parietal and temporal lobes. Microscopically, the most frequent features include both extracellular deposits of Aβ which forms the central core of senile plaques (SP) and intracellular deposits of hyperphosphorylated tau protein, the latter being a microtubule-associated protein, which forms neurofibrillary tangles [2, 6]. These pathological lesions selectively affect neurons in specific brain regions, in particular the neocortex, entorhinal area, hippocampus, amygdala, basal nucleus of the anterior portion of the thalamus and several brainstem monoaminergic nuclei [7]. These brain areas are endowed with high cholinergic activity and this likely contributes to the impairments of cognitive and memory functions characteristic of AD patients.

Amyloid-β-peptide contains 39-43 amino acids and is produced by serial cleavages of the APP by β- and γ-secretases (for further information see below). Once formed, Aβ tends to form spontaneous aggregates in the form of oligomers or fibrils [2, 6, 8]. Amyloid-β oligomers and fibrils can be destroyed by neurons *via* a ubiquitin-proteasome-dependent process known as the *unfolded protein response* [9, 10]. In addition, Aβ can be effluxed from the brain by the low density lipoprotein receptor-related protein 1 (LRP-1), which in AD is oxidized and dysfunctional [11]. When these measures are not sufficiently efficient, there is an excessive build-up of Aβ that can trigger the onset of AD. This idea is known

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as the "amyloid hypothesis" that posits that fragment Aβ(1-42), and less likely Aβ(1-40), are both responsible for the onset of AD through their toxicity to cells. In addition, Aβ deposition increases in the brain of people with Down syndrome because the gene codifying for APP is located on chromosome 21, which is present in three copies in people with this genetic disease [1]. This explains the early onset of AD in people with trisomy 21 [1].

Tau is a soluble protein whose role is to assure a correct assembly and stability of microtubules and vesicle transport. In AD, tau undergoes hyperphosphorylation and experimental data obtained in cultured cells and transgenic mice suggest that kinases like the p25/cdk5 complex [12] or GSK-3β [13] are involved. As a result, the protein becomes insoluble, its affinity for microtubules declines, tau aggregates with a double-helix secondary structure and forms neurofibrillary tangles. As with Aβ, phosphorylated tau that is not efficiently destroyed by the proteasome accumulates and exerts neurotoxic effects [14].

Amyloid-β-peptide induces neuronal damage through several mechanisms, the main one of which depends on the induction of oxidative and nitrosative stress [8, 15, 16]. In addition to its direct cytotoxic effects, Aβ synergizes with products of lipid peroxidation, such as isoprostanes and 4-hydroxy-2-nonenal (4-HNE), to promote tau phosphorylation and aggregation [2] (Figs. **1**, **2**). It is well known that Aβ stimulates the production of reactive oxygen species (ROS) which can induce neuronal damage either directly or by reacting with NO, generated by the inducible isoform of nitric oxide synthase (iNOS or NOS-2) up-regulated in microglial cells, thus forming reactive nitrogen species (RNS) such as peroxynitrite [2, 17] (Fig. **1**). Both ROS and RNS oxidize protein, lipids and nucleic acids thus compromising cell function. Several brain proteins undergo oxidative modifications, indexed by increased carbonyl levels, protein-bound 4-HNE and glutathionylation, and contribute to cell damage. Among these dysfunctional proteins it is noteworthy to mention those involved in energy production (α-enolase, aconitase, aldolase, ATP-synthase, creatine kinase BB), antioxidant defense (Mn-superoxide dismutase, peroxyredoxins, heat shock cognate 71), synaptic and cholinergic functions (α-tubulin, DRP-2), excitotoxicity (glutamine synthase) and proteasomal activity (ubiquitin carboxy-terminal hydrolase L1) [18-20].

Preclinical studies in several models of AD have led to the proposal that mitochondrial impairment is a potential mechanism leading to neuronal cell death. It is thought that cell death might be related either to a direct toxic effect of hyperphosphorylated tau and Aβ on enzymes involved in the respiratory chain (complex I and complex IV) and Krebs-cycle (α-ketoglutarate and pyruvate dehydrogenases) (Figs. **1**, **2**) or to the effect of toxic 4-HNE [2]. Due to the alterations of the respiratory chain, hyperphosphorylated tau increases the generation of superoxide radical which activates members of the mitogen-activated protein kinases (JNK and p38) and initiates caspase-3-dependent apoptotic cell death (Fig. **2**). Finally, Aβ damages mitochondrial DNA which is responsible for cytotoxicity. As a consequence of mitochondrial impairment, a collapse in mitochondrial membrane potential and the opening of membrane transition pores leading to cytochrome *c* release, caspase-3 activation and apoptotic cell death occur [2]. Another mechanism responsible for neuronal damage in AD involves increased concentrations of transition metals, including iron and copper, which can directly induce oxidative stress or promote hyperphosphorylation and aggregation of tau [2]. The divalent cation zinc, is considered as a toxin in AD for several reasons. It has been shown that zinc (*i*) increases the levels of secreted APP, (*ii*) inbits α-secretase, thus shifting APP through the amyloidogenic pathway and (*iii*) favors Aβ aggregation and precipitation [21]. However, zinc, at low micromolar concentrations, protects from Aβ cytotoxicity by blocking the calcium channel pore formed by Aβ within the membrane or competing with iron or copper and thus reducing the intracellular formation of hydrogen peroxide [2, 21].

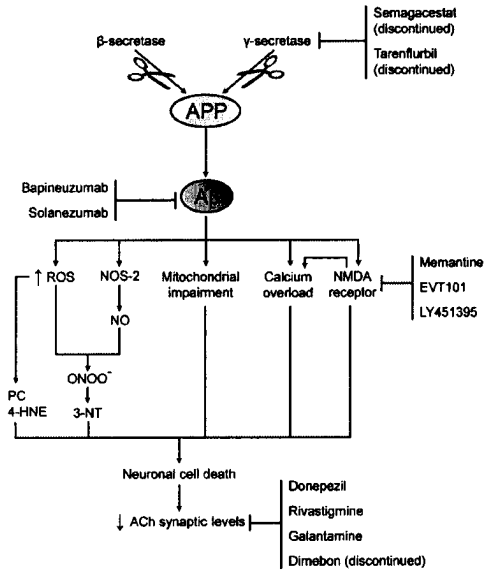

Fig. (1). Pleiotropic mechanisms of harm and drugs acting on amyloid precursor protein (APP) cleavage. Following the proteolytic cleavage of APP by β- and γ-secretases, the formation of amyloid-β-peptide(1-42) (Aβ) occurs. Amyloid-β-peptide aggregates and forms oligomers and fibrils and exerts its toxic effects through (a) increases in the formation of both reactive oxygen species (ROS), mainly the superoxide anion, and activation of inducible nitric oxide synthase (NOS-2) leading to nitric oxide generation, (b) mitochondrial impairment by inhibiting important enzymes involved in the respiratory chain and Krebs-cycle and causing mitochondrial DNA fragmentation, and (c) stimulation of the ionotropic glutamate NMDA receptor resulting in an intracellular Ca^{2+} overload thus leading to excitotoxic cell death . As a consequence of the first mechanism, NO can react with superoxide generating peroxynitrite ($ONOO^-$). Both ROS and $ONOO^-$ contribute to cell death by oxidizing or nitrating proteins and lipids which generate protein carbonyls (PC), 4-hydroxy-2-nonenal (4-HNE) and 3-nitrotyrosine (3-NT) protein adducts. The opening of mitochondrial membrane transition pores, as depicted in b), causes cytochrome *c* release, caspase-3 activation and apoptotic cell death. This cascade of events could be blocked at least at three levels: semagacestat and tarenflurbil by inhibiting γ-secretase block the APP cleavage into Aβ; bapineuzumab and solanezumab are monoclonal antibodies which accelerate the brain clearance of insoluble and soluble Aβ whereas memantine, ECT 101 and LY451395 modulate NMDA and AMPA receptors. Finally, drugs including donepezil, rivastigmine and galantamine inhibit acetylcholinesterase thus increasing acetylcholine concentration in the synaptic cleft and improving cognitive functions.

Amyloid-β-peptide alters also the intracellular ion homeostasis by increasing the entry of both sodium and calcium mediated by the ion pump Na^+/K^+-ATPase, the ionotropic glutamate receptor N-methyl-D-aspartate (NMDA) and the membrane attack complex of the complement. A direct consequence of this ion imbalance is a Ca^{2+} overload and excitotoxic cell death [2] (Fig. **1**).

NITRIC OXIDE, NITROSATIVE STRESS AND AD

The Nitric Oxide Synthase/NO System

Nitric oxide is generated by the NOS family of enzymes, which catalyse the conversion of L-arginine to L-citrulline and NO. Three main isoforms of NOS were identified so far and named (a) neuronal NOS (nNOS, type 1) (b) endothelial NOS (eNOS; type 3), and (c) inducible NOS (iNOS, type 2) [22, 23]. Activation of different isoforms of NOS requires various factors and co-factors. Formation of calcium/calmodulin complexes is a prerequisite

before the functional active dimer exhibits NOS activity, which additionally depends on cofactors such as tetrahydrobiopterin (BH_4), flavins and NADPH [24-26]. In contrast to nNOS and eNOS, iNOS can bind to calmodulin even at very low concentration of intracellular calcium, thus iNOS can exert its activity in a calcium-independent manner [26-28].

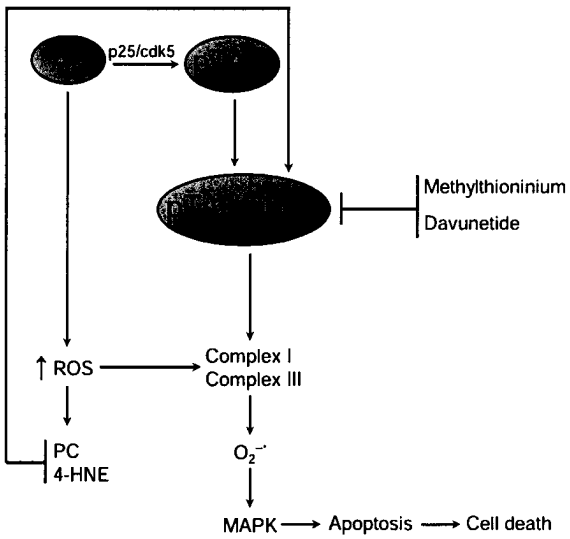

Fig. (2). Drugs acting on the phosphorylation of tau protein. Through specific kinases, such as the p25/cdk5 system, amyloid-β-peptide(1-42) (Aβ) increases the phosphorylation of tau protein. As a consequence of this modification, hyperphosphorylated tau becomes insoluble and forms fibrils with a double-helix secondary structure, known as neurofibrillary tangles. In addition, the formation of tau fibrils is stimulated by protein carbonyls and 4-hydroxy-2-nonenal (4-HNE) protein adducts produced as consequence of oxidative stress. Once formed, tau fibrils contribute to the destruction of mitochondrial respiratory chain complex I/III which, in turn, increases the formation of the superoxide anion, the activation of several members of the mitogen-activated protein kinase (MAPK) family and ultimately induces neuronal cell death. Methylthioninium and davunetide block the aggregation of tau oligomers and dissolve tau aggregates into short truncated monomers which are further cleared efficiently through the proteasomal system.

In the brain nNOS is localized in neuronal populations in the cerebral *cortex*, *striatum*, *hippocampus* (CA1 region and dentate gyrus), lateral dorsal and peduncolopontine tegmental nuclei, *cerebellum* (granule cells) and in the hypothalamic paraventricular and supraoptic nuclei [29]. Neuronal NOS immunoreactivity has also been detected in astrocytes, cerebral blood vessels and in the posterior pituitary [29, 30]. Endothelial NOS is expressed in cerebral endothelial cells and regulates cerebral blood flow; however, eNOS immunoreactivity has also been observed in *cerebellum*, olfactory bulb, cerebellar *cortex*, dentate gyrus of the *hippocampus* and the bed nucleus of the *stria terminalis* [24]. Inducible NOS levels in the central nervous system (CNS) are generally fairly low; however, an increased expression of iNOS in astrocytes and microglia occurs following viral infection and trauma [31]. Activation of iNOS requires gene transcription, and the induction can be influenced by amyloid-β-peptide, lipopolysaccharide and pro-inflammatory cytokines (Table **1**). This activation can be blocked by anti-inflammatory drugs, inhibitory cytokines, prostaglandins, tissue growth factors or inhibitors of protein synthesis [30, 32] (see also Table **1**).

Whether using Valence-Bond Theory (in which the NO molecule is viewed as having 11 valence electrons—5 from N and 6 from O) or Molecular Orbital Theory (in which an unpaired electron occupies a π* antibonding orbital), NO has one unpaired electron. Consequently, this molecule is a free radical. Removal from or addition to NO of electrons results in different redox-related forms. In fact, the removal of the unpaired electron from NO generates nitrosonium ion, whereas the addition of another electron to the π* orbital of NO forms nitroxyl anion [33]. Finally, in the presence of superoxide radical anion, radical-radical recombination with NO occurs at a diffusion-controlled rate, forming peroxynitrite [33]. Nitric oxide reactions include formation of dinitrogen trioxide (N_2O_3), which can modify thiol groups (e.g. cysteine) to form S-nitrosothiols [33]. In addition, NO covalently binds to certain metal centers, particularly iron [34].

Early studies into NO-mediated signalling indicated that this gas is able to interact with soluble guanylyl cyclase and, in doing so, stimulate its activity [35-37]. The consequent increase in the intracellular levels of cyclic GMP can influence synaptic plasticity [38, 39], smooth muscle relaxation [40-42], neurosecretion [43-47] and neurotransmission [48-50]. NO has subsequently been shown to interact with other members of the haemoprotein family such as cycloxygenase [51, 52] and heme oxygenase-1 [53, 54].

Nitric oxide also regulates the Akt kinase pathway and the transcription factor cyclic-AMP-response-element-binding protein (CREB), two pathways that promote cell survival and neuroprotection [55, 56]. Finally, NO has been demonstrated to regulate cell signalling by S-nitrosylation — its ability to complex with the thiol groups of proteins and non-protein molecules [57-59]. By this reaction NO exerts both neuroprotective and neurotoxic effects (see below).

Nitrosative Stress and AD

The involvement of nitrosative stress in the development of neurodegenerative disorders is now widely accepted. In these diseases, NO is produced in excess by iNOS induction due to the pro-inflammatory response, the latter being a common feature of neurodegenerative disorders. Moreover, NO is much more harmful under pathological conditions involving the production of ROS such as superoxide anion and the final formation of the toxic product peroxynitrite [60, 61]. Nitrotyrosine formation, as a marker of nitrosative stress, has been documented in AD, Parkinson's disease and amyotrophic lateral sclerosis [62-64].

Redox proteomics techniques have been used recently to determine which proteins are post-translationally modified to 3-nitrotyrosine (3-NT) in AD brain [65]. Ten proteins were identified which exhibited increased specific 3-NT immunoreacivity: α-enolase, triosophosphate isomerase, neuropolypeptide h3, β-actin, L-lactate dehydrogenase, carbonic anhydrase II, glyceraldehyde 3-phosphate dehydrogenase, ATP synthase alpha chain, voltage-dependent anion channel protein 1, and γ-enolase [63, 66].

Alpha-enolase had previously been identified as specifically oxidized in AD brain [19], and is one of the subunits composing the enzyme enolase. Enolase catalyzes the reversible conversion of 2 phosphoglycerate to phosphoenolpyruvate in glycolysis. Taken together with the increased nitration of triosephosphate isomerase, which interconverts dihydroxyacetone phosphate and 3-phophoglyceraldehyde in glycolysis, these results indicate a possible mechanism to explain the altered glucose tolerance and metabolism exhibited in AD [67, 68]. In addition to its role in glycolysis, enolase is involved in other pathways including induction of plasminogen (leading to plasmin, one of whose proteolytic substrates is Aβ) and induction of ERK 1/2 [69]. Neuropolypeptide h3, also known as phosphatidylethanolamine-binding protein, hippocampal cholinergic neurostimulating peptide and raf-kinase inhibitor protein, has a variety of functions in the brain. Among them is *in vitro* up-regulation of the production of choline acetyltrasferase in cholinergic neurons following NMDA receptor activation [70]. Choline acetyltransferase activity is known to be decreased in AD patients, and cholinergic deficits are prominent in the brains of these patients [71, 72]. Nitration of

Table 1. Main Factors Regulating Nitric Oxide Production Through Effects Exerted on Nitric Oxide Synthase Isoforms. (Reprinted, with Permission, from Reference [47])

nNOS		eNOS		iNOS	
Factor	**Mechanism[a]**	**Factor**	**Mechanism[a]**	**Factor**	**Mechanism[a]**
Bilirubin	↑ expression	Aβ	↑ expression	Aβ	↑ expression
Calcium/calmodulin	↑ activity	Calcium/calmodulin	↑ activity	CHX	↓ expression
Dephosphorylation		Dephosphorylation		DEX	↓ expression
PP1(S847[b])	↑ activity	PP2A (S1177[c])	↓ activity	IFN-γ	↑ expression
Hsp90	↑ activity	PP1 (T495[c])	↑ activity	IL-1	↑ expression
NMDA	↑ activity	Hypoxia	↑ expression	IL-2	↑ expression
NOSIP	↓ activity	LPS	↑ expression	IL-4	↓ expression
Phosphorylation		Phosphorylation		IL-10	↓ expression
CaMKII (S847[b])	↓ activity	Akt (S615[c], S1177[c])	↑ activity	PGA_2	↓ expression
CaMKI (S741[b])	↓ activity	CaMKII (S1177[c])	↑ activity	TNF-α	↑ expression
Parkinson's disease	↑ expression	PKC (S114[c], T495[c])	↓ activity		
PSD95	↑ activity	Physical exercise	↑ expression		
		TNF-α	↑ expression		

[a]Upregulation (↑) / downregulation (↓) of NOS gene *expression* or NOS *activity*.
[b]Studies performed in rat cortical and hippocampal neurons.
[c]The amino acid position number refers to the sequence of human eNOS.
Abbreviations: Aβ, amyloid-β-peptide; CaMK, calcium/calmodulin-dependent protein kinase; CHX, cycloheximide; DEX, dexamethasone; Hsp90, heat shock protein 90; IFN-γ, interferon-γ; IL, interleukin; LPS, bacterial lipopolysaccharide; NMDA, N-methyl-D-aspartate; NOSIP, nitric oxide synthase-interactive protein; PG, prostaglandin; PKC, protein kinase C; PP, protein phosphatase; PSD 95, postsynaptic density protein 95; Ser, serine residue; T, threonine residue; TNF-α, tumor necrosis factor-α.

neuropolypeptide h3 [73], and the consequent lack of neurotrophic action on cholinergic neurons of the hippocampus and basal forebrain might help to explain the decline in cognitive function. Carbonic anhydrase II is critical for maintence of pH and control of carbon dioxide levels, and its activity is altered in AD [74]. Glyceraldehyde 3-phosphate dehydrogenase is not only important in ATP production, but also plays a role as a NO sensor [75, 76]. ATP synthase alpha chain is clearly important in energy metabolism, and voltage-dependent anion channel protein 1 is involved in the mitochondrial permeability transition pore, with consequent apoptotic considerations, as well as in mitochondrial calcium ion homeostasis [66].

DRUG TARGETS IN AD

Acetylcholinesterase

Acetylcholinesterase (AChE) hydrolizes the neurotransmitter acetylcholine (ACh) to choline and acetic acid. Inhibitors of AChE were the first drugs developed for the treatment of AD. The rationale for the use of AChE inhibitors in AD is to delay the degradation of ACh in the synaptic cleft thereby increasing the concentration of this neurotransmitter at both muscarinic and nicotinic receptors. Tacrine was the earliest AChE inhibitor shown to have any benefit in AD [77, 78]. However, due to the occurrence of significant adverse effects, primarily nausea and vomiting, and potentially dangerous hepatic toxicity, tacrine was withdrawn from the market [79-81]. Donepezil, rivastigmine and galantamine are the AChE inhibitors currently used in AD therapy. Some of these drugs are characterized by a multifaceted mechanism of action which includes not only the inhibition of AChE and/or butyrylcholinesterase but also the activation of cytoprotective pathways and the blockade of cytotoxic systems. Donepezil and galantamine act as allosteric potentiation ligands on α4- and α7-nicotinic ACh receptors. Through this mechanism, donepezil and galantamine activate pro-survival pathways such as the proto-oncogene Akt and protein Bcl-2 and down-regulate calcium-induced activation of NOS and the further increase of cytotoxic RNS, including peroxynitrite [82]. These drugs have an excellent bioavailability after oral administration and only donepezil is tightly bound to plasma proteins [83]. Both rivastigmine and galantamine have short elimination half-lives (1.5-2 h and 5-7 h, respectively) and this requires multiple daily dosing scheme of administration; donepezil has a half-life of about 70 h and can be administered once daily [83]. Importantly, donepezil and galantamine are metabolized by the liver enzyme isoforms 3A4 and 2C6 of the cytochrome-P-450 (CYP) and this implies a potential reduction of drug plasma concentrations and therapeutic effect if inducers of CYP3A4 and 2C6 are concomitantly administered [84]. Among the adverse effects of AChE inhibitors worthy of mention are fatigue, nausea, emesis, muscle cramps, dizziness, sinusitis [85, 86].

Acetylcholinesterase inhibitors have been shown to produce some degree of improvement in cognitive functions, but their effects are confined largely to patients with mild to moderate AD-like dementia, and the most marked effects are observed during the first year or so of treatment [87, 88]. Thereafter, their efficacy declines progressively and disappears entirely after 2 or 3 years. Attempts have been made to increases the efficacy of AChE inhibitors by combining them with other drugs such as memantine or statins, but it remains to be seen whether these associations are more effective than the drugs alone [89, 90].

Latrepirdine, also known as dimebon or dimebolin, was initially developed as an orally active anti-histamine drug, but due to the development of newer and safer antihistamines, it was withdrawn from the market. The interest on latrepirdine was renewed at the beginning of the 2000s when this agent was shown to exhibit strong neuroprotective effects in preclinical models of AD and Huntington disease [91-94]. The findings of neuroprotection prompted neurologists to study dimebon in subjects with mild-moderate AD. Unfortunately, although there were promising results in earlier undersized clinical studies, a recent randomized, double-blind,

placebo controlled trial in which 598 patients were recruited, clearly demonstrated that dimebon (5-20 mg 3 times a day) did not improve cognitive and global functions in mild-moderate AD subjects over 6 months of treatment and the development of dimebon was discontinued [95].

Glutamate Receptors

The ionotropic glutamate receptor, NMDA, is a ligand-gated ionic channel, which, if opened, allows the influx of calcium ions from the extracellular space into the neuron. As a consequence of this event, the intracellular calcium concentration increases and activates several pathways leading to the activation of physiologic functions or cytotoxicity [96, 97]. Neuronal NOS is activated by calcium and this results in an increased production of NO [98]. The increased intraneuronal level of NO is not necessarily a *noxa* for the cell since it is a main regulatory system for the induction and maintenance of long-term potentiation, necessary for synaptic plasticity [38]. However, in the case of a sustained and prolonged NMDA activation, as during chronic neuroinflammation, the intracellular levels of L-arginine are progressively reduced, and, during its catalytical cycle, nNOS generates superoxide radicals which react with NO forming the toxic RNS peroxynitrite [99, 100]. The formation of peroxynitrite together with the calcium-related mitochondrial impairment results in excitotoxic cell death in AD [2]. On this basis, the blockade of NMDA receptor was hypothesized to be a useful mechanism to counteract or, at least delay, the progression of AD.

Memantine (3,5-dimethyladamantan-1-amine) is a non-competitive NMDA blocker. After oral administration, memantine is almost completely absorbed, reaches peak plasma values in 6-8 h and its half-life is 60-80 h [101]. Almost half of the drug is excreted unchanged by the kidney whereas the remainder is metabolized *via* conjugation with glucuronic acid and excreted in the urine [101]. The main side effects of memantine are dizziness, constipation, cataracts, nausea, dyspnea, confusion, headache, and urinary incontinence [102]. Memantine is currently used, either in monotherapy or combined with donepezil or rivastigmine, for the treatment of patients with moderate-severe AD [103-106]. Several clinical studies showed that memantine improved activities of daily living, global outcome and behavior, whereas the beneficial effect on cognitive function is still under debate [103-105, 107]. However, similarly to AChE inhibitors, the clinical efficacy of memantine decreases within about 6 months of therapy.

EVT 101 (initially developed by Evotec) and LY451395 (Ely-Lilly) are two modulators of glutamate receptor still under development. EVT101 is an antagonist at the NR2B subunit of the NMDA receptor, whereas LY451395 is an AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) receptor potentiator [108, 109]. Earlier clinical studies demonstrated that EVT101 improved cerebral blood flow in specific region of the cortex but this effect was not paralleled by a significant amelioration of cognitive functions in healthy volunteers [110]. Evotec planned to carry out phase 2 clinical trials of EVT 101 in AD patients over the next years in order to understand the potential neuroprotective role of this agent. With regard to LY451395, this compound did not exhibit any effect on cognitive function in patients suffering from mild-moderate AD but demonstrated some potentially beneficial effects on neuropsychiatric functions and behaviors [111].

β- and γ- Secretases

These enzymes play a main role in the formation of Aβ. In amyloidogenic processing, APP is initially cleaved by β-secretase, also known as β-site APP-cleaving enzyme 1, which releases an extracellular soluble fragment leaving in the cell membrane a 99-residue carboxy-terminal fragment. This fragment represents the substrate for γ-secretase, an aspartyl protease, which generates Aβ and a membrane-bound fragment called AICD. In addition, *in vitro* studies demonstrated that the inhibition of γ-secretase reduced the generation of both NO and ROS thus suggesting that NO/RNS-induced neurotoxicity is positively correlated with the activity of γ-secretase [112]. That said, it is straightforward to hypothesize that the blockade of γ-secretase could reduce the formation of Aβ and the cytotoxic events downstream.

Semagacestat (Ely-Lilly) and tarenflurbil (developed by Loma Linda University Medical Center and later licensed to Encore Pharmaceuticals) are two γ-secretase inhibitors. Both these agent are well adsorbed after oral administration, reach peak plasma values within 0.5-3 h and their half-lives range from 2.5 h (semagacestat) to 2-8 h (tarenflurbil) [113, 114]. Phase 2 clinical studies demonstrated that semagacestat markedly reduced Aβ plasma concentration in mild-moderate AD patients [115, 116]. However, despite this indirect evidence of a reduced Aβ formation, semagacestat did not improve cognitive functions in these patients. In August 2010, Eli-Lilly decided to discontinue the clinical development of semagacestat. Results from two phase 3 trials demonstrated that mild-moderate AD patients treated with semagacestat (60-140 mg/day *per os* for 21 months) did not exhibit any beneficial effect on cognitive function relative to the placebo group and patients in the active group had an increased risk to develop skin cancer [117]. This last side effect was due to the inhibition by semagacests of pathways different from γ-secretase, such as Notch [117]. For this reason, semagacestat was discontinued.

With regard to tarenflurbil, early studies showed a significant improvement in the activities of daily living and global function only in patients suffering from mild AD [118]. No positive effects on cognitive function were found in mild to moderate AD subjects [118]. This evidence was recently confirmed by a large phase 3 trial [119] and, therefore, the development of tarenflurbil was discontinued.

AMYLOID-β-PEPTIDE

Once formed by the activity of β- and γ- secretases, Aβ tends to form oligomers and fibrils [8]. The importance of Aβ fibrils to trigger intracellular cytotoxic pathways is no longer a matter of discussion. Both Aβ(1–40) and (1–42) were found in AD brain and a number of *in vitro* and *in vivo* studies showed that Aβ(1–42), a primary component of SP, is more toxic than Aβ(1–40) [120, 121]. In addition, small oligomers of Aβ were suggested to be the actual toxic species rather than fibrillar Aβ [122-124]. In order to avoid the formation of both oligomers and fibrils, monoclonal antibodies against Aβ were developed.

Bapineuzumab (initially developed by Wyeth/Pfizer and Elan but later acquired by Janssen) is a humanized monoclonal antibody designed to target the N-terminus of Aβ in the brain. Bapineuzumab strongly binds to fibrillar Aβ and removes Aβ from the brain and thereby potentially preventing or reversing the progression of AD [125-127]. Little data on the pharmacokinetics of bapineuzumab in humans are available. In a recent phase 2 clinical trial in mild-moderate AD subjects, bapineuzumab was well tolerated, the only significant adverse effect reported being vasogenic oedema [128]. The administration of bapineuzumab significantly decreased cortical fibrillar Aβ and showed demonstrable clinical benefits on both cognitive and functional endpoints in non-carriers ApoE4 AD patients [128]. In June 2009, phase 3 clinical studies of bapineuzumab in mild-moderate AD were initiated and the results will be released in the near future.

Solanezumab (Ely-Lilly) is the humanized analog of the of the murine antibody m266.2 and differs from bapineuzumab mainly in the mechanism of action. Solanezumab binds not only full-length Aβ but also other truncated forms of the peptide [126, 129]. In addition, solanezumab selectively binds soluble Aβ with very low affinity for the fibrillar form [125, 126]. Solanezumab significantly increased Aβ brain clearance in subjects suffering from mild-

moderate AD [130]. However, despite this effect, this compound did not improve cognitive functions in these patients [130]. Importantly, solanezumab administration did not result in meningoencephalitis [130]. Two phase 3 clinical trials of solanezumab are being conducted by Ely-Lilly but they will be completed in late 2012.

TAU PROTEIN

As mentioned earlier, hyperphosphorylated tau becomes insoluble and forms aggregates with a double-helix secondary structure, known as neurofibrillary tangles. An intriguing aspect related to tau phosphorylation is that it occurs concurrently with NOS up-regulation and is inhibited by the addition of NOS inhibitor in an *in vitro* co-culture of astrocytes with primary hippocampal neurons [131]. An important finding of this study was the evidence of a prominent role of GSK-3β as an intermediate in NO-induced tau hyperphosphorylation [132]. The contribution of the single NOS isoforms to NO effects in AD is still debated. The overexpression of NOS-3 was associated with increased oxidative stress and mitochondrial dysfunction leading to neuronal damage [133] in Long Evans rat pups. On the contrary, NOS-2 gene deletion was shown to increase pathological hyperphosphorylation of tau, caspase-3 activation and neuronal cell death [134, 135]. The evidence of a cytoprotective role of NO was also supported by Cappelletti *et al.* [136] who reported on a physiological nitration of tau protein as an important process in neuronal growth and differentiation.

Methyltioninium (TauRx Therapeutics) and davunetide (Allon Therapeutics) are new molecules still under development which act on tau protein. Methyltioninium blocks the aggregation of tau oligomers and their conversion into paired helical filaments [137]. Furthermore, this agent dissolves tau aggregates into short truncated monomers which are further cleared efficiently through the proteasomal system [138]. In patients affected by mild-moderate AD, oral methylthioninium 60 mg 3-times daily significantly increased cognitive performance over 50 weeks of treatment [139]. This is perhaps not surprising given that the severity of cognitive deficits correlates much better with the extent of neurofibrillary tangles than with plaque formation.

Davunetide is a peptide derived from an endogenous brain protective protein (Activity-Dependent Neuroprotective Protein) and it is able to cross blood-brain-barrier [140]. Several preclinical studies demonstrated a neuroprotective role for davunetide due to a significant reduction in both hyperphosphorylated and insoluble tau [141, 142]. In addition, davunetide reduced Aβ(1-40) and Aβ(1-42) protein levels in the brain of a transgenic mouse model of AD [143, 144]. Recent clinical trials showed that intranasal davunetide improved recognition and short-term memory in patients with amnestic-mild cognitive impairment (MCI) [145], which is considered to be a precursor to full-blown AD. Furthermore, davunetide up to 15 mg exhibited a good safety profile in both healthy adults [146, 147] and MCI subjects [145].

NITRIC OXIDE SYNTHASE

Although NO, or other molecules acting on the NO-cGMP pathway, are used to treat important disorders such as impotence [148, 149], respiratory diseases [150, 151] and pulmonary hypertension [152], current data indicate that an NO-based therapy for treating neurodegenerative disorders, including AD, would not be appropriate. This pessimism arises from the intrinsic difficulty of delivering NO in the CNS without side-effect, as well as from the dual role — neurotoxic or neuroprotective — of NO in the brain [100]. In order to appreciate much better the difficulties that could derive from an uncontrolled inhibition of the NOS/NO pathway in the CNS, it is necessary to recall some of the physiologic action of this gaseous neurotransmitter as well as the mechanisms involved in its neuroprotective effects.

The first evidence of NO's activity as a neurotransmitter was reported in 1988 by Garthwaite *et al.* [50]. They demonstrated that glutamate stimulation of cerebellar NMDA receptors caused neurons to release a diffusible molecule with features very similar to those of endothelium-dependent relaxing factor. In the years that followed, multiple functions in the CNS were documented for NO, including the roles it played in cognitive function [50, 153, 154], the control of sleep [155-157], appetite [158, 159], body temperature [160-163] and neurosecretion [47, 164, 165]. In the peripheral nervous system, NO was shown to regulate the nonadrenergic, noncholinergic relaxation of smooth muscle in the corpora cavernosa and the gastrointestinal tract [166, 167].

Furthermore, NO exerts neuroprotective effects *via* the modulation of many intracellular pathways. Through the stimulation of the sGC/cGMP system, NO regulates the Akt and CREB transcription factors in brain cells; notably, both proteins are important as signal transducers of neurotrophin-mediated survival and protection against various neurodegenerative challenges, and this similarity contributes to the neuroprotective role of NO [55, 56, 168, 169]. Nitric oxide protects against such excitotoxicity by nitrosating the NR1 and NR2 subunits of the NMDA receptor [170, 171], which reduces the intracellular Ca^{2+} influx responsible for neuronal death [172]. Consequently, it is possible to conclude that NO formed during excessive NMDA activation nitrosates the NMDA subunits, and thereby diminishes either the formation of peroxynitrite or Ca^{2+} influx to promote neuronal survival. Nicholson *et al.* showed that NO-mediated inhibition of NMDA receptor activity in a subpopulation of *substantia gelatinosa* neurons in adult rat spinal cord is mediated, at least in part, *via* cGMP, thus providing data about an alternate mechanism involved in NO-NMDA interaction [173]. Furthermore, NO-related species, such as nitroxyl anion, could react with critical thiols in the NMDA receptor and generate an R-SNH-OH derivative which inhibits NMDA receptor activity [174]. Nitric oxide can also bind the cysteines of the catalytic-site of caspases thus conferring cytoprotection [175, 176]. The latter mechanism has been shown to reduce the activity of caspases in several cell lines, including neurons [176-179]. The controversial role of NO is also confirmed by *in vitro* and *in vivo* studies in many experimental models of AD. Data from preclinical studies demonstrated that the inactivation of NOS-2 increases both Aβ deposition and tau phosphorylation thus supporting a neuroprotective role of NOS-2 derived NO [134, 135].

The role of NOS-2 in AD prompted the investigators to study the role of microglial cells, characterized by a marked NOS-2 expression and activity. Blood-derived microglial cells are associated with Aβ(1-40)/(1-42) and prevent the formation of oligomers and fibrils through a phagocytosis-dependent mechanism [180]. That said, it is plausible to hypothesize that changes in microglial reactivity during senescence as well as the prevalence of resident microglia (which is not able to clear Aβ) [180], might have a permissive role in the brain damage characteristic of AD. With regard to NOS-3, the upregulation of this isoform leads to the activation of the apoptotic machinery and neuronal cell death [133]. That said, any drug targeted for the NOS/NO system should not only be specific for NOS but also selective, in the sense that it should inhibit NOS-3 without affecting NOS-2 activities.

CONCLUSIONS AND PERSPECTIVES

Over the last 10-15 years several efforts have been made by researchers to design and develop new drugs to improve cognitive functions in patients suffering from AD. Unfortunately, AChE inhibitors and the NMDA antagonist memantine did not have any effect on the clinical course of AD and only slightly improved cognitive functions, especially in mild-moderate AD patients. In addition, another promising class of agents, secretase inhibitors, were discontinued from clinical development due to a substantial lack of beneficial effect on cognitive function and the appearance of severe side effects. The only agents, whose clinical development is

still ongoing, are the monoclonal antibodies against Aβ and tau inhibitors.

Very controversial is the potential therapeutic effect on AD obtainable by pharmacological manipulation of the NO/NOS system. As noted above, the effect of NO in the CNS is something of a double-edged sword, since this gaseous compound has important physiological effects but it may become toxic if produced in excess or under pro-oxidant conditions which trigger the formation of RNS. Therefore, drug research should focus on hits/leads that modulate NO generation and reactivity in a dose-dependent manner. With compounds of this sort, one would be able to titrate the dose to achieve the desired pharmacologic effect without provoking toxicity. Unfortunately, this goal is more difficult than it sounds. First of all, we do not have enough data to calculate the therapeutic index for NO in the whole brain or in those brain areas involved in cognitive or autonomic functions and second, the sensitivity to the therapeutic and/or toxic effect of NO depends on the redox status of the cell. This potential limitation might be overcome by the development of new delivery systems that can selectively drive substances in the brain and modulate NO generation. A possible way to resolve this problem is the synthesis of a pro-drug composed by a molecular probe which senses the NO level within the cell and an active component which, once released, increase/decrease the activation of NOS. Although plausible from a theoretical point of view, this goal is hard to achieved. The main obstacle is represented by the blood-brain-barrier which reduces the possibility for drugs to reach pharmacological concentrations in the brain. To go through this restriction, the administration of modulators of NO synthesis by the intrathecal route could be proposed; however, this route of administration is not ideal for chronic therapeutic use.

Regarding the administration of NO donors or stimulators/inhibitors of NO synthesis by sublingual or oral routes (much more feasible for chronic treatment and currently used for the administration of organic nitrates), its use results in the onset of harmful systemic effects on the cardiovascular system, such as changes in blood pressure and platelet aggregation, which should be avoided in the elderly suffering from AD. The last possibility could be the administration of gaseous NO by inhalation. If administered through this route, NO exerts its main effect locally by reducing arterial pulmonary pressure and improving lung perfusion. Similarly to other gaseous compounds, the possibility for inhaled NO to reach pharmacological concentrations in the CNS, is based on several factors, such as the degree of solubility in the blood (measured as the blood/gas partition coefficient), the concentration of the gas in the inspired air, the pulmonary ventilation and pulmonary blood flow. The first two factors could be easily handled, but the latter are characterized by a high individual variability. For this reasons, it is not possible to predict the amount of inhaled NO to reach the brain.

In conclusion, pharmacological manipulation of NO synthesis and release is not easy to achieve and this is more difficult if the main target of the gaseous compound is the brain. The translation of basic pharmacologic issues of NO to the bedside is a challenging goal, however, and one that will require close, active collaboration by pharmacologists, chemists, and clinicians.

CONFLICT OF INTEREST

The Authors declare no conflict of interest.

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ABBREVIATIONS

3-NT	=	3-nitrotyrosine
4-HNE	=	4-hydroxy-2-nonenal
Aβ	=	Amyloid-β-peptide
AD	=	Alzheimer disease
APP	=	Amyloid precursor protein
NMDA	=	N-methyl-D-aspartate
NO	=	Nitric oxide
NOS	=	Nitric oxide synthase
PC	=	Protein carbonyls
RNS	=	Reactive nitrogen species
ROS	=	Reactive oxygen species
sGC	=	Soluble guanylyl cyclase.

REFERENCES

[1] Bekris, L.M.; Yu, C.E.; Bird, T.D.; Tsuang, D.W. Genetics of Alzheimer's disease. *J. Geriatr. Psychiatry Neurol.*, **2010**, *23*(4), 213-227.

[2] Querfurth, H.W.; LaFerla, F.M. Alzheimer's disease. *N. Engl. J. Med.*, **2010**, *362*(4), 329-344.

[3] Mayeux, R. Clinical practice: early Alzheimer's disease. *N. Engl. J. Med.*, **2010**, *362*(23), 2194-2201.

[4] Seripa, D.; Panza, F.; Franceschi, M.; D'Onofrio, G.; Solfrizzi, V.; Dallapiccola, B.; Pilotto, A. Non-apolipoprotein E and apolipoprotein E genetics of sporadic Alzheimer's disease. *Ageing Res. Rev.*, **2009**, *8*(3), 214-236.

[5] Schipper, H.M. Apolipoprotein E: implications for AD neurobiology, epidemiology and risk assessment. *Neurobiol. Aging*, **2011**, *32*(5), 778-790.

[6] Citron, M. Alzheimer's disease: strategies for disease modification. *Nat. Rev. Drug. Discov.*, **2010**, *9*(5), 387-398.

[7] Zubenko, G.S.; Moossy, J.; Martinez, A.J.; Rao, G.; Claassen, D.; Rosen, J.; Kopp, U. Neuropathologic and neurochemical correlates of psychosis in primary dementia. *Arch. Neurol.*, **1991**, *48*(6), 619-624.

[8] Sultana, R.; Perluigi, M.; Butterfield, D.A. Oxidatively modified proteins in Alzheimer's disease (AD), mild cognitive impairment and animal models of AD: role of Abeta in pathogenesis. *Acta Neuropathol.*, **2009**, *118*(1), 131-150.

[9] Hoozemans, J.J.; Stieler, J.; van Haastert, E.S.; Veerhuis, R.; Rozemuller, A.J.; Baas, F.; Eikelenboom, P.; Arendt, T.; Scheper, W. The unfolded protein response affects neuronal cell cycle protein expression: implications for Alzheimer's disease pathogenesis. *Exp. Gerontol.*, **2006**, *41*(4), 380-386.

[10] Hoozemans, J.J.; Veerhuis, R.; Van Haastert, E.S.; Rozemuller, J.M.; Baas, F.; Eikelenboom, P.; Scheper, W. The unfolded protein response is activated in Alzheimer's disease. *Acta Neuropathol.*, **2005**, *110*(2), 165-172.

[11] Owen, J.B.; Sultana, R.; Aluise, C.D.; Erickson, M.A.; Price, T.O.; Bu, G.; Banks, W.A.; Butterfield, D.A. Oxidative modification to LDL receptor-related protein 1 in hippocampus from subjects with Alzheimer disease: implications for Aβ accumulation in AD brain. *Free Radic. Biol. Med.*, **2010**, *49*(11), 1798-1803.

[12] Iqbal, K.; Alonso Adel, C.; Chen, S.; Chohan, M.O.; El-Akkad, E.; Gong, C.X.; Khatoon, S.; Li, B.; Liu, F.; Rahman, A.; Tanimukai, H.; Grundke-Iqbal, I. Tau pathology in Alzheimer disease and other tauopathies. *Biochim. Biophys. Acta*, **2005**, *1739*(2-3), 198-210.

[13] Sperber, B.R.; Leight, S.; Goedert, M.; Lee, V.M. Glycogen synthase kinase-3 beta phosphorylates tau protein at multiple sites in intact cells. *Neurosci. Lett.*, **1995**, *197*(2), 149-153.

[14] Oddo, S. The ubiquitin-proteasome system in Alzheimer's disease. *J. Cell. Mol. Med.*, **2008**, *12*(2), 363-373.

[15] Mangialasche, F.; Polidori, M.C.; Monastero, R.; Ercolani, S.; Camarda, C.; Cecchetti, R.; Mecocci, P. Biomarkers of oxidative and nitrosative damage in Alzheimer's disease and mild cognitive impairment. *Ageing Res. Rev.*, **2009**, *8*(4), 285-305.

[16] Calabrese, V.; Sultana, R.; Scapagnini, G.; Guagliano, E.; Sapienza, M.; Bella, R.; Kanski, J.; Pennisi, G.; Mancuso, C.; Stella, A.M.; Butterfield, D.A. Nitrosative stress, cellular stress response, and thiol

homeostasis in patients with Alzheimer's disease. *Antioxid. Redox Signal.*, **2006**, *8*(11-12), 1975-1986.

[17] Brown, G.C.; Neher, J.J. Inflammatory neurodegeneration and mechanisms of microglial killing of neurons. *Mol. Neurobiol.*, **2010**, *41*(2-3), 242-247.

[18] Castegna, A.; Aksenov, M.; Aksenova, M.; Thongboonkerd, V.; Klein, J.B.; Pierce, W.M.; Booze, R.; Markesbery, W.R.; Butterfield, D.A. Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part I: creatine kinase BB, glutamine synthase, and ubiquitin carboxy-terminal hydrolase L-1. *Free Radic. Biol. Med.*, **2002**, *33*(4), 562-571.

[19] Castegna, A.; Aksenov, M.; Thongboonkerd, V.; Klein, J.B.; Pierce, W.M.; Booze, R.; Markesbery, W.R.; Butterfield, D.A. Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part II: dihydropyrimidinase-related protein 2, alpha-enolase and heat shock cognate 71. *J. Neurochem.*, **2002**, *82*(6), 1524-1532.

[20] Perluigi, M.; Sultana, R.; Cenini, G.; Di Domenico, F.; Memo, M.; Pierce, W.M.; Coccia, R.; Butterfield, D.A. Redox proteomics identification of 4-hydroxynonenal-modified brain proteins in Alzheimer's disease: Role of lipid peroxidation in Alzheimer's disease pathogenesis. *Proteomics Clin. Appl.*, **2009**, *3*(6), 682-693.

[21] Cuajungco, M.P.; Faget, K.Y. Zinc takes the center stage: its paradoxical role in Alzheimer's disease. *Brain Res. Brain Res. Rev.*, **2003**, *41*(1), 44-56.

[22] Bredt, D.S.; Hwang, P.M.; Snyder, S.H. Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature*, **1990**, *347*(6295), 768-770.

[23] Busse, R.; Mülsch, A. Calcium-dependent nitric oxide synthesis in endothelial cytosol is mediated by calmodulin. *FEBS Lett.*, **1990**, *265*(1-2), 133-136.

[24] Bredt, D.S.; Snyder, S.H. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. *Proc. Natl. Acad. Sci. USA*, **1990**, *87*(2), 682-685.

[25] Mayer, B.; John, M.; Heinzel, B.; Werner, E.R.; Wachter, H.; Schultz, G.; Böhme, E. Brain nitric oxide synthase is a biopterin- and flavin-containing multi-functional oxido-reductase. *FEBS Lett.*, **1991**, *288*(1-2), 187-191.

[26] Stuehr, D.J.; Cho, H.J.; Kwon, N.S.; Weise, M.F.; Nathan, C.F. Purification and characterization of the cytokine-induced macrophage nitric oxide synthase: an FAD- and FMN-containing flavoprotein. *Proc. Natl. Acad. Sci. USA*, **1991**, *88*(17), 7773-7777.

[27] Schulz, R.; Nava, E.; Moncada, S. Induction and potential biological relevance of a Ca(2+)-independent nitric oxide synthase in the myocardium. *Br. J. Pharmacol.*, **1992**, *105*(3), 575-580.

[28] Xie, Q.W.; Cho, H.J.; Calaycay, J.; Mumford, R.A.; Swiderek, K.M.; Lee, T.D.; Ding, A.; Troso, T.; Nathan, C. Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. *Science*, **1992**, *256*(5054), 225-228.

[29] Dawson, T.M.; Snyder, S.H. Gases as biological messengers: nitric oxide and carbon monoxide in the brain. *J. Neurosci.*, **1994**, *14*(9), 5147-5159.

[30] Guix, F.X.; Uribesalgo, I.; Coma, M.; Muñoz, F.J. The physiology and pathophysiology of nitric oxide in the brain. *Prog. Neurobiol.*, **2005**, *76*(2), 126-152.

[31] Dawson, V.L.; Dawson, T.M. Nitric oxide neurotoxicity. *J. Chem. Neuroanat.*, **1996** *10*(3-4), 179-190.

[32] Sharma, H.S. Neurobiology of the nitric oxide in the nervous system. Basic and clinical perspectives. *Amino Acids*, **1998**, *14*(1-3), 83-85.

[33] Stamler, J.S.; Singel, D.J.; Loscalzo, J. Biochemistry of nitric oxide and its redox-activated forms. *Science*, **1992**, *258*(5090), 1898-1902.

[34] Pellat, C.; Henry, Y.; Drapier, J.C. IFN-gamma-activated macrophages: detection by electron paramagnetic resonance of complexes between L-arginine-derived nitric oxide and non-heme iron proteins. *Biochem. Biophys. Res. Commun.*, **1990**, *166*(1), 119-125.

[35] Arnold, W.P.; Mittal, C.K.; Katsuki, S.; Murad, F. Nitric oxide activates guanylate cyclase and increases guanosine 3':5'-cyclic monophosphate levels in various tissue preparations. *Proc. Natl. Acad. Sci. USA*, **1977**, *74*(8), 3203-3207.

[36] Braughler, J.M.; Mittal, C.K.; Murad, F. Purification of soluble guanylate cyclase from rat liver. *Proc. Natl. Acad. Sci. USA*, **1979**, *76*(1), 219-222.

[37] Lewicki, J.A.; Brandwein, H.J.; Mittal, C.K.; Arnold, W.P.; Murad, F. Properties of purified soluble guanylate cyclase activated by nitric oxide and sodium nitroprusside. *J. Cyclic Nucleotide Res.*, **1982**, *8*(1), 17-25.

[38] Haley, J.E.; Wilcox, G.L.; Chapman, P.F. The role of nitric oxide in hippocampal long-term potentiation. *Neuron*, **1992**, *8*(2), 211-216.

[39] Shibuki, K.; Okada, D. Endogenous nitric oxide release required for long-term synaptic depression in the cerebellum. *Nature*, **1991**, *349*(6307), 326-328.

[40] Gruetter, C.A.; Barry, B.K.; McNamara, D.B.; Gruetter, D.Y.; Kadowitz, P.J.; Ignarro, L. Relaxation of bovine coronary artery and activation of coronary arterial guanylate cyclase by nitric oxide, nitroprusside and a carcinogenic nitrosoamine. *J. Cyclic Nucleotide Res.*, **1979**, *5*(3), 211-224.

[41] Murad, F.; Waldman, S.; Molina, C.; Bennett, B.; Leitman, D. Regulation and role of guanylate cyclase-cyclic GMP in vascular relaxation. *Prog. Clin. Biol. Res.*, **1987**, *249*, 65-76.

[42] Gruetter, C.A.; Gruetter, D.Y.; Lyon, J.E.; Kadowitz, P.J.; Ignarro, L.J. Relationship between cyclic guanosine 3':5'-monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite and nitric oxide: effects of methylene blue and methemoglobin. *J. Pharmacol. Exp. Ther.*, **1981**, *219*(1), 181-186.

[43] Uretsky, A.D.; Weiss, B.L.; Yunker, W.K.; Chang, J.P. Nitric oxide produced by a novel nitric oxide synthase isoform is necessary for gonadotropin-releasing hormone-induced growth hormone secretion *via* a cGMP-dependent mechanism. *J. Neuroendocrinol.*, **2003**, *15*(7), 667-676.

[44] Brunetti, L.; Preziosi, P.; Ragazzoni, E.; Vacca, M. Involvement of nitric oxide in basal and interleukin-1 beta-induced CRH and ACTH release *in vitro*. *Life Sci.*, **1993**, *53*(13), PL219-PL222.

[45] Costa, A.; Trainer, P.; Besser, M.; Grossman, A. Nitric oxide modulates the release of corticotropin-releasing hormone from the rat hypothalamus *in vitro*. *Brain Res.*, **1993**, *605*(2), 187-192.

[46] Yasin, S.; Costa, A.; Trainer, P.; Windle, R.; Forsling, M.L.; Grossman, A. Nitric oxide modulates the release of vasopressin from rat hypothalamic explants. *Endocrinology*, **1993**, *133*(3), 1466-1469.

[47] Mancuso, C.; Navarra, P.; Preziosi, P. Roles of nitric oxide, carbon monoxide, and hydrogen sulfide in the regulation of the hypothalamic-pituitary-adrenal axis. *J. Neurochem.*, **2010**, *113*(3), 563-575.

[48] Rajfer, J.; Aronson, W.J.; Bush, P.A.; Dorey, F.J.; Ignarro, L.J. Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. *N. Engl. J. Med.*, **1992**, *326*(2), 90-94.

[49] Koch, K.W.; Lambrecht, H.G.; Haberecht, M.; Redburn, D.; Schmidt, H.H. Functional coupling of a Ca2+/calmodulin-dependent nitric oxide synthase and a soluble guanylyl cyclase in vertebrate photoreceptor cells. *EMBO J.*, **1994**, *13*(14), 3312-3320.

[50] Garthwaite, J.; Charles, S.L.; Chess-Williams, R. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature*, **1988**, *336*(6197), 385-388.

[51] Salvemini, D.; Misko, T.P.; Masferrer, J.L.; Seibert, K.; Currie, M.G.; Needleman, P. Nitric oxide activates cyclooxygenase enzymes. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*(15), 7240-7244.

[52] Mollace, V.; Muscoli, C.; Masini, E.; Cuzzocrea, S.; Salvemini, D. Modulation of prostaglandin biosynthesis by nitric oxide and nitric oxide donors. *Pharmacol. Rev.*, **2005**, *57*(2), 217-252.

[53] Willis, D.; Tomlinson, A.; Frederick, R.; Paul-Clark, M.J.; Willoughby, D.A. Modulation of heme oxygenase activity in rat brain and spleen by inhibitors and donors of nitric oxide. *Biochem. Biophys. Res. Commun.*, **1995**, *214*(3), 1152-1156.

[54] Motterlini, R.; Foresti, R.; Intaglietta, M.; Winslow, R.M. NO-mediated activation of heme oxygenase: endogenous cytoprotection against oxidative stress to endothelium. *Am. J. Physiol.*, **1996**, *270*(1 Pt 2), H107-H114.

[55] Contestabile, A.; Ciani, E. Role of nitric oxide in the regulation of neuronal proliferation, survival and differentiation. *Neurochem. Int.*, **2004**, *45*(6), 903-914.

[56] Riccio, A.; Alvania, R.S.; Lonze, B.E.; Ramanan, N.; Kim, T.; Huang, Y.; Dawson, T.M.; Snyder, S.H.; Ginty, D.D. A nitric oxide signaling pathway controls CREB-mediated gene expression in neurons. *Mol. Cell.*, **2006**, *21*(2), 283-294.

[57] Foster, M.W.; McMahon, T.J.; Stamler, J.S. S-nitrosylation in health and disease. *Trends Mol. Med.*, **2003**, *9*(4), 160-168.

[58] Stamler, J.S.; Simon, D.I.; Osborne, J.A.; Mullins, M.E.; Jaraki, O.; Michel, T.; Singel, D.J.; Loscalzo, J. S-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds. *Proc. Natl. Acad. Sci. USA*, **1992**, *89*(1), 444-448.

[59] Simon, D.I.; Mullins, M.E.; Jia, L.; Gaston, B.; Singel, D.J.; Stamler, J.S. Polynitrosylated proteins: characterization, bioactivity, and functional consequences. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*(10), 4736-4741.

[60] Beckman, J.S.; Beckman, T.W.; Chen, J.; Marshall, P.A.; Freeman, B.A. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA*, **1990**, *87*(4), 1620-1624.

[61] Radi, R.; Beckman, J.S.; Bush, K.M.; Freeman, B.A. Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. *J. Biol. Chem.*, **1991**, *266*(7), 4244-4250.

[62] Good, P.F.; Hsu, A.; Werner, P.; Perl, D.P.; Olanow, C.W. Protein nitration in Parkinson's disease. *J. Neuropathol. Exp. Neurol.*, **1998**, *57*(4), 338-342.

[63] Castegna, A.; Thongboonkerd, V.; Klein, J.B.; Lynn, B.; Markesbery, W.R.; Butterfield, D.A. Proteomic identification of nitrated proteins in Alzheimer's disease brain. *J. Neurochem.*, **2003**, *85*(6), 1394-1401.

[64] Abe, K.; Pan, L.H.; Watanabe, M.; Kato, T.; Itoyama, Y. Induction of nitrotyrosine-like immunoreactivity in the lower motor neuron of amyotrophic lateral sclerosis. *Neurosci. Lett.*, **1995**, *199*(2), 152-154.

[65] Dalle-Donne, I.; Scaloni, A.; Butterfield, D.A. Redox Proteomics: From Protein Modifications to Cellular Dysfunctions and Diseases. John Wiley & Sons, Hoboken, NJ, **2006**.

[66] Sultana, R.; Poon, H.F.; Cai, J.; Pierce, W.M.; Merchant, M.; Klein, J.B.; Markesbery, W.R.; Butterfield, D.A. Identification of nitrated proteins in Alzheimer's disease brain using a redox proteomics approach. *Neurobiol. Dis.*, **2006**, *22*(1), 76-87.

[67] Messier, C.; Gagnon, M. Glucose regulation and brain aging. *J. Nutr. Health Aging*, **2000**, *4*(4), 208-213.

[68] Vanhanen, M.; Soininen, H. Glucose intolerance, cognitive impairment and Alzheimer's disease. *Curr. Opin. Neurol.*, **1998**, *11*(6), 673-677.

[69] Butterfield, D.A.; Lange, M.L. Multifunctional roles of enolase in Alzheimer's disease brain: beyond altered glucose metabolism. *J. Neurochem.*, **2009**, *111*(4), 915-933.

[70] Ojika, K.; Tsugu, Y.; Mitake, S.; Otsuka, Y.; Katada, E. NMDA receptor activation enhances the release of a cholinergic differentiation peptide (HCNP) from hippocampal neurons *in vitro*. *Brain Res. Dev. Brain Res.*, **1998**, *106*(1-2), 173-180.

[71] Rossor, M.N.; Svendsen, C.; Hunt, S.P.; Mountjoy, C.Q.; Roth, M.; Iversen, L.L. The substantia innominata in Alzheimer's disease: an histochemical and biochemical study of cholinergic marker enzymes. *Neurosci. Lett.*, **1982**, *28*(2), 217-222.

[72] Giacobini, E. Cholinergic function and Alzheimer's disease. *Int. J. Geriatr. Psychiatry*, **2003**, *18*(Suppl 1), S1-S5.

[73] Reed, T.T.; Pierce, W.M. Jr.; Turner, D.M.; Markesbery, W.R.; Butterfield, D.A. Proteomic identification of nitrated brain proteins in early Alzheimer's disease inferior parietal lobule. *J. Cell. Mol. Med.*, **2009**, *13*(8B), 2019-2029.

[74] Sun, M.K.; Alkon, D.L. Carbonic anhydrase gating of attention: memory therapy and enhancement. *Trends Pharmacol. Sci.*, **2002**, *23*(2), 83-89.

[75] Chuang, D.M.; Hough, C.; Senatorov, V.V. Glyceraldehyde-3-phosphate dehydrogenase, apoptosis, and neurodegenerative diseases. *Annu. Rev. Pharmacol. Toxicol.*, **2005**, *45*, 269-290.

[76] Butterfield, D.A.; Hardas, S.S.; Lange, M.L. Oxidatively modified glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and Alzheimer's disease: many pathways to neurodegeneration. *J. Alzheimers Dis.*, **2010**, *20*(2), 369-393.

[77] Summers, W.K.; Viesselman, J.O.; Marsh, G.M.; Candelora, K. Use of THA in treatment of Alzheimer-like dementia: pilot study in twelve patients. *Biol. Psychiatry*, **1981**, *16*(2), 145-153.

[78] Summers, W.K.; Majovski, L.V.; Marsh, G.M.; Tachiki, K.; Kling, A. Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type. *N. Engl. J. Med.*, **1986**, *315*(20), 1241-1245.

[79] Blackard, W.G. Jr.; Sood, G.K.; Crowe, D.R.; Fallon, M.B. Tacrine. A cause of fatal hepatotoxicity? *J. Clin. Gastroenterol.*, **1998**, *26*(1), 57-59.

[80] Watkins, P.B.; Zimmerman, H.J.; Knapp, M.J.; Gracon, S.I.; Lewis, K.W. Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease. *JAMA*, **1994**, *271*(13), 992-998.

[81] Farlow, M.; Gracon, S.I.; Hershey, L.A.; Lewis, K.W.; Sadowsky, C.H.; Dolan-Ureno, J. A controlled trial of tacrine in Alzheimer's disease. The Tacrine Study Group. *JAMA*, **1992**, *268*(18), 2523-2529.

[82] Takada-Takatori, Y.; Kume, T.; Izumi, Y.; Ohgi, Y.; Niidome, T.; Fujii, T.; Sugimoto, H.; Akaike, A. Roles of nicotinic receptors in acetylcholinesterase inhibitor-induced neuroprotection and nicotinic receptor up-regulation. *Biol. Pharm. Bull.*, **2009**, *32*(3), 318-324.

[83] Jann, M.W.; Shirley, K.L.; Small, G.W. Clinical pharmacokinetics and pharmacodynamics of cholinesterase inhibitors. *Clin. Pharmacokinet.*, **2002**, *41*(10), 719-739.

[84] Correia, M.A. In: *Katzung BG. Basic and Clinical Pharmacology*, 10th Ed.; McGraw Hill, **2007**; pp. 50-63.

[85] Román, G.C.; Rogers, S.J. Donepezil: a clinical review of current and emerging indications. *Expert Opin. Pharmacother.*, **2004**, *5*(1), 161-180.

[86] Lockhart, I.A.; Mitchell, S.A.; Kelly, S. Safety and tolerability of donepezil, rivastigmine and galantamine for patients with Alzheimer's disease: systematic review of the 'real-world' evidence. *Dement. Geriatr. Cogn. Disord.*, **2009**, *28*(5), 389-403.

[87] Birks, J.; Grimley Evans, J.; Iakovidou, V.; Tsolaki, M.; Holt, F.E. Rivastigmine for Alzheimer's disease. *Cochrane Database Syst. Rev.*, **2009**, (2), CD001191.

[88] Benjamin, B.; Burns, A. Donepezil for Alzheimer's disease. *Expert Rev. Neurother.*, **2007**, *7*(10), 1243-1249.

[89] Thomas, S.J.; Grossberg, G.T. Memantine: a review of studies into its safety and efficacy in treating Alzheimer's disease and other dementias. *Clin. Interv. Aging*, **2009**, *4*, 367-377.

[90] Hsiung, G.Y. Feldman HH. Pharmacological treatment in moderate-to-severe Alzheimer's disease. *Expert Opin. Pharmacother.*, **2008**, *9*(15), 2575-2582.

[91] Bachurin, S.; Bukatina, E.; Lermontova, N.; Tkachenko, S.; Afanasiev, A.; Grigoriev, V.; Grigorieva, I.; Ivanov, Y.; Sablin, S.; Zefirov, N. Antihistamine agent Dimebon as a novel neuroprotector and a cognition enhancer. *Ann. NY Acad. Sci.*, **2001**, *939*, 425-435.

[92] Bachurin, S.O.; Shevtsova, E.P.; Kireeva, E.G.; Oxenkrug, G.F.; Sablin, S.O. Mitochondria as a target for neurotoxins and neuroprotective agents. *Ann. NY Acad. Sci.*, **2003**, *993*, 334-344.

[93] Lermontova, N.N.; Redkozubov, A.E.; Shevtsova, E.F.; Serkova, T.P.; Kireeva, E.G.; Bachurin, S.O. Dimebon and tacrine inhibit neurotoxic action of beta-amyloid in culture and block L-type Ca(2+) channels. *Bull. Exp. Biol. Med.*, **2001**, *132*(5), 1079-1083.

[94] Grigorev, V.V.; Dranyi, O.A.; Bachurin, S.O. Comparative study of action mechanisms of dimebon and memantine on AMPA- and NMDA-subtypes glutamate receptors in rat cerebral neurons. *Bull. Exp. Biol. Med.*, **2003**, *136*(5), 474-477.

[95] Jones, R.W. Dimebon disappointment. *Alzheimers Res. Ther.*, **2010**, *2*(5), 25.

[96] Brown, T.H.; Chapman, P.F.; Kairiss, E.W.; Keenan, C.L. Long-term synaptic potentiation. *Science*, **1988**, *242*(4879), 724-728.

[97] Lafon-Cazal, M.; Pietri, S.; Culcasi, M.; Bockaert, J. NMDA-dependent superoxide production and neurotoxicity. *Nature*, **1993**, *364*(6437), 535-537.

[98] Mayer, B.; Klatt, P.; Böhme, E.; Schmidt, K. Regulation of neuronal nitric oxide and cyclic GMP formation by Ca2+. *J. Neurochem.*, **1992**, *59*(6), 2024-2029.

[99] Mungrue, I.N.; Bredt, D.S. nNOS at a glance: implications for brain and brawn. *J. Cell. Sci.*, **2004**, *117*(Pt 13), 2627-2629.

[100] Calabrese, V.; Mancuso, C.; Calvani, M.; Rizzarelli, E.; Butterfield, D.A.; Stella, A.M. Nitric oxide in the central nervous system: neuroprotection versus neurotoxicity. *Nat. Rev. Neurosci.*, **2007**, *8*(10), 766-775.

[101] McKeage, K. Memantine: a review of its use in moderate to severe Alzheimer's disease. *CNS Drugs*, **2009**, *23*(10), 881-897.

[102] Rossom, R.; Adityanjee Dysken, M. Efficacy and tolerability of memantine in the treatment of dementia. *Am. J. Geriatr. Pharmacother.*, **2004**, *2*(4), 303-312.

[103] Reisberg, B.; Doody, R.; Stöffler, A.; Schmitt, F.; Ferris, S.; Möbius, H.J. Memantine Study Group. Memantine in moderate-to-severe Alzheimer's disease. *N. Engl. J. Med.*, **2003**, *348*(14), 1333-1341.

[104] Tariot, P.N.; Farlow, M.R.; Grossberg, G.T.; Graham, S.M.; McDonald, S.; Gergel, I. Memantine Study Group. Memantine treatment in patients with moderate to severe Alzheimer disease already receiving donepezil: a randomized controlled trial. *JAMA*, **2004**, *291*(3), 317-324.

[105] van Dyck, C.H.; Tariot, P.N.; Meyers, B.; Malca Resnick, E. for the Memantine MEM-MD-01 Study Group. A 24-week randomized, controlled trial of memantine in patients with moderate-to-severe Alzheimer disease. *Alzheimer Dis. Assoc. Disord.*, **2007**, *21*(2), 136-143.

[106] Farlow, M.R.; Alva, G.; Meng, X.; Olin, J.T. A 25-week, open-label trial investigating rivastigmine transdermal patches with concomitant memantine in mild-to-moderate Alzheimer's disease: a post hoc analysis. *Curr. Med. Res. Opin.*, **2010**, *26*(2), 263-269.

[107] Modrego, P.J.; Fayed, N.; Errea, J.M.; Rios, C.; Pina, M.A.; Sarasa, M. Memantine versus donepezil in mild to moderate Alzheimer's disease: a randomized trial with magnetic resonance spectroscopy. *Eur. J. Neurol.*, **2010**, *17*(3), 405-412.

[108] Skolnick, P.; Popik, P.; Trullas, R. Glutamate-based antidepressants: 20 years on. *Trends Pharmacol. Sci.*, **2009**, *30*(11), 563-569.

[109] Jhee, S.S.; Chappell, A.S.; Zarotsky, V.; Moran, S.V.; Rosenthal, M.; Kim, E.; Chalon, S.; Toublanc, N.; Brandt, J.; Coutant, D.E.; Ereshefsky, L. Multiple-dose plasma pharmacokinetic and safety study of LY450108 and LY451395 (AMPA receptor potentiators) and their concentration in cerebrospinal fluid in healthy human subjects. *J. Clin. Pharmacol.*, **2006**, *46*(4), 424-432.

[110] Evotec AG. http://www.evotec.com (accessed Mar 28, 2008).

[111] Chappell, A.S.; Gonzales, C.; Williams, J.; Witte, M.M.; Mohs, R.C.; Sperling, R. AMPA potentiator treatment of cognitive deficits in Alzheimer disease. *Neurology*, **2007**, *68*(13), 1008-1012.

[112] Sheng, B.; Gong, K.; Niu, Y.; Liu, L.; Yan, Y.; Lu, G.; Zhang, L.; Hu, M.; Zhao, N.; Zhang, X.; Tang, P.; Gong, Y. Inhibition of gamma-secretase activity reduces Abeta production, reduces oxidative stress, increases mitochondrial activity and leads to reduced vulnerability to apoptosis: Implications for the treatment of Alzheimer's disease. *Free Radic. Biol. Med.*, **2009**, *46*(10), 1362-1375.

[113] Yi, P.; Hadden, C.; Kulanthaivel, P.; Calvert, N.; Annes, W.; Brown, T.; Barbuch, R.J.; Chaudhary, A.; Ayan-Oshodi, M.A.; Ring, B.J. Disposition and metabolism of semagacestat, a {gamma}-secretase inhibitor, in humans. *Drug Metab. Dispos.*, **2010**, *38*(4), 554-565.

[114] Galasko, D.R.; Graff-Radford, N.; May, S.; Hendrix, S.; Cottrell, B.A.; Sagi, S.A.; Mather, G.; Laughlin, M.; Zavitz, K.H.; Swabb, E.; Golde, T.E.; Murphy, M.P.; Koo, E.H. Safety, tolerability, pharmacokinetics, and Abeta levels after short-term administration of R-flurbiprofen in healthy elderly individuals. *Alzheimer Dis. Assoc. Disord.*, **2007**, *21*(4), 292-299.

[115] Siemers, E.R.; Quinn, J.F.; Kaye, J.; Farlow, M.R.; Porsteinsson, A.; Tariot, P.; Zoulnouni, P.; Galvin, J.E.; Holtzman, D.M.; Knopman, D.S.; Satterwhite, J.; Gonzales, C.; Dean, R.A.; May, P.C. Effects of a gamma-secretase inhibitor in a randomized study of patients with Alzheimer disease. *Neurology*, **2006**, *66*(4), 602-604.

[116] Fleisher, A.S.; Raman, R.; Siemers, E.R.; Becerra, L.; Clark, C.M.; Dean, R.A.; Farlow, M.R.; Galvin, J.E.; Peskind, E.R.; Quinn, J.F.; Sherzai, A.; Sowell, B.B.; Aisen, P.S.; Thal, L.J. Phase 2 safety trial targeting amyloid beta production with a gamma-secretase inhibitor in Alzheimer disease. *Arch. Neurol.*, **2008**, *65*(8), 1031-1038.

[117] Extance, A. Alzheimer's failure raises questions about disease modifying strategies. *Nat. Rev. Drug Discov.*, **2010**, *9*(10), 749-751.

[118] Wilcock, G.K.; Black, S.E.; Hendrix, S.B.; Zavitz, K.H.; Swabb, E.A.; Laughlin, M.A. Tarenflurbil Phase II Study investigators. Efficacy and safety of tarenflurbil in mild to moderate Alzheimer's disease: a randomised phase II trial. *Lancet Neurol.*, **2008**, *7*(6), 483-493.

[119] Green, R.C.; Schneider, L.S.; Amato, D.A.; Beelen, A.P.; Wilcock, G.; Swabb, E.A.; Zavitz, K.H. Tarenflurbil Phase 3 Study Group. Effect of tarenflurbil on cognitive decline and activities of daily living in patients with mild Alzheimer disease: a randomized controlled trial. *JAMA*, **2009**, *302*(23), 2557-2564.

[120] Boyd-Kimball, D.; Sultana, R.; Mohmmad-Abdul, H.; Butterfield, D.A. Neurotoxicity and oxidative stress in D1M-substituted Alzheimer's A beta(1–42): relevance to N-terminal methionine chemistry in small model peptides. *Peptides*, **2005**, *26*(4), 665-673.

[121] Boyd-Kimball, D.; Sultana, R.; Poon, H.F.; Lynn, B.C.; Casamenti, F.; Pepeu, G.; Klein, J.B.; Butterfield, D.A. Proteomic identification of proteins specifically oxidized by intracerebral injection of amyloid beta-peptide (1-42) into rat brain: implications for Alzheimer's disease. *Neuroscience*, **2005**, *132*(2), 313-324.

[122] Demuro, A.; Mina, E.; Kayed, R.; Milton, S.C.; Parker, I.; Glabe, C.G. Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. *J. Biol. Chem.*, **2005**, *280*(17), 17294-17300.

[123] Drake, J.; Link, C.D.; Butterfield, D.A. Oxidative stress precedes fibrillar deposition of Alzheimer's disease amyloid beta-peptide (1–42) in a transgenic *Caenorhabditis elegans* model. *Neurobiol. Aging*, **2003**, *24*(3), 415-420.

[124] Oda, T.; Wals, P.; Osterburg, H.H.; Johnson, S.A.; Pasinetti, G.M.; Morgan, T.E.; Rozovsky, I.; Stine, W.B.; Snyder, S.W.; Holzman, T.F.; Krafft, A.G.; Finch E.C. Clusterin (apoJ) alters the aggregation of amyloid beta-peptide (A beta 1–42) and forms slowly sedimenting Abeta complexes that cause oxidative stress. *Exp. Neurol.*, **1995**, *136*(1), 22-31.

[125] Seubert, P.; Barbour, R.; Khan, K.; Motter, R.; Tang, P.; Kholodenko, D.; Kling, K.; Schenk, D.; Johnson-Wood, K.; Schroeter, S.; Gill, D.; Jacobsen, J.S.; Pangalos, M.; Basi, G.; Games, D. Antibody capture of soluble Abeta does not reduce cortical Abeta amyloidosis in the PDAPP mouse. *Neurodegener. Dis.*, **2008**, *5*(2), 65-71.

[126] Kerchner, G.A.; Boxer, A.L. Bapineuzumab. *Expert Opin. Biol. Ther.*, **2010**, *10*(7), 1121-1230.

[127] Rinne, J.O.; Brooks, D.J.; Rossor, M.N.; Fox, N.C.; Bullock, R.; Klunk, W.E.; Mathis, C.A.; Blennow, K.; Barakos, J.; Okello, A.A.; Rodriguez Martinez de Liano, S.; Liu, E.; Koller, M.; Gregg, K.M.; Schenk, D.; Black, R.; Grundman, M. 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. *Lancet Neurol.*, **2010**, *9*(4), 363-372.

[128] Salloway, S.; Sperling, R.; Gilman, S.; Fox, N.C.; Blennow, K.; Raskind, M.; Sabbagh, M.; Honig, L.S.; Doody, R.; van Dyck, C.H.; Mulnard, R.; Barakos, J.; Gregg, K.M.; Liu, E.; Lieberburg, I.; Schenk, D.; Black, R.; Grundman, M. Bapineuzumab 201 Clinical Trial Investigators. A phase 2 multiple ascending dose trial of bapineuzumab in mild to moderate Alzheimer disease. *Neurology*, **2009**, *73*(24), 2061-2070.

[129] DeMattos, R.B.; Racke, M.M.; Gelfanova, V. Identification, characterization, and comparison of amino-terminally truncated Abeta42 peptides in Alzheimer's disease brain tissue and in plasma from Alzheimer's patients receiving solanezumab immunotherapy treatment. *Alzheimers Dement.*, **2009**, *5*(4 Suppl 1), P156-P157.

[130] Siemers, E.R.; Friedrich, S.; Dean, R.A.; Gonzales, C.R.; Farlow, M.R.; Paul, S.M.; DeMattos, R.B. Safety and changes in plasma and cerebrospinal fluid amyloid beta after a single administration of an amyloid beta monoclonal antibody in subjects with Alzheimer disease. *Clin. Neuropharmacol.*, **2010**, *33*(2), 67-73.

[131] Saez, T.E.; Pehar, M.; Vargas, M.; Barbeito, L.; Maccioni, R.B. Astrocytic nitric oxide triggers tau hyperphosphorylation in hippocampal neurons. *In Vivo*, **2004**, *18*(3), 275-280.

[132] Zhang, Y.J.; Xu, Y.F.; Liu, Y.H.; Yin, J.; Wang, J.Z. Nitric oxide induces tau hyperphosphorylation *via* glycogen synthase kinase-3beta activation. *FEBS Lett.*, **2005**, *579*(27), 6230-6236.

[133] de la Monte, S.M.; Jhaveri, A.; Maron, B.A.; Wands, J.R. Nitric oxide synthase 3-mediated neurodegeneration after intracerebral gene delivery. *J. Neuropathol. Exp. Neurol.*, **2007**, *66*(4), 272-283.

[134] Colton, C.A.; Vitek, M.P.; Wink, D.A.; Xu, Q.; Cantillana, V.; Previti, M.L.; Van Nostrand, W.E.; Weinberg, J.B.; Dawson, H. NO synthase 2 (NOS2) deletion promotes multiple pathologies in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA*, **2006**, *103*(34), 12867-12872.

[135] Colton, C.A.; Wilcock, D.M.; Wink, D.A.; Davis, J.; Van Nostrand, W.E.; Vitek, M.P. The effects of NOS2 gene deletion on mice expressing mutated human AbetaPP. *J. Alzheimers Dis.*, **2008**, *15*(4), 571-587.

[136] Cappelletti, G.; Tedeschi, G.; Maggioni, M.G.; Negri, A.; Nonnis, S.; Maci, R. The nitration of tau protein in neurone-like PC12 cells. *FEBS Lett.*, **2004**, *562*(1-3), 35-39.

[137] Wischik, C.M.; Edwards, P.C.; Lai, R.Y.; Roth, M.; Harrington, C.R. Selective inhibition of Alzheimer disease-like tau aggregation by phenothiazines. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*(20), 11213-11218.

[138] Medina, D.X.; Caccamo, A.; Oddo, S. Methylene Blue Reduces Abeta Levels and Rescues Early Cognitive Deficit by Increasing Proteasome Activity. *Brain Pathol.*, **2011**, 21(2), 140-149.

[139] Medpage Today. http://www.medpagetoday.com/MeetingCoverage/ICAD/10320

[140] Gozes, I.; Divinski, I.; Piltzer, I. NAP and D-SAL: neuroprotection against the beta amyloid peptide (1-42). *BMC Neurosci.*, **2008**, *9*(Suppl 3), S3.

[141] Matsuoka, Y.; Jouroukhin, Y.; Gray, A.J.; Ma, L.; Hirata-Fukae, C.; Li, H.F.; Feng, L.; Lecanu, L.; Walker, B.R.; Planel, E.; Arancio, O.; Gozes, I.; Aisen, P.S. A neuronal microtubule-interacting agent, NAPVSIPQ, reduces tau pathology and enhances cognitive function in a mouse model of Alzheimer's disease. *J. Pharmacol. Exp. Ther.*, **2008**, *325*(1), 146-153.

[142] Vulih-Shultzman, I.; Pinhasov, A.; Mandel, S.; Grigoriadis, N.; Touloumi, O.; Pittel, Z.; Gozes, I. Activity-dependent neuroprotective protein snippet NAP reduces tau hyperphosphorylation and enhances learning in a novel transgenic mouse model. *J. Pharmacol. Exp. Ther.*, **2007**, *323*(2), 438-449.

[143] Matsuoka, Y.; Gray, A.J.; Hirata-Fukae, C.; Minami, S.S.; Waterhouse, E.G.; Mattson, M.P.; LaFerla, F.M.; Gozes, I.; Aisen, P.S. Intranasal NAP administration reduces accumulation of amyloid peptide and tau hyperphosphorylation in a transgenic mouse model of Alzheimer's

disease at early pathological stage. *J. Mol. Neurosci.*, **2007**, *31*(2), 165-170.

[144] Ashur-Fabian, O.; Segal-Ruder, Y.; Skutelsky, E.; Brenneman, D.E.; Steingart, R.A.; Giladi, E.; Gozes, I. The neuroprotective peptide NAP inhibits the aggregation of the beta-amyloid peptide. *Peptides*, **2003**, *24*(9), 1413-1423.

[145] Allon Therapeutics Inc. http://www.allontherapeutics.com (accessed Sep 27, 2010).

[146] Allon Therapeutics Inc. http://www.allontherapeutics.com (May 27, 2005).

[147] Allon Therapeutics Inc. http://www.allontherapeutics.com (Aug 28, 2006).

[148] Boolell, M.; Allen, M.J.; Ballard, S.A.; Gepi-Attee, S.; Muirhead, G.J.; Naylor, A.M.; Osterloh, I.H.; Gingell, C. Sildenafil: an orally active type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction. *Int. J. Impot. Res.*, **1996**, *8*(2), 47-52.

[149] Boolell, M.; Gepi-Attee, S.; Gingell, J.C.; Allen, M.J. Sildenafil, a novel effective oral therapy for male erectile dysfunction. *Br. J. Urol.*, **1996**, *78*(2), 257-261.

[150] Redington, A.E. Modulation of nitric oxide pathways: therapeutic potential in asthma and chronic obstructive pulmonary disease. *Eur. J. Pharmacol.*, **2006**, *533*(1-3), 263-276.

[151] Griffiths, M.J.; Evans, T.W. Inhaled nitric oxide therapy in adults. *N. Engl. J. Med.*, **2005**, *353*(25), 2683-2695.

[152] Hemnes, A.R.; Champion, H.C. Sildenafil, a PDE5 inhibitor, in the treatment of pulmonary hypertension. *Expert Rev. Cardiovasc. Ther.*, **2006**, *4*(3), 293-300.

[153] Boulton, C.L.; Southam, E.; Garthwaite, J. Nitric oxide-dependent long-term potentiation is blocked by a specific inhibitor of soluble guanylyl cyclase. *Neuroscience*, **1995**, *69*(3), 699-703.

[154] Chien, W.L.; Liang, K.C.; Teng, C.M.; Kuo, S.C.; Lee, F.Y.; Fu, W.M. Enhancement of long-term potentiation by a potent nitric oxide-guanylyl cyclase activator, 3-(5-hydroxymethyl-2-furyl)-1-benzyl-indazole. *Mol. Pharmacol.*, **2003**, *63*(6), 1322-1328.

[155] Datta, S.; Patterson, E.H.; Siwek, D.F. Endogenous and exogenous nitric oxide in the pedunculopontine tegmentum induces sleep. *Synapse*, **1997**, *27*(1), 69-78.

[156] Cavas, M.; Navarro, J.F. Effects of selective neuronal nitric oxide synthase inhibition on sleep and wakefulness in the rat. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **2006**, *30*(4), 56-67.

[157] Clement, P.; Sarda, N.; Cespuglio, R.; Gharib, A. Potential role of inducible nitric oxide synthase in the sleep-wake states occurrence in old rats. *Neuroscience*, **2005**, *135*(2), 347-355.

[158] Vozzo, R.; Wittert, G.A.; Horowitz, M.; Morley, J.E.; Chapman, I.M. Effect of nitric oxide synthase inhibitors on short-term appetite and food intake in humans. *Am. J. Physiol.*, **1999**, *276*(6 Pt 2), R1562-R1568.

[159] Su, Y.C.; Vozzo, R.; Doran, S.; Leelakusolvong, S.; Rayner, C.K.; Chapman, I.M.; Jones, K.L.; Horowitz, M. Effects of the nitric oxide synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME) on antropyloroduodenal motility and appetite in response to intraduodenal lipid infusion in humans. *Scand. J. Gastroenterol.*, **2001**, *36*(9), 948-954.

[160] Mathai, M.L.; Hjelmqvist, H.; Keil, R.; Gerstberger, R. Nitric oxide increases cutaneous and respiratory heat dissipation in conscious rabbits. *Am. J. Physiol.*, **1997**, *272*(6 Pt 2), R1691-R1697.

[161] Mills, P.C.; Scott, C.M.; Marlin, D.J. Effects of nitric oxide inhibition on thermoregulation during exercise in the horse. *Ann. NY Acad. Sci.*, **1997**, *813*, 591-599.

[162] Lacerda, A.C.; Marubayashi, U.; Coimbra, C.C. Nitric oxide pathway is an important modulator of heat loss in rats during exercise. *Brain Res. Bull.*, **2005**, *67*(1-2), 110-116.

[163] Fabris, G.; Anselmo-Franci, J.A.; Branco, L.G. Role of nitric oxide in hypoxia-induced hyperventilation and hypothermia: participation of the locus coeruleus. *Braz. J. Med. Biol. Res.*, **1999**, *32*(11), 1389-1398.

[164] Karanth, S.; Lyson, K.; McCann, S.M. Role of nitric oxide in interleukin 2-induced corticotropin-releasing factor release from incubated hypothalami. *Proc. Natl. Acad. Sci USA*, **1993**, *90*(8), 3383-3387.

[165] Rivier, C. Role of nitric oxide in regulating the rat hypothalamic-pituitary-adrenal axis response to endotoxemia. *Ann. NY Acad. Sci.*, **2003**, *992*, 72-85.

[166] Van Geldre, L.A.; Lefebvre, R.A. Interaction of NO and VIP in gastrointestinal smooth muscle relaxation. *Curr. Pharm. Des.*, **2004**, *10*(20), 2483-2497.

[167] Toda, N.; Ayajiki, K.; Okamura, T. Nitric oxide and penile erectile function. *Pharmacol. Ther.*, **2005**, *106*(2), 233-266.

[168] Culmsee, C.; Gerling, N.; Landshamer, S.; Rickerts, B.; Duchstein, H.J.; Umezawa, K.; Klumpp, S.; Krieglstein, J. Nitric oxide donors induce neurotrophin-like survival signaling and protect neurons against apoptosis. *Mol. Pharmacol.*, **2005**, *68*(4), 1006-1017.

[169] Karaçay, B.; Li, G.; Pantazis, N.J.; Bonthius, D.J. Stimulation of the cAMP pathway protects cultured cerebellar granule neurons against alcohol-induced cell death by activating the neuronal nitric oxide synthase (nNOS) gene. *Brain Res.*, **2007**, *1143*, 34-45.

[170] Jaffrey, S.R.; Erdjument-Bromage, H.; Ferris, C.D.; Tempst, P.; Snyder, S.H. Protein S-nitrosylation: a physiological signal for neuronal nitric oxide. *Nat. Cell. Biol.*, **2001**, *3*(2), 193-197.

[171] Choi, Y.B.; Tenneti, L.; Le, D.A.; Ortiz, J.; Bai, G.; Chen, H.S.; Lipton, S.A.Molecular basis of NMDA receptor-coupled ion channel modulation by S-nitrosylation. *Nat. Neurosci.*, **2000**, *3*(1), 15-21.

[172] Lipton, S.A.; Singel, D.J.; Stamler, J.S. Nitric oxide in the central nervous system. *Prog. Brain Res.*, **1994**, *103*, 359-364.

[173] Nicholson, R.; Spanswick, D.; Lee K. Nitric oxide inhibits NMDA currents in a subpopulation of substantia gelatinosa neurons of the adult rat spinal cord. *Neurosci. Lett.*, **2004**, *359*(3), 180-184.

[174] Choi, Y.B.; Lipton, S.A. Redox modulation of the NMDA receptor. *Cell. Mol. Life Sci.*, **2000**, *57*(11), 1535-1541.

[175] Melino, G.; Bernassola, F.; Knight, R.A.; Corasaniti, M.T.; Nistico, G.; Finazzi-Agro, A. S-nitrosylation regulates apoptosis. *Nature*, **1997**, *388*(6641), 432-433.

[176] Liu, L.; Stamler, J.S. NO: an inhibitor of cell death. *Cell Death Differ.*, **1999**, *6*(10), 937-942.

[177] Mannick, J.B.; Schonhoff, C.; Papeta, N.; Ghafourifar, P.; Szibor, M.; Fang, K.; Gaston B. S-Nitrosylation of mitochondrial caspases. *J. Cell. Biol.*, **2001**, *154*(6), 1111-1116.

[178] Tenneti, L.; D'Emilia, D.M.; Lipton, S.A. Suppression of neuronal apoptosis by S-nitrosylation of caspases. *Neurosci. Lett.*, **1997**, *236*(3), 139-142.

[179] Mannick, J.B.; Hausladen, A.; Liu, L.; Hess, D.T.; Zeng, M.; Miao, Q.X.; Kane, L.S.; Gow, A.J.; Stamler, J.S. Fas-induced caspase denitrosylation. *Science*, **1999**, *284*(5414), 651-654.

[180] Simard, A.R.; Rivest, S. Neuroprotective properties of the innate immune system and bone marrow stem cells in Alzheimer's disease. *Mol. Psychiatry*, **2006**, *11*(4), 327-335.

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