

Invited review

Cross-talk between oxidative stress and modifications of cholinergic and glutaminergic receptors in the pathogenesis of Alzheimer's disease¹

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Key words

Alzheimer's disease; oxidative stress; nicotinic receptor; muscarinic receptor; *N*-methyl-*D*-aspartate receptor

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Abstract

Alzheimer's disease (AD) is the most common neurodegenerative disorder, and its pathogenesis is likely to be associated with multiple etiologies and mechanisms in which oxidative stress and deficits of neurotransmitter receptors may play important roles. It has been indicated that a high level of free radicals can influence the expressions of nicotinic receptors (nAChRs), muscarinic receptors (mAChRs), and *N*-methyl-*D*-aspartate (NMDA) receptors, exhibiting disturbances of cellular membrane by lipid peroxidation, damages of the protein receptors by protein oxidation, and possible modified gene expressions of these receptors by DNA oxidation. nAChRs have shown an antioxidative effect by a direct or an indirect pathway; mAChR stimulation may generate reactive oxygen species, which might be a physiological compensative reaction, or improve oxidative stress; and high stimulation to NMDA receptors can increase the sensitivity of oxidative stress of neurons. This review may provide complementary information for understanding the correlation between oxidative stress and changed cholinergic and glutaminergic receptors in AD processing, and for revealing the underlying molecular mechanisms of these factors in the multiple etiologies and pathophysiology of the disorder.

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder of the elderly. The disease occurs both in sporadic and hereditary forms, and the main clinical features of this disease are progressive memory loss, decline in language skills, and other cognitive impairments^[1]. The major pathological hallmarks in AD are senile plaques (SP) and neurofibrillary tangles (NFT), which have for the most part been regarded as central mediators of neuronal cell death leading to cognitive decline and eventual demise^[2,3]. The core of SP is abundantly deposited with β -amyloid peptides (A β), a 39–43 amino acid peptide, which is the result of proteolytic cleavage of the transmembrane amyloid precursor protein (APP). The NFT contain paired helical filaments that are mostly composed of a hyperphosphorylated form of the microtubule-associated tau protein.

Although the initiating causes leading to AD are unknown, it is clear that its pathophysiology is complex and most likely involves multiple distinct and overlapping path-

ways of neuronal damage, including genetic deficit, oxidative stress, impaired neurotransmitter receptors, and other biological or biochemical abnormalities (Figure 1). Since the pathogenesis of AD remains elusive, there is no efficient cure of the disorder, and targeting the disruption of neurotransmission is the most viable therapeutic strategy at present^[4].

Oxidative stress in AD

Oxidative stress, defined as a disturbance in the balance between the production of free radicals and antioxidative defense, may play an important role in several pathological conditions of the central nervous system (CNS)^[5,6]. There is a great deal of evidence to suggest that the damage induced by free radicals may be an important pathogenesis in AD^[7]. Free radicals, such as superoxide, hydroxyl ions, and nitric oxide, cause cell injury by damaging lipids, protein, and DNA when they are generated in excess or antioxidative defense is impaired^[8]. The brain is more vulnerable to oxradical-

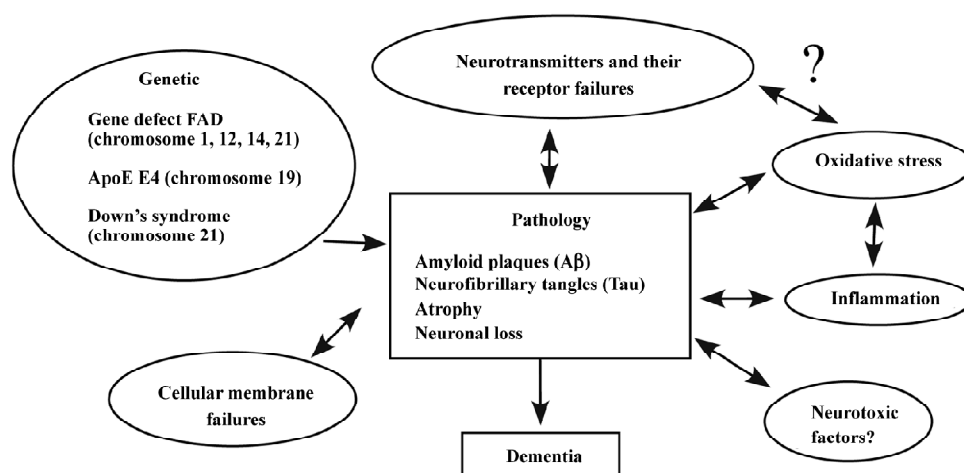


Figure 1. Etiology and pathogenesis of AD. Arrow with double directions means both agents can be initiating reason or secondary result.

mediated injury because its cellular membranes are preferentially enriched in oxyradical-sensitive polyunsaturated fatty acids that are the substrates of lipid peroxidation, and damaged adult neurons cannot be replaced.

Multiple lines of evidence link oxidative stress and AD. In brain tissues from AD, carbonyls derived from protein oxidation were increased in the neuronal cytoplasm and nuclei of neurons and glial cells^[9]. Moreover, NFT were strongly labeled by carbonyls. Transgenic animals with A β overexpression show the same type of oxidative damage found in AD, and this damage directly correlates with the presence of A β deposits^[10]. To diagnose AD in clinical examinations, oxidative stress markers, such as 3-nitrotyrosine, 8-hydroxy-2'-deoxyguanosine, and isoprostanes, are found to be increased in cerebrospinal fluid in AD patients^[11]. Interestingly, the elevation of lipid peroxidation in transgenic mice with APP mutations has been observed to precede the surge in the A β level and amyloid plaque formation^[12,13].

Reduced glucose metabolism and mitochondrial abnormalities are associated with AD, as mitochondrial abnormalities are considered to be the source of oxidative stress in AD^[14]. Mitochondrial abnormalities have been associated with deficiencies in enzyme activities, specifically the α -keto-glutarate dehydrogenase complex, pyruvate dehydrogenase complex, and cytochrome oxidase in AD neurons. Oxidative phosphorylation produces superoxide radicals as a byproduct associated with electron transport. Heightened superoxide radical formation in AD also correlates with heightened superoxide dismutase levels that may allow the release of H₂O₂ from the mitochondria to the cytoplasm^[15].

Recent studies of living patients and transgenic mod-

els of the disease have shed light on the central issue of whether oxidative stress is a result of the pathology of AD or whether it is an initiator of pathological damage. From our studies relating to oxidative stress in AD, we have shown decreased cellular membrane phospholipids and polyunsaturated fatty acids in the AD brain, in which these specific lipid modifications strongly support the involvement of free radicals in the pathogenesis of AD^[16]. A closed correlation has been found between the high level of lipid peroxidation and the decreased number of nicotinic receptors in the AD brain^[17]. We have suggested that oxidative stress is an early event in AD and is likely to play a more active role in the pathogenesis than previously hypothesized^[18]. The mechanisms of oxidative stress in AD are shown in Figure 2.

Aggregated A β is toxic in a variety of neuronal cell preparations, and the overexpression of A β in transgenic mice leads to increased neuronal oxidative stress. Interestingly, recent evidence, however, has revealed that A β and NTF might have a compensatory response induced by reactive oxygen species (ROS) and have neurotrophic and potentially cytoprotective properties^[19].

Changed cholinergic and glutaminergic receptors in AD

There is a great deal of evidence that suggests that in the AD brain, a number of neurotransmitter receptor systems are defective. Such abnormalities include cholinergic-, glutaminergic-, dopaminergic-, and γ -amino butyric acid-receptor (GABA) systems. Of all the neurotransmitter systems studied, cholinergic and glutaminergic receptors are

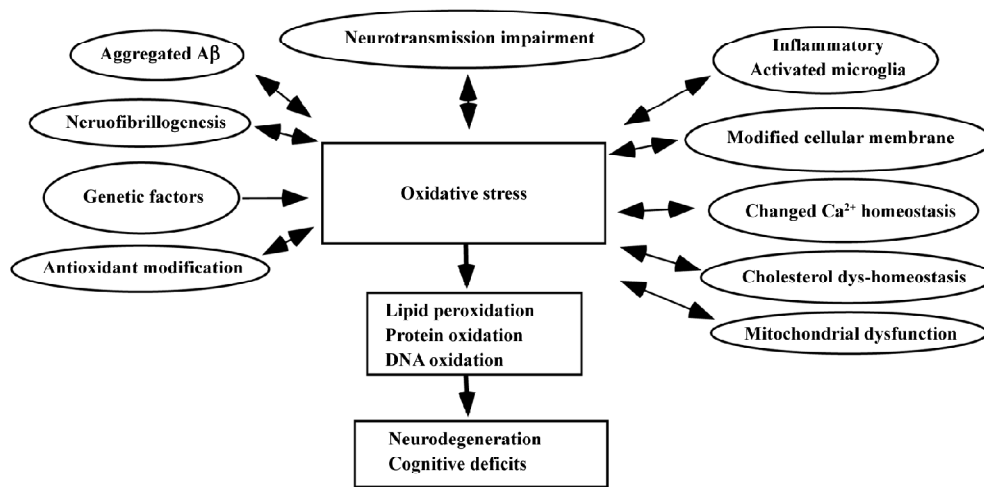


Figure 2. Mechanism of oxidative stress in AD. Arrow with double directions means both agents can be initiating reason or secondary result.

more noticeably involved in the pathogenesis of AD.

Neuronal nicotinic acetylcholine receptors in AD

In cholinergic transmission, neuronal nicotinic acetylcholine receptors (nAChRs) have been extensively investigated during the progress of AD. The nAChRs belong to a gene super family of homologous receptors, including nicotinic, GABA, glycine, serotonin, and glutamate receptors^[20]. The nAChRs in the brain are composed of 2 types of subunits, α and β . The gene family for neuronal nAChRs contains at least 9 α subunits ($\alpha 2$ – $\alpha 10$) and 3 β subunits ($\beta 2$ – $\beta 4$). Like the muscle type of nAChRs, the agonist recognition site of the neuronal nAChRs is on the interface between the α and β subunits. The expression of combinations of different α subunits with β subunits may produce functionally distinct neuronal nAChRs in different brain regions. The $\alpha 4\beta 2$ and $\alpha 7$ subtypes are the most common patterns of nAChRs in the brain^[20].

Interestingly, nAChRs are involved in many brain functions, such as cognition, memory, and neuroprotection, and a pathogenic role of the receptors has been established in AD^[21]. Studies of AD patients with positron emission tomography have shown significant deficits in nAChRs early in the course of the disease, which is related to cognitive function. Significant reductions in the number of nAChRs were also observed in various autopsy brain cortical regions of the Swedish 670/671 APP mutation family^[22]. In our previous investigations, a decreased level of nAChR subunit proteins, such as $\alpha 4$, $\alpha 7$, and $\alpha 3$, were detected in AD brains^[23,24]. A large loss of the receptors in AD brains and in a cellular

model provoked interest in developing therapeutic agonists for specific nAChR subtypes. In a recent study, we found that suppressed gene expression of nAChRs resulted from treatment by nanomolar $A\beta_{25-35}$ and $A\beta_{40}$ in PC12 cells^[25].

The $\alpha 7$ nAChR is of importance in an AD study due to its neuroprotective function^[26]. The $\alpha 7$ nAChR is highly expressed on neurons of the hippocampus and cholinergic projection neurons from the basal forebrain. A number of recent studies have convincingly demonstrated an interaction between the $\alpha 7$ nAChR and $A\beta$ *in vitro* and on neurons. For instance, it was shown that $A\beta_{1-42}$ co-immunoprecipitates with the $\alpha 7$ nAChR in the samples from a postmortem AD hippocampus, and $A\beta$ can activate $\alpha 7$ nAChR^[27]. A recent study has shown a selective increased $\alpha 7$ nAChR in APP_{SWE} transgenic mice, which is not consistent with earlier changes in AD, and suggested important protective compensatory mechanisms in response to deficits in synaptic plasticity induced by elevated soluble $A\beta$ ^[28]. We observed an increase in the $\alpha 7$ subunit in astrocytes and a decrease in the same receptor subtype in neurons in the hippocampus and the temporal cortex of AD patients who died carrying the APP_{SWE} mutation^[24]. The findings suggest different regulatory mechanisms and roles of the $\alpha 7$ nAChR in astrocytes and neurons.

Muscarinic acetylcholine receptors in AD

The muscarinic acetylcholine receptors (mAChRs) mediate most of the actions of the neurotransmitter acetylcholine in the CNS and peripheral nervous system, as well as in the end organs of parasympathetic nerves^[29]. In mammals, 5 distinct mAChR subtypes (M1–M5) have been identified, with each receptor subtype being the product of a different

gene. The mAChR belongs to the super family of 7 transmembrane receptors, which activate signal transduction pathways through their interaction with GTP binding proteins (G-proteins). mAChRs are stimulated by agonists, such as acetylcholine, which activates G-protein and evokes typically slow and modulatory second message responses. In cholinergic transmission, mAChRs have been implicated in higher brain functions, such as learning and memory, and the loss of muscarinic cholinergic synapses may contribute to the symptoms of AD^[30].

Among the 5 types of mAChRs, the M1 mAChR is predominant in the cerebral cortex and hippocampus and has attracted great interest for its major function in cognitive processing relevant to AD, in particular short-term memory. An early study showed that a significantly reduced number of [³H] quinuclidinyl benzilate binding sites was obtained in AD dementia groups. A significant negative correlation was observed in AD brains between the histopathological dementia score and the reduction in the activity of the acetylcholine synthesizing enzyme choline acetyltransferase^[31]. A disturbance of the muscarinic receptor–G-protein coupling in AD has been suggested, which might be impaired by the formation of A β . This can lead to decreased signal transduction, a decreased secreted form of APP, and increased generation of A β s, and can further aggravate the cholinergic deficiency. M1 agonists can elevate APP, decrease tau protein phosphorylation/hyperphosphorylation, and restore cognitive impairments in several animal models for AD^[32,33]. A significant decrease of M1 mAChR mRNA, but with no changes of M2, M3, or M4, in the temporal cortex of AD has been observed^[34]. The M1 mAChR protein was decreased in the cortex and hippocampus despite unchanged levels of the M1 binding sites in the same regions^[35]. The impaired neurotransmission of mAChRs might be involved in the pathogenesis of AD.

N-methyl-D-aspartate receptors in AD

Glutamatergic neurons form the major excitatory system in the brain and play an important role in brain functions. Glutamate activates several classes of metabotropic receptors, including 3 major types of ionotropic receptors, such as N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid, and kainic acid receptors^[36]. Essentially, the NMDA receptor has important functions in synaptic transmission, synaptic plasticity, synaptogenesis, and excitotoxicity^[37].

There is growing evidence that disturbances of glutamatergic neurotransmission may contribute to the pathophysiological mechanism and cognitive deficits of AD. The colocalization of glutamatergic neurons and NFT or SP

in the AD brain has been observed^[38]. It has been reported that NMDA receptors are decreased in the cortical regions and hippocampus in AD brains^[39]. A reduction in NMDA receptor subunit 1 mRNA in the hippocampus and an increase in the frontal and temporal cortices have also been observed^[40]. In *in vitro* studies, the addition of A β enhances glutamate release from primary cultured microglia of rats^[15] and increases the toxicity of glutamate^[41]. The increase of vulnerability to glutamate-induced excitotoxicity has been found in the hippocampal neurons prepared from presenilin-1 mutation mice^[42]. The studies by using transgenic mice with APP mutations have shown an enhanced sensitivity to glutamatergic agonists, such as NMDA and kainic acid^[43]. Although almost all of the data have reported a decrease or no change of NMDA receptor subunits in AD, there is still ongoing debate as to whether NMDA receptor changes cause excitotoxicity or hypoactivity in AD^[44]. This interest has been increased due to the drug approval of memantine, a partial NMDA antagonist, that can reduce clinical deterioration in moderate to severe AD^[45].

Correlations between oxidative stress and modifications of cholinergic and glutamatergic receptors connected with AD

Both cholinergic and glutamatergic receptors are cellular membrane proteins that are located on the structure, which consists of membrane lipids. Studies on protein–lipid interactions have shown that functions of neurotransmitter receptors are highly dependent on the environment of lipid compositions^[46]. Brain tissue is more vulnerable to free radical-mediated injury, because the organ utilizes high amounts of oxygen and its cellular membranes are preferentially enriched in oxyradical-sensitive polyunsaturated fatty acids^[47]. Sequentially, the surrounding lipids of receptors in the brain are very sensitive to attack by free radicals. Oxidative stress has been implicated as a contributing factor to neurodegeneration in AD and has a significant connection with the deficits of cholinergic and glutamatergic receptors in the CNS.

Oxidative stress and nAChRs

Recently, we observed that lipid peroxidation in cellular membranes can induce a reduction in [³H]epibatidine or [¹²⁵I] α -bungarotoxin (α -BTX) binding of nAChR in PC12 cells^[18]. In addition, the free radical insult (FeSO₄) we used, which it could induce concentration-dependent increases in lipid peroxidation, but did not result in

apoptosis in PC12 cells, significantly induced reductions at the protein level for $\alpha 3$ and $\alpha 7$ nAChR subunits, and at the mRNA level for the $\alpha 7$ subunit^[18], indicating an effect of free radicals on the gene expression of nAChR. The pretreatment of cultural cells with antioxidants, such as vitamin E and reduced glutathione, can prevent the inhibitory effect of free radicals on [³H]epibatidine and [¹²⁵I] α -BTX-binding sites.

The correlation between oxidative stress and the loss of the $\alpha 4$ nAChR subunit has been investigated in the temporal cortex from patients with AD, and the results showed a significant correlation between increased levels of lipid peroxidation and decreased numbers of the $\alpha 4$ nAChR subunit protein in AD brains^[17]. In a recent study, we also found that lipid peroxidation induced directly by A β might be involved in the deficits of nAChR^[48]. In this study, PC12 cells were treated by the addition of 5 mmol/L A β_{25-35} and A β_{1-40} , respectively, with or without an antioxidant, vitamin E. An increased lipid peroxidation and significant reductions in [³H]epibatidine and [¹²⁵I] α -BTX binding sites and in the protein levels of the $\alpha 3$ and $\alpha 7$ nAChR subunits were observed in the cells treated with A β . Interestingly, the decreases of the nAChR binding sites and subunit proteins resulting from A β were mostly prevented by pretreatment with the antioxidant. A β s damage and kill neurons possibly through an effect on membrane lipid peroxidation, impaired ion-motive ATPases, glucose, and glutamate transporters making nerve cells vulnerable to the excitotoxic effects of glutamate^[49]. These findings suggest that lipid peroxidation induced by A β might trigger the loss of nAChR in AD^[48].

Interestingly, the $\alpha 7$ nAChR subtype may have an antioxidative function. When being exposed to ethanol, PC12 cells exhibited an increase in intracellular oxidative stress, whereas after selectively activating $\alpha 7$ nAChR by a receptor agonist, 3-(2,4)-dimethoxybenzylidene anabaseine, the action of ethanol for stimulating oxidative stress can be attenuated^[50]. These results suggest that the cytoprotection conferred by the $\alpha 7$ nAChR agonist may be mediated at least in part by reducing the formation or accumulation of ROS^[50]. Recently, we observed that the decreased expression of $\alpha 7$ nAChR in SH-SY5Y cells by employing small interference RNA that are specifically targeted towards the receptor can enhance lipid peroxidation and stimulate the toxicity exerted by A β ^[51], suggesting that $\alpha 7$ nAChR plays a significant antioxidative role in connection with the pathogenesis of AD. The antioxidative properties connected with a mechanism through the activation of nAChR have also been demonstrated in PC12 cells by treatment with varying

amounts of nicotine^[52].

Oxidative stress and mAChR

Early studies have shown that oxidative stress can reduce the number of mAChR in the rat brain and canine heart^[53]. The effects may be due to receptor destruction or inactivation resulting from membrane damage mediated by free radicals.

Recent findings have indicated that M1, M2, or M4 mAChR-transfected COS-7 cells show greater oxidative stress sensitivity than those transfected with M3 or M5, and similar findings have also been observed when the cells were exposed to A β_{25-35} and A β_{1-40} , suggesting an attack on the receptors by free radicals induced by A β ^[54]. An endogenous inhibitor with low molecular weight from the AD brain inactivates the human brain mAChR via a decrease of ligand binding to the receptor^[55]. The prevention of inactivation of the mAChR with antioxidants suggests that the endogenous inhibitor with low molecular weight generates damage of the receptor due to oxidative stress.

It has been demonstrated that ROS are generated after mAChR stimulation^[56]. Blocking mAChR can prevent increased levels of oxidative stress. Since stimulation of the mAChR leads to the activation of multisignaling pathways, including the muscarinic-induced, mitogen-activated protein kinases pathways and ROS production, and to the upregulation of the binding activity of transcription factors, such as NF- κ B and activator protein-1 (AP-1), ROS (over a narrow concentration range) might function as second messengers in cell-signaling pathways^[57]. ROS may not merely cause damage, but may also be mediators of physiological functions.

Oxidative stress and NMDA receptors

It has been indicated that A β could place glutamate-sensitive neurons at risk by enhancing glutamate and oxygen free radical production by monocyte-derived cells, in which such mechanisms might contribute to the pathogenesis of AD. In glia cultures, A β inhibits glutamate uptake, probably connected with an increased production of free radicals^[58]. Lipid peroxidation induced by homocysteine can be completely inhibited by the NMDA receptor antagonist, dizocilpine (MK-801), suggesting that oxidative injury to nerve terminals involves NMDA receptor stimulation^[59]. Acute ammonia intoxication diminishes the activities of antioxidative enzymes and increases superoxide formation in rat brains, and the ammonia-induced oxidative stress is mediated by the excessive activation of NMDA receptors^[60].

The protective action of stobadine, an antioxidant, can prevent the decreased number of NMDA binding sites elicited by hypoxia/reoxygenation in the rat hippocampus by its antioxidative and antiradical effects^[61]. A low-affinity antagonist of NMDA receptors, sulfasalazine, can have neuroprotective effects by preventing Ca^{2+} influx and accumulation through blocking NMDA receptors^[62]. Pyridostigmine bromide, a reversible cholinesterase inhibitor, can induce apoptosis in rat cerebellar granule cells by producing ROS generation, which can be blocked by MK-801 and atropine, a muscarinic receptor antagonist^[63].

The mechanism of the influence of oxidative stress on membrane receptors might be involved in: (1) reactive oxygen metabolites affecting the binding of ligands to membrane receptors and also the coupling of receptors to G-proteins and effector enzymes; (2) the peroxidation of membrane lipids altering the viscosity of the plasma membrane, which affects receptor coupling; (3) ROS interaction with thiol/disulfide moieties on receptor proteins or on other factors in the receptor system, which is responsible for alterations in receptor binding or coupling; (4) the association of lipid peroxidation with the modification of fatty acids by the phospholipase A2 pathway, in which arachidonic acid binds directly to the receptors or perturbs the local environment to indirectly affect receptor function; (5) oxidative stress leading to a disturbance in cellular Ca^{2+} homeostasis, which might be related to an effect on Ca^{2+} -mobilizing receptors; (6) ROS interference with actions of nitric oxide, thus affecting another pharmacological messenger system; and (7) oxidative stress influencing the pro-

cessing of transcription or translation of these neurotransmitter receptors by modulating the expression at the protein or mRNA levels.

Conclusion

Both oxidative stress and the modifications of nAChR, mAChR, and NMDA receptors play important roles in the pathogenesis of AD. In addition, there is a closed relationship between oxidative stress and these receptors (Figure 3). A high level of free radicals can induce the disturbance of the cellular membrane by lipid peroxidation and attack protein receptors by protein oxidation, as well as possibly damaging the gene expression of these receptors by DNA oxidation, which might be an important mechanism for the receptor deficit in the AD process. nAChRs have also shown an antioxidative effect by a direct or indirect pathway, whereas high stimulation to NMDA receptors can increase the sensitivity of oxidative stress of neurons. mAChR stimulation can generate ROS, which might be a physiological compensative reaction, or improve oxidative stress. When considering the therapeutic strategy for AD, 2 more approaches with antioxidative properties and neurotransmitter receptor regulation might ultimately be combined to provide important and efficient roles in drug action.

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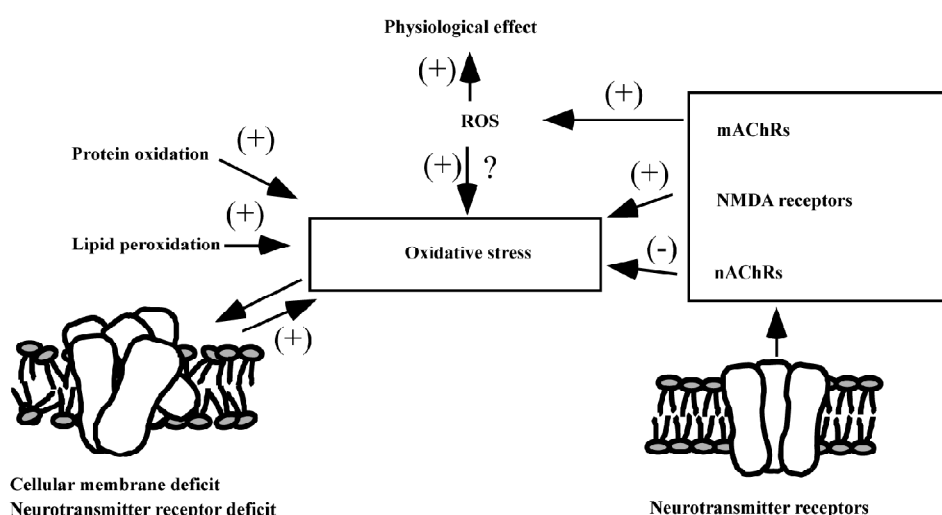


Figure 3. Relationship between oxidative stress and neurotransmitter receptors. The symbol (+) means the effect of stimulation and (-) means inhibition.

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