Neuroprotection

Edited by Raymond Chuen-Chung Chang and Yuen-Shan Ho

Neuroprotection is a strategy to prevent or delay the progression of chronic neurodegenerative diseases, acute neurological disorders, or even mental disorders. The major aim of this book is to focus on different approaches to achieve neuroprotection. In this book, there are chapters discussing imidazoline ligands and opioid ligands in Alzheimer's disease, the beneficial effects of adenosine A2A receptor antagonist, adrenergic receptor agonists and antagonists modulating microglial responses, and different approaches to achieve neuroprotection against aging-associated macular degeneration. This book will give insights to scientists in the field to stimulate their research, medical professionals to review their clinical practices, and others who would like to learn more about different neuroprotective approaches.
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Meet the editor

Dr. Chang is the Lab Chief for the Laboratory of Neurodegenerative Diseases in the School of Biomedical Sciences, LKS Faculty of Medicine, the University of Hong Kong. Dr. Chang has organized the International Alzheimer’s Disease Conference every year since 2000. He has published over 142 peer-reviewed papers, 14 book chapters, and edited 3 books on neurodegenerative diseases. Dr. Chang is the chief editor for the *American Journal of Alzheimer’s Disease and Other Dementias*, senior editor for the *Journal of Neuroimmune Pharmacology*, and associate handling editor for *Frontiers in Neurology/Neurosciences/Psychiatry*. He is on the Scientific Advisory Board of the International AD/PD Symposium, a member of the editorial boards of more than 20 different journals, and a grant reviewer for different grant agencies/foundations.

Dr. Ho has been working for some time on aging-associated neurodegenerative diseases. She is a registered Chinese medicine practitioner with rich experience in both laboratory and clinical research. She has worked as an assistant professor in Macau and is now at the School of Nursing in Hong Kong Polytechnic University. Her research interest includes the use of Chinese herbal medicine and acupuncture to prevent neurodegeneration (neuroprotection). Dr. Ho focuses her research on topics including, but not limited to, disease progression and risk factors leading to neurodegenerative diseases. She has expertise in combining Western and Chinese medicine to elicit holistic effects on the body to exert neuroprotection. She is a member of the editorial boards of four journals and a constant grant reviewer for different journals and funding agencies.
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Neuroprotection is a key approach to maintaining brain health to prevent the retraction of synapses, accumulation of neurodegenerative proteins (tau, \(\beta\)-amyloid peptide, \(\alpha\)-synuclein, huntingtin, or any misfolded proteins), activation of neuroinflammation, or even the breakdown of the blood-brain barrier. Neuroprotection can be applied to chronic neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, Lewy's body diseases, Huntington's disease, amyotrophic lateral sclerosis, or even brain tumors), acute neurological disorders (brain trauma, stroke, transient ischemia, epilepsy, or cerebral hemorrhage), or even mental disorders (psychosis, depression, or schizophrenia). The traditional view of neuroprotection is often restricted to the use of agonists or antagonists of neurotransmitter receptors. While this is still a great approach to safeguard neurons, neuroprotection strategy is not limited to modulating neurotransmitter receptors. Neuroprotection can be accomplished by (1) natural products, (2) herbal medicine, (3) different forms of exercise, (4) learning new skills or languages, or (5) even good sleeping patterns. From all these new perspectives, the concept of neuroprotection is limited not only to the protection of neurons in the pathogenesis of diseases but also to the prevention of any detrimental factors leading to neuronal cell loss.

The major aim of this book is to focus on different approaches to achieve neuroprotection. In this book, most of our authors review the advantages of neurotransmitter receptors. Mititelu-Tartau and Bogdan's group reviews the imidazoline ligands and opioid ligands in Alzheimer's disease. They review the neuroprotective effects of agmatine on memory. In addition, they also summarize the findings of using different opioid receptor ligands to elicit neuroprotection. Similarly, Yanuar's group reviews the beneficial effects of the adenosine A2A receptor antagonist. It has been reported that the adenosine receptor antagonist elicits neuroprotective effects, partially because of its signaling pathways to activate the cytosolic fraction of cAMP. Yanuar's group reviews how A2A antagonists provide neuroprotection.

Apart from modulating neurotransmitter receptors, Flood's group reviews another very important pathological factor leading to neurodegeneration: neuroinflammation. Microglial cells are the major line of cells to be activated to produce cytokines and free radicals to damage neurons. Interestingly, Flood's group reviews how different adrenergic receptor agonists and antagonists modulate microglial responses. This independent review suggests that modulation of neuroinflammation can also be achieved by intervening neurotransmitter receptors and their associated signaling events.

Neuroprotection can be disease specific but use different approaches. Nian and Lo review the neuroprotection of aging eyes, in which aging-associated macular degeneration often occurs in the elderly. With the progression of the disease and identification of biological targets at different states of the disease, the strategy of neuroprotection can be changed.

Taken together, neuroprotection receives increasing attention from different approaches in different states of neurodegenerative diseases, acute neurological disorders, mental disorders, and even age-related diseases.
Preface

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Taken together, neuroprotection receives increasing attention from different approaches in different states of neurodegenerative diseases, acute neurological
disorders, and even mental disorders. Even in disease progression, neuroprotection can prevent the spread of neuronal damage or neuronal loss from one region to other regions. This book will give insights to scientists in the field to stimulate their research, medical professionals to review their clinical practices, and others who would like to learn more about different neuroprotective approaches.

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Section 1

Neurotransmitter Receptor as Target for Neuroprotection
Chapter 1
Introductory Chapter: Concept of Neuroprotection - A New Perspective
Raymond Chuen-Chung Chang and Yuen-Shan Ho

1. Introduction
Neuroprotection is an approach to preserve neurons so that neurons cannot be hurt by different pathological factors in neurodegenerative diseases. It can be an approach before the onset of the disease so that neurons cannot be affected by any risk factors. It can also be an approach during the progression of the disease to prevent spreading of injury from one neuron to neighboring neurons. Therefore, neuroprotection can also be one approach as "disease-modifying agent" to delay and even stop progressive neurodegeneration.

The concept of neuroprotection has long been confined to intervene neurotransmitter receptors via agonists and antagonists. A very well-known example is the neuroprotective effect of caffeine via adenosine receptor, because caffeine is an A2 receptor antagonist. Caffeine can exert neuroprotection via adenosine A2 receptor to protect dopaminergic neurons in an experimental model of Parkinson's disease (PD) by using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as a toxin agent [1]. Therefore, drinking coffee has been considered to be neuroprotection. Apart from adenosine receptor on neurons, it is now known that microglial cells also express A2 receptor, and A2 receptor antagonist or caffeine can reduce activation of microglial cells [2]. Since caffeine or A2 receptor antagonists can protect neurons and minimize activation of microglial cells, research is still very active in this direction. Indeed, investigation of other neurotransmitter receptor antagonists, agonists, blockers, or even partial blockers is still a very active research area in neuroprotection.

While it is exciting to reveal differential neuroprotective effects via modulating receptors for different neurotransmitters, we should not restrict ourselves to this perspective only. In fact, a wider scope of neuroprotection has been evolved. The concept of neuroprotection can now be categorized into three groups: (A) pharmacological intervention, (B) non-pharmacological intervention, and (C) cellular and genetic approaches (Figure 1).

In group A neuroprotective approaches, pharmacological intervention includes modulation of neurotransmitter receptors (as introduced above), anti-oxidative stress, and anti-inflammatory responses [3]. These are classical pharmacological approaches. These methods have been explored for decades and can be used for both neuroprotection and as disease-modifying agents. Their effects can be target-specific for one single protein (e.g., receptor for neurotransmitter or one type of cytokines) or pathway-specific (anti-oxidative responses using nuclear factor erythroid 2-related factor 2, Nrf2). Since single biological target may limit the beneficial effects and may not be able to intervene the complexity of the disease
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progression, neuroprotective effects from herbal medicine or natural products receive increasing attention in scientific research because of their multiple effects on different biological targets [4–6]. For example, it has been shown that triterpenoids extracted from *Prunella vulgaris* has anti-inflammatory effects [7]. We have proved that polysaccharide part of *Prunella vulgaris* can modulate the immune responses of microglia and macrophages [8, 9]. Therefore, *Prunella vulgaris* exerts neuroprotection toward neurodegenerative diseases against neuroinflammation.

In group B neuroprotective approaches, non-pharmacological approaches receive increasing attention. Since it is quite difficult to ask a healthy adult or even elderly to constantly take medicine even though it is herbal, non-pharmacological approaches to earn neuroprotection are most welcome. Among these different methods, exercise is the best to prevent nearly all kinds of diseases. Exercise can be further divided into aerobic and resistance exercises, in which aerobic exercise is suitable for motor-healthy adults or elderly. However, there is a large population of elderly who have difficulty in their motor functions. Resistance exercise becomes an excellent tool for them. Both aerobic and resistance exercises can affect the metabolisms of the body and induce trophic factors like myokines, released from muscles and liver [10, 11]. Since body metabolism is important, diet control is also important to reduce any risk factors leading to the development of neurodegenerative diseases, such as diabetes, hyperlipidemia, or hypercholesterolemia. All of these risk factors are related to vascular components, which may further add on vascular dementia [12–14]. Apart from exercise, it is well-known that, maintaining social activity is essential to keep our brain healthy [15]. In addition, sleeping can promote clearing of pathological factors, such as β-amyloid (Aβ) peptide and phosphorylated tau protein [16, 17]. Also, it has been recently shown that learning second language or bilingualism can be neuroprotective [18]. Furthermore, increasing lines of evidence have shown that acupuncture can help adjusting the body metabolism and immunity [18, 19]. Therefore, acupuncture can also be considered to be one method in non-pharmacological approaches. One key point should be noticed that all these neuroprotective approaches are multiple targets. This may be why they are so effective in minimizing neurodegeneration to preserve neurons.

Neuroprotective approaches in group C are the new extension of multiple effects from trophic factors secreted by genetically engineered cells or viral vector. Alternatively, stimulating the proliferation and differentiation of endogenous stem

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**Figure 1.**
*A summary of different neuroprotective approaches.*
cells, application of induced pluripotent stem cells (iPSC), or mesenchymal stem cells are popular trends in scientific research to prevent neurodegeneration and neuronal loss [20–22].

Since there are several chapters about the effects of neurotransmitter receptors, we will focus on some neuroprotective approaches only, as this is an introductory chapter.

2. Neuroprotective effects of herbal medicine and natural products

This direction of neuroprotection is the most controversial approach among different pharmacological tools. This is because most of the herbal medicine or natural products have a wide array of effects. If we use traditional way of thinking to target a specific protein or signaling pathway, herbal medicine fails to do so. However, if we are aware of neurodegenerative diseases that are usually multifactorial, we can then accept that herbal medicine should be the direction to prevent neuronal loss and spreading of neurodegeneration. Our group has long been working on discovering herbal medicine as neuroprotective agents and investigating their underlying mechanisms. Since most of the herbal medicines are in decoction form, for oral consumption, the effective components in different herbs are not limited to one single chemical. The small molecules being extracted from one herb can also be found from other herbs. There are many laboratories investigating those small molecules and have Research and Development (R&D) to be commercial products. In contrast, big molecules such as polysaccharides have been an unexplored area. In fact, a large portion of components from herbs is polysaccharides. When we use hot water to prepare decoction, what we can easily absorb is polysaccharides. Therefore, we have investigated polysaccharides extracted from different herbs [23].

We have first found that polysaccharides from *Nerium indicum* exert neuroprotective effects against β-amyloid (Aβ) neurotoxicity cultured neurons [24, 25]. Then, we also found that neuroprotective effects of *Verbena officinalis* and *Ganoderma lucidum* [26, 27]. Having investigated different herbal medicine, we found that the polysaccharides from *Lycium barbarum* (Wolfberry) are potent [28, 29]. Polysaccharides can be extracted in hot water or from alkaline condition and elicit neuroprotection [30]. It should be noted that not all polysaccharides, but only some sub-fractions provide neuroprotective effects. Apart from neurons in the brain, polysaccharides from wolfberry can also protect the retina against experimental glaucoma and stroke [31–33]. As harvesting and planting processes have good agricultural practice and the extraction can be done under stringent control of good manufacturing practice, we choose wolfberry as our long-term study. Wolfberry is indeed an anti-aging Chinese herbal medicine. We would like to understand the concept of “anti-aging.” Therefore, we have investigated its application in different aging-associated neurodegenerative diseases [34]. In fact, not all aging-associated neurodegenerative diseases can be attenuated by the polysaccharide fraction of Wolfberry. In Parkinson’s disease (PD), more potent antioxidants are required to provide neuroprotection.

In experimental PD using 6-hydroxydopamine (6-OHDA) as toxin agent, we employed oxyresveratrol, which is a natural product but a structural analog of resveratrol, and found that it can attenuate neurodegeneration [35, 36]. For this kind of neurodegenerative disease requiring high levels and high capacity of antioxidant, another method is to employ pro-drug approach so that oxidant cleave the precursor and the product becomes potent antioxidant. Interestingly, polyphenol (-)-epigallocatechin-3-gallate (EGCG) from green tea falls in this category [37].

The advantages of using herbal medicine or natural products are to make use of their multiple effects. In neurodegenerative diseases, a wide array of stress responses is stimulated because of free radicals or accumulation of misfolded or
badly folded proteins. Most of the herbal medicine and natural products attenuate many stress kinases. They may not be a good candidate to clear accumulation of bad proteins; however, they can inhibit the cascades of stress responses (e.g., activation of c-Jun-N-terminal kinase, JNK; endoplasmic reticulum stress pathways), which usually lead to neuronal apoptosis. Some of the herbal medicine or natural products can even strengthen the survival signaling pathways such as mTOR or Akt pathways.

In addition to their direct effects on neurons, there are many herbal medicine or natural products that can modulate body immune responses. For example, polysaccharides fraction from Prunella vulgaris L. can modulate innate immune responses of macrophages [8, 9]. It has been increasingly aware that activation of body (systemic) immune responses are the origin of our sickness responses leading to low appetites and even fever [38]. Long-term effects of systemic immune responses can even result in psychological depression or acute delirium [39]. Experimentally, we have shown that infection in the body or immune responses triggered by surgery (laparotomy) can modulate cognitive functions because of stimulation of neuroimmune responses [40, 41]. Therefore, modulation of body immunity can be a powerful method to minimize neuroimmune responses and then reduce cognitive dysfunctions.

3. Aerobic and resistance exercises

As emphasized above, the advantages of herbal medicine and natural products are their multiples and wide array effects. Similarly, exercise is another method to achieve this goal. Exercise can stimulate production of different trophic/growth factors, e.g., vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1), peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α)/neuronal fibronectin type III domain-containing protein 5 (FNDC5). Some trophic factors can be secreted by muscles or liver, which is collectively called myokines. It has been shown that muscle cells can produce PGC-1α, irisin, cathepsin B, myostatin (growth differentiation factor 8), fibroblast growth factor 21 (FGF-21), IL-6, IL-15, myonectin, and SPARC (osteonectin) as novel brain-beneficial myokines [10, 42]. Some of them, such as FGF21, can also be secreted by the liver or even in the brain upon exercise [43]. They all can affect muscle, liver, adipose tissues, and brain cells, leading to preservation of cognitive functions, modulating the lipid metabolism, and immune responses [44]. This kind of myokine can pass through the blood-brain barrier.

For motor-healthy subject, aerobic exercise is beneficial to majority of people. As discussed above, myokines can be induced so that they provide neuroprotective effects to the brain and attenuate any systemic immune responses. While aerobic exercise is known to be good, a great number of elderly and demented patients could not enjoy the benefit of aerobic exercise. Those patients may have prior knee replacement or fall down hurting the legs. They may be lying down on the bed. No matter how we advocate the beneficial effects of aerobic exercise, this will not be beneficial to this group of patients. Does it mean that they will not have any beneficial effects of exercise? This question leads us to investigate the beneficial effects of resistance exercise.

In a systematic review, aerobic exercise shows beneficial effects on cognitive functions and executive functions, which is better than that from resistance exercise [45]. Furthermore, aerobic exercise seems to elicit strong anti-inflammatory effect than that of resistance exercise [46]. However, resistance exercise seems to particularly increase the volume of hippocampus [47]. A study in laboratory animal
has shown that aerobic exercise can markedly increase BDNF; whereas, resistance exercise can significantly increase IGF-1 [48]. Although resistance exercise may have some limitations, this is still a good choice for those elderly and dementia patients to have this form of exercise. IGF-1 can still elicit multiple functions in the brain to preserve neurons.

4. Concluding remarks

Since this is an introductory chapter, we do not intend to discuss every single method of neuroprotection in detail. We should keep in mind that neurodegenerative diseases are multiple-hit processes. Therefore, no single biological target can afford all neuroprotective needs. Approaches in using herbal medicine and natural products remind us that multiple biological targets may be the way to exert effective neuroprotection. Therefore, any non-pharmacological approaches including exercise and even the new stem cell approaches exert multiple effects. This can be the way for our effective neuroprotective strategies.

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Chapter 2

Current Therapeutic Approaches
from Imidazoline and Opioid Receptors Modulators in Neuroprotection

Liliana Mititelu-Tartau, Maria Bogdan, Victor Gheorman, Liliana Foia, Ancuta Goriuc, Gabriela Rusu, Beatrice Buca, Liliana Pavel, Ana Cristofor, Cosmin-Gabriel Tartau and Gratiela Eliza Popa

Abstract

Due to brain plasticity, the nervous system is capable of manifesting behavioral variations, adapted to the influences from both external and internal environment. Multiple neurotransmitters are involved in the mediation of pathological processes at the molecular, cellular, regional, and interregional levels participating in cerebral plasticity, their intervention being responsible for various structural, functional, and behavioral disturbances. The current therapeutic strategies in neuroprotection aim at blocking on different levels, the molecular cascades of the pathophysiological mechanisms responsible for neuronal dysfunctions and ultimately for neuronal death. Different agents influencing these neurotransmitters have demonstrated beneficial effects in neurogenesis and neuroprotection, proved in experimental animal models of focal and global ischemic injuries. Serotonin, dopamine, glutamate, N-methyl-D-aspartate, and nitric oxide have been shown to play a significant role in modulating nervous system injuries. The imidazoline system is one of the important systems involved in human brain functioning. Experimental investigations have revealed the cytoprotective effects of imidazoline I2 receptor ligands against neuronal injury induced by hypoxia in experimental animals. The neuroprotective effects were also highlighted for kappa and delta receptors, whose agonists demonstrated the ability to reduce architectural lesions and to recover neuronal functions of animals with experimentally induced brain ischemia.

Keywords: neuroprotection, neurodegenerative diseases, ischemic stroke, imidazoline, opioids, nitric oxide

1. Introduction

Increase in life expectancy has led to aging of the population and consequently to an expansion of the prevalence of neurodegenerative diseases (NDDs) [1].
Chapter 2

Current Therapeutic Approaches from Imidazoline and Opioid Receptors Modulators in Neuroprotection

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1. Introduction

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Neurodegeneration represents a loss of neurons and their structural components (dendrites, axons, and synapses) with a corresponding gradual atrophy in neuronal function [2].

<table>
<thead>
<tr>
<th>Clinical type</th>
<th>Disease/disorder</th>
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| Signs of progressive dementia with no other neurological signs (absent/inconspicuous) | • Alzheimer’s disease  
• Frontotemporal dementias  
• Some cases of Lewy-body disease  
• Posterior cortical atrophy (visuospatial dementia) |
| Signs of progressive dementia accompanied by other neurological abnormalities | • Huntington’s disease (chorea)  
• Lewy-body disease (Parkinsonian features)  
• Some cases of Parkinson’s disease  
• Corticobasal ganglionic degeneration  
• Cortical-striatal-spinal degeneration (Jakob’s disease)  
• Dementia-Parkinson-amyotrophic lateral sclerosis complex  
• Cerebroglobular degeneration  
• Familial dementia with spastic paraparesis, amyotrophy, or myoclonus  
• Polyglucosan body disease  
• Frontotemporal dementia with parkinsonism or ALS |
| Signs of movement disorders or other posture abnormalities | • Parkinson’s disease  
• Multiple system atrophy  
• Essential tremor  
• Progressive supranuclear palsy  
• Dystonia musculorum deformans  
• Huntington’s disease (chorea)  
• Acanthocytosis with chorea  
• Corticobasal ganglionic degeneration  
• Lewy-body disease  
• Restricted dystonia |
| Signs of progressive ataxia | • Spinocerebellar ataxias  
• Cerebellar cortical ataxias  
• Complicated hereditary and sporadic cerebellar ataxias |
| Signs of slowly developing muscular weakness and atrophy | • Motor disorders with amyotrophy  
• Spastic paraplegia without amyotrophy |
| Sensory and sensorimotor disorders | • Hereditary sensorimotor neuropathies  
• Pure or predominantly sensory or motor neuropathic  
• Riley-Day autonomic degeneration |
| Signs of progressive blindness with or without other neurological disorders | • Pigmentary degeneration of retina  
• Stargardt’s disease  
• Age-related macular degeneration |
| Signs characterized by degenerative neurosensory deafness | • Pure neurosensory deafness  
• Hereditary hearing loss with retinal diseases  
• Hereditary hearing loss with system atrophies of the nervous system |

NDDs (e.g., Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, dementia with Lewy bodies) determine cognitive and memory deterioration or alteration of the ability to move, speak, and breathe. These chronic and progressive disorders are an important cause of reduced quality of life, morbidity, caregiver burden and also, of the increase in total healthcare expenditure [3–5]. **Table 1** lists the main clinical types of NDDs [6].

Neuroprotection can be defined as a “relative preservation of neuronal structure and/or function” or as an action that aims “to prevent neuronal damage over time (either acute or chronic)” [7]. Neuroprotective action is primary if it is exerted directly on the neuron, or secondary if it appears from an activity on an intermediary that endangers neuronal function [8].

The mechanisms by which most agents with efficiency in NDDs act are not fully elucidated, requiring multiple and in-depth experimental and clinical studies.

Several experimental investigations highlight the multiple and various interrelations between adrenergic, serotoninergic, dopaminergic, glutamatergic, opioid, imidazoline systems, and the nitric oxide pathways, which may elucidate the effects of different compounds involved in the mediation of pathogenic mechanisms responsible for numerous structural, functional, and behavioral disturbances.

This chapter presents a brief overview of the most studied mechanisms related to neuroprotection and details the possibilities to pharmacologically influence through the main known neurotransmitters the pathophysiological mechanisms linked to various NDDs.

## 2. The imidazoline system

Imidazoline receptors are located not only in the mammalian central nervous system (CNS) cells but also in the peripheral nervous system [9], being involved in the mediation of various physiological processes in the body. It is currently known that there are four types of imidazoline receptors: I1, I2, I3, I4 (non I1-non I2), from which the first three have been mostly studied [10].

It has been emphasized that these receptors play an essential role in cell proliferation, regulation of adipose tissue formation, body temperature maintenance, mediation of gastrointestinal motility, neuroprotection, inflammation, nociceptive sensitivity, and some neurological or psychiatric disorders (such as depression) [11]. Moreover, it is known that these imidazoline receptor subtypes exert control over the activity of the hypothalamic-pituitary-adrenal and noradrenergic axis [12, 13].

A number of different endogenous ligands have been characterized: agmatine, the best known and largely studied, harmalane and harmaline (derivatives of the beta-carboline group), and the newly discovered ribotide (acetic acid imidazole). Agmatine, the potent neurotransmitter of the imidazoline system, has an important role in the mediation of body’s response to stress, analgesia, drug addiction, and abstinence syndrome, in modulation of seizures development, and in neuroprotection [14, 15].

Endogenous agmatine is produced in response to stress (in conditions of ischemia, prolonged exposure to cold) and/or to inflammation [16]. It is assumed that agmatine is also an effective neurotransmitter, due to its concentration in the brain similar to classical neurotransmitters [17, 18].

Literature data have revealed that agmatine stimulates the activity of endothelial nitric oxide synthase [16], this effect being also proved by its level in the rat brain after cerebral ischemia [19, 20].
Along with evidence of its neuromodulatory and neuroprotective properties, there are numerous preclinical studies demonstrating the beneficial effects of exogenous administration of agmatine in depression, anxiety, hypoxic ischemia, pain, morphine tolerance, memory impairment, Parkinson's disease, Alzheimer's disease, epilepsy, and other related conditions with traumatic brain injuries (Figure 1) [21–23]. All these are arguments in favor of the potential of agmatine as a new pharmacological agent for the treatment of various neurological diseases and NDDs [24].

3. The involvement of the imidazoline system in the mediation of cognitive functions

Electrophysiological studies involving various brain areas, performed on laboratory animals with experimentally induced cerebral alterations, have demonstrated the neurotropic effects of agmatine [25].

In vitro experimental researches have shown that activation of I2 receptors via the agmatine endogenous ligand exerts neuroprotective effects by increasing the

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Figure 1.

Neuroprotective effects of agmatine and their first discovery.
expression of glial fibrillary acidic protein in astrocyte cultures and by inhibiting MAO activity [26, 27]. Moreover, the beneficial effects of agmatine have been observed on ischemic-hypoxic lesions, on glutamate-induced neurotoxicity by activating the imidazoline receptors [28, 29]. It was also demonstrated that agmatine administration improves learning activity and memory of rats in experimental models of Alzheimer’s disease and streptozotocin-induced type two diabetes mellitus [22, 30].

Other experimental investigations highlight the neuroprotective effect of intranasal administration of agmatine in elderly female rats, with a significant improvement of neurological status and increase of survival rate [28, 29].

The neuroprotective effects of agmatine on the morphological changes determined by repeated induced stress on medial prefrontal cortex and hippocampus of the rat were also investigated [31]. It was emphasized that under constant stress conditions, morphological alterations of the brain are associated with the reduction of endogenous agmatine levels [measured by high-performance liquid chromatography (HPLC)] and with an increase of arginine-decarboxylase level in the prefrontal cortex, hippocampus, striatum, and hypothalamus [32].

The exogenous administration of agmatine lowers brain morphological impairment, suggesting thus its neuroprotective effects against structural changes in the rat brain, under recurrent stress circumstances [32]. Moreover, elevated levels of agmatine have been evidenced in the blood, cortex, hippocampus, and hypothalamus, immediately after brain hypoxic ischemia. Other studies emphasize the neuroprotective influences of agmatine, highlighted by the increase of its brain levels, in rats subjected to prolonged cold-exposure stress conditions [33].

The neuroprotective potential of agmatine was also highlighted in the experimental model of 1-methyl-4-phenyltetrahydropyridine (MPTP)-induced Parkinson’s disease in mice [29]. The use of agmatine attenuates the loss of cellular dopamine from the black substance and repeated treatment improves short-term memory impairment induced by MPTP in elderly mice. The behavioral benefits of agmatine are associated with the decrease in MPTP-induced glutamate capture in the hippocampal area, suggesting thus its involvement in modulation of glutamate recapture, the possible mechanisms responsible for lowering glutamate extracellular levels, thereby alleviating its neurotoxicity [29].

It is known that alteration of spatial memory in Parkinson’s disease and schizophrenia is attributed to several factors, including hypofunction of glutamate and reduction of hippocampal volume [34]. Literature data report that the administration of the N-methyl-D-aspartate (NMDA) receptor antagonists (phencyclidine, also coded MK801) frequently impairs the late alternation performance in a standardized behavioral model of cognitive functions alteration similar to schizophrenia in laboratory animals [34]. The use of the glutamate/NMDA receptor antagonist phencyclidine induces a spectrum of behavioral, neurochemical, and anatomical changes, manifested by locomotor hyperactivity, motor-negative deficits, and cognition alterations (with memory impairment and visual attention) in laboratory animals. This substance was used to induce the experimental schizophrenia in laboratory animals [35, 36]. Agmatine attenuates cognitive and behavioral deficiency in rats with experimental phencyclidine-induced schizophrenic manifestations [37].

The effects of agmatine on memory alterations similar to those found in Alzheimer’s disease have been evaluated in rats; in the pathogenesis of this degenerative disorder (which causes cognitive deficits in rodents), the fragment beta amyloid Aβ2 25–35 plays an essential role. Studies have shown that agmatine significantly reduces the alterations in memory and spatial learning induced by the beta amyloid Aβ2 25–35 fragment (the neurotoxic component of beta amyloid Aβ 1–42) in various behavioral experimental models, such as: the swimming test, the radial arm maze test, and the object recognition test [38].
It has been revealed that agmatine diminishes the activation of hippocampal caspase-3 (the early indicator of neuronal apoptosis) and prevents the alteration of spatial memory induced by lipopolysaccharides, in the swimming test in rat [39], suggesting its neuroprotective effects.

The neurotropic activity of agmatine has been also evidenced in the structural and cognitive alterations after the administration of NMDA (N-methyl-D-aspartate) in rats [40]. The use of high performance liquid chromatography (HPLC) and electrochemical detection allowed highlighting that the treatment with NMDA is associated with low concentrations of monoamines (epinephrine, norepinephrine, dopamine, and serotonin) in rat PC12 cells [29, 40]. In this experimental model (swimming test), agmatine protects against NMDA-induced PC12 cell lesions, augmenting the levels of epinephrine, norepinephrine, and dopamine, but not influencing serotonin values, together with lowering intracellular Ca^{2+} overload. These results indicate that the neuroprotective action of agmatine may be related to NMDA-receptor modulation and/or to controlling the decrease in monoamine content and NMDA-induced intracellular Ca^{2+} overload [40].

Immunohistochemical studies and electrophysiological investigations performed on the brain have validated the neuroprotective actions of both imidazoline receptor antagonists idazoxan and efaroxan in rats with cerebral damages caused by the use of quinolinic acid [41], and also in mice with experimentally induced autoimmune encephalomyelitis, confirming the improvement of brain structural alterations and blood brain barrier lesions curtail [42].

A new (+)-2-(ethyl-2,3-dihydrobenzofuranyl)-2-imidazoline derivative—dexefaroxan—the (+) enantiomer of efaroxan has been characterized. It has a potent and selective α2 antagonist activity, with facultative effects on cognitive functions in the passive avoidance test in rats with memory-deficiency induced by scopolamine, diazepam, or by the 2-adrenergic agonist UK 14,304.

Dexefaroxan improves the cognitive performances in the passive avoidance test, facilitates spatial memory in the Morris swimming test in rats, and increases the object recognition ability in the specific behavioral test in mice [43, 44]. It has also proved to ameliorate the animal’s memory deficits in these tests, particularly by attenuation of spontaneous memory loss, and to improve their spatial recognition ability, rather than through the acquisition skills or various other non-cognitive effects.

After subcutaneous administration of dexefaroxan, its pharmacodynamic effects persist for about 21–25 days, indicating that tolerance does not occur during prolonged treatment. Moreover, it was emphasized that dexefaroxan exerts protective effects on the spatial memory deficit caused by cortical devascularization in the Morris swimming test in rats [43].

Dexefaroxan has also been shown to exhibit neuroprotective effects on the devascularization-induced neurodegeneration, to ameliorate the structural changes in the hippocampus, and to remove the cognitive deficits induced by cerebral ischemia in rats [45, 46]. Its neuroprotective effects were present also in the excitotoxic lesions produced at the region of the basal magnocellular nucleus, increasing the olfactory discriminative capacity of rats, suggesting thus the possibility of its use in the treatment of memory disturbances in Alzheimer’s disease [43].

Studies performed on genetically modified animals revealed that dexefaroxan improves cognitive performance in knockout mice with Alzheimer’s disease [47].

Literature data regarding the neuroprotective action of imidazoline agonist and antagonist agents in human studies are only few, and the mechanisms involved in these effects are not completely deciphered.

Some investigations suggested that agmatine manifests protective activity against brain cell injury in different in vivo models of Parkinson’s disease, as well as
in vitro studies, performed on human-derived dopaminergic neuroblastoma cell lines. It was postulated that the neuromodulatory properties of agmatine are related to the protective effects on the dopaminergic neurons, to NMDA receptor blocking, and to the decrease in oxidative stress, due to the inhibition of nitric oxide synthase (NOS) activity [48, 23].

Other clinical trials highlighted that the treatment with agmatine was associated with cytoprotective actions, in patients with spinal cord injury, proved by lessening the glial weal construction, decreasing the collagen scar zone, relieving the neuronal alterations, and recovering remyelination [49]. Moreover, the beneficial effects of agmatine have been demonstrated in various CNS lesions such as: cerebrovascular accident, brain trauma, neuropathic pain, lumbar degenerative disc disease, and different other types of neuropathy [50–52].

The administration of dexmedetomidine has also shown neuroprotective effects in humans with acute cerebral lesions [53].

In patients with dementia due to brain frontal lesions, idazoxan alleviates attentional and executive dysfunctions evoked by classical cognitive function tests [54].

4. The interrelation between the adrenergic and the imidazoline systems in the mediation of cognitive functions

Clonidine, both a non-specific α2 adrenergic and imidazoline receptor agonist, decreases the cognitive function alterations induced by phencyclidine and MK801, facilitating spatial memory in the radial arm maze test in rats [35], but does not influence the behavioral and cognitive deficits in the experimental NMDA-induced excitotoxic dorsal hippocampal lesions [35]. Such findings indicate that clonidine improves memory alterations caused by glutamate hypofunction, but not by hippocampal injury, implying that multiple and distinct mechanisms are involved in the development of memory disorders.

The administration of the α2 adrenergic receptor agonist clonidine or guanfacine prevents some of the behavioral effects of NMDA antagonists, proving that the monoaminergic system mediates a number of aspects of the cognitive deficit. Clonidine and guanfacine improve the lack of visual attention and spatial memory induced by phencyclidine in rats [34, 36]. It was demonstrated that low doses of clonidine recover the animal’s ability to accurately choose the object and prevent the performance deficit induced by phencyclidine. At high doses, clonidine decreases the response time and induces a lack of the choice accuracy. These results indicate that clonidine treatment can alleviate phencyclidine-induced deficit of attention and of working memory, probably by preventing some of the neurochemical and anatomical effects of this psychotomimetic drug [34].

On the other hand, the use of only the selective α2 adrenergic receptor antagonists does not impair the animal’s spatial memory, but dramatically aggravates the phencyclidine-induced memory deficit [36]. These data demonstrate that α2 adrenergic receptors mediate the inhibition of spatial memory disturbances, suggesting their important role in cognitive deficits associated to NMDA receptor hypofunction [34, 36].

The role of moxonidine (an α2 adrenergic imidazoline I1 receptor agonist) on cognitive function in rats with Huntington’s disease experimentally induced with 3-nitropropionic acid (3-NPA) was investigated in the Morris swimming test and in the elevated plus maze test. The administration of 3-NPA induces degenerative brain damage, progressive motor dysfunction, loss of grip force, emotional disturbances, weight loss, anxiety, and impairment of learning activity and memory. An increase in cerebral acetylcholinesterase level, enhancement of oxidative stress, and
impairment of the activity of mitochondrial enzyme complexes I, II, and IV were also noted [28]. The treatment with moxonidine resulted in the alleviation of disturbances caused on animal weight, motor activity, gripping ability, anxiety, impairment of learning ability and memory, and biochemical disturbances, thus indicating that substances modifying the activity of I1 receptors may be potential pharmacological agents for the treatment of degenerative brain disorders [55].

The effects of clonidine have also been evaluated in mice with subacute brain ischemia obtained after permanent ligation of common carotid arteries. The subsequent brain damages consisted of expansion of cerebral infarction areas, assessed by computed tomography scans. This experimentally induced chronic cerebral hypoperfusion was associated with a significant impairment of animal’s learning ability and memory in the Morris swimming test [25, 28]. Subacute treatment with clonidine for 7 days increases the expression of neuronal nuclei, glutamic acid-decarboxylase-67, and gamma-aminobutyric acid (GABA) B receptor (GABAB1) in hippocampal subregion cornu amonis (CA3) but does not influence the level of these elements in the hippocampal area CA3, nor in the dentate gyrus. These data support the idea that clonidine exerts neuroprotective effects on chronic cerebral ischemic lesions, by regulating GABAB1 receptors and the activity of glutamic acid-decarboxylase-67 [25].

Additionally, the decrease in superoxide dismutase (SOD), catalase (CAT), and glutathione levels as well as the increase of both malondialdehyde (MDA) level and cerebral acetylcholinesterase activity were noted in animals with brain ischemic lesions [28].

Both moxonidine and clonidine have shown a decrease in histopathological changes, oxidative stress, central cholinesterase activity, as well as a reduction in memory disturbances and learning deficits in mice with vascular dementia induced by subacute ischemia after permanent bilateral cerebral artery ligation [28, 40].

In vitro cell culture studies from the rat frontal cortex with glutamate-induced neurotoxicity revealed the partial neuroprotective effects of moxonidine, with a significant decrease in the number of dead cells [26]. Moxonidine has shown beneficial effects on cerebral spasm in an experimental rabbit model of subarachnoid hemorrhage [40, 56].

5. The interrelations between the imidazoline system and the oxidative stress in the mediation of cognitive functions

Different pathological conditions of the body, as well as the physiological process of aging, can cause cognitive impairment and free oxygen radicals production, being responsible for abnormal functioning and cell death. Subsequently, a new idea has emerged claiming that nitric oxide (NO), along with the free radicals, plays a key role in the aging process, due to neurotoxic effects on the brain exerted by its excessive levels [57]. Nitric oxide is generated from L-arginine under the action of nitric oxide synthase (NOS). The three isoforms of NOS have different roles in the body: neuronal NOS (nNOS) is responsible for synaptic plasticity, learning and memory processes; endothelial NOS (eNOS) provides stabilization and regulation of vascular micro-environment and contributes to neuroplasticity [58]; and inducible NOS (iNOS) is involved in various pathophysiological conditions [57].

Numerous experimental researches reveal that NOS activity is significantly elevated in the brain of elderly rats, being associated with existing cognitive alterations [57, 59]. Mediated by the competitive inhibition of nNOS and iNOS, and correlated with the stimulation of NOS, agmatine contributes to the improvement of cognitive functions [19, 60, 61], while exhibiting neuroprotective effects [38, 39].
Literature data have shown that agmatine eliminates neuroinflammation and lipopolysaccharide-induced memory impairment (which is known to stimulate iNOS activity and, implicitly, the NO production) in laboratory animals. It prevents cognitive alterations, probably as a result of inhibition of iNOS activity [39]. Other researchers have disproved these results by showing that agmatine can cause cognitive impairment due to the inhibition of NMDA receptors and of NO, important elements in the modulation of learning and memory processes [62, 63].

On the other hand, it is known that the central cholinergic system plays a crucial role in the mediation of cognitive functions. Cognitive deficits have been induced in laboratory animals by using an anticholinergic agent, scopolamine, its administration producing a significant reduction in NOS activity, and an increase in arginase activity, of L-ornithine and putrescine levels in the hippocampus [51]. It has been observed that agmatine eliminates the scopolamine-induced alterations of memory and learning capacity [64, 65].

Although glutamatergic activity is required for cognitive processes, it is assumed that the increase of glutamate levels or of NMDA activity would also be responsible for the scopolamine-induced cognitive disturbances [66].

Knowing that agmatine blocks NMDA receptors and also interferes with the pathways of NO, NOS, and L-arginine, it was assumed that the removal of scopolamine-induced cognitive deficits can be attributed to its modulating effect on NO/NOS activity, on L-arginine, and also to the antagonization of NMDA receptors, with subsequent suppression of excessive glutamatergic activity [61, 65].

Abnormal release and disturbances of neuromodulatory activities due to variation in cerebral agmatine levels may be correlated to different CNS diseases (such as schizophrenia). Interactions of agmatine with other central neurotransmitter systems (such as glutamatergic and nitrergic) appear to be particularly important in the pathophysiological mechanisms of CNS disorders associated with brain damage and cognitive functions deficit.

6. The opioid system

Neurodegeneration can be caused by chronic disease progression or by acute injury (cerebral ischemia—stroke or trauma) [67]. Ischemic stroke represents a vascular ailment with neurological consequences produced by the obstruction of the arteries in a part of the brain, therefore by blood supply privation [68]. Stroke can determine long-term neurological and psychiatric impairments, its therapy being focused on confining secondary injury processes [67].

In a recent review article, Chamorro presented that ischemic stroke is “the first cause of permanent disability in adult people, the second single most frequent cause of death for people older than 60 years, the second most common cause of dementia, representing approximately 3% to 7% of the total health-care expenditure in high income countries” [69].

A superpose of pathologies in different neurological disorders was proposed since NDDs and ischemic stroke are frequently concomitant, hence the neuroprotective therapy could be similar [70].

Opioids are substances with morphine-like action binding to specific opioid receptors (ORs). In the early 1990s, three important opioid receptor families [μ (MOR), κ (KOR), and δ (DOR)] were identified, and in 1994 another opioid receptor was discovered [nociceptin, orphanin FQ receptor (NOP), or the opioid receptor-like orphan receptor (ORL)]. ORs are found in the nervous system, lungs, heart, liver, and gastrointestinal and reproductive tracts. They have been intensively studied and it was emphasized that they not only are related to...
antinociceptive action, but also have a role in cell proliferation, ionic homeostasis, emotional response, immune function, epileptic seizures, feeding, obesity, respiratory and cardiovascular control, hibernation, and neuroprotection [71, 72].

In the last decades, researches have pointed out that the opioid system can be promising to get neuroprotective treatment in the event of stroke, through OR agonists at lower doses, to avoid tolerance and/or physical dependency. DOR agonists followed by KOR agonists have revealed the most intense neuroprotective efficacy [64]. Major OR agonists tested for neuroprotection are listed in Table 2 [71, 72].

DOR activation is beneficial against ischemic, hypoxic, and excitotoxic injuries [73]; recent studies promote DOR and especially DADLE (an analog of endogenous delta-opioid enkephalin) as promising targets for treating NDDs like stroke and PD [74–76].

DADLE alleviates apoptotic pathways, supports not only cell survival of peripheral organs (such as lung, heart, kidney, and liver) but also neuronal survival, and protects neurons and glial cells from ischemia-induced cell death [76–78].

In a cellular model of PD, DADLE administration augmented cell survivability with concurrent downregulation of the unfolded protein response stress sensors and protein aggregates [79].

Findings from a rat middle cerebral artery occlusion (MCAO) stroke model proposed that neuroprotection of DADLE treatment was based on the activation of PI3K-Akt pathway by reducing nerve cell apoptosis [80].

Non-selective opioid receptor agonists were also tested: LYS739 (fluorinated enkephalin-fentanyl derivative) and the most promising compound—biphalin—which proved to be effective both in vitro and in vivo stroke models [72].

The latter is a dimeric enkephalin analog (Tyr-D-Ala-Gly-Phe-NH₂)₂ with high potency and affinity for MOR and DOR and low affinity for KOR. Biphalin crosses blood-brain barrier reaching spinal and supraspinal sites expressing OR and produces less physical dependence and tolerance compared to morphine [72, 81–84].

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<th>Opioid receptor</th>
<th>Agonist</th>
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<tr>
<td>δ</td>
<td>DADLE [D-Ala², D-Leu⁵]-enkephalin</td>
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<td>DPDPE (D-Pen²,D-Pen⁵)-enkephalin</td>
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<td>Salvinorin A</td>
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<td></td>
<td>Dynorphin</td>
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<tr>
<td>μ</td>
<td>DAMGO [D-Ala²,N-MePhe⁴,Gly-ol]-enkephalin</td>
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<td></td>
<td>Endorphin 1 and 2 (EM 1/2),</td>
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<td>Morphone</td>
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Table 2.
Opioid receptors and their agonists tested for neuroprotective action.
Different studies using a mouse MCAO stroke model reported that biphalin reduced brain edema and infarction, ROS production, and NMDA-induced excitotoxicity. It also increased locomotor activities and neurological score after stroke resembled to saline-treated animals [72, 81, 85, 86]. Biphalin notably diminished penumbral expression of Na⁺, K⁺, 2Cl⁻ co-transporter (NKCC), and the translocation of the conventional isoforms of protein kinase C [81].

It has been hypothesized that biphalin’s neuroprotective effects are more intense compared to subtype-selective agonists due to concomitant activation of the three types of OR [72, 85].

7. Conclusion

Various pharmacological substances influencing the pathways of the main neurotransmitters have confirmed valuable effects in neurogenesis and neuroprotection, being validated in different in vitro researches and in vivo experimental animal models of limited or extensive ischemic brain lesions.

Deciphering the roles of the neurotransmitters in central nervous system activity other than the signaling function will represent a starting point to deepen the knowledge about the complex mechanisms of the brain functions and to obtain new agents useful for protection of ischemic neurons and for preventing their irreversible damage.
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Chapter 3

The Role and Development of the Antagonist of Adenosine A2A in Parkinson’s Disease

Widya Dwi Aryati, Nabilah Nurtika Salamah, Rezi Riadhi Syahdi and Arry Yanuar

Abstract

Adenosine is a neuromodulator that regulates the body’s response to dopamine and another neurotransmitter in the brain that is responsible for motoric, emotion, learning, and memory function. Adenosine is a G-protein-coupled receptor and has four subtypes, which are A1, A2A, A2B, and A3. Adenosine A2A is located in the striatum of the brain. Antagonist interferes with GABA releasing, modulates acetylcholine and releases dopamine, and also facilitates dopamine receptor’s signaling. Therefore, it can reduce motoric symptoms in Parkinson’s disease. Adenosine A2A antagonist is also believed to have neuroprotective effects. Several compounds have been reported and have undergone clinical test as selective adenosine A2A antagonists, including istradefylline, preladenant, tozadenant, vipadenant, ST-1535, and SYN-115. Nonselective adenosine A2A antagonists from natural compounds are caffeine and theophylline.

Keywords: adenosine A2A, selective adenosine A2A antagonists, Parkinson’s disease, neuroprotective, natural compounds

1. Introduction

Adenosine is a neuromodulator that coordinates responses to dopamine and other neurotransmitters in areas of the brain responsible for motor function, mood, learning, and memory [1]. Adenosine consists of four receptor subtypes: A1, A2A, A2B, and A3 belonging to the superfamily of G-protein-coupled receptor. Adenosine A1 and A3 receptors are coupled to inhibitory G proteins, while A2A and A2B receptors are coupled to stimulatory G proteins [2].

Adenosine A1 receptor can be found in adipose tissue, heart muscle, and inflammatory cells. The receptor mostly expressed by the central nervous system such as neocortex, cerebellum, hippocampus, and dorsal horn of the spinal cord [3]. The pre- and postsynaptic nerve terminals, mast cells, airway smooth muscle, and circulating leukocytes are the places where adenosine A2 receptor can be found. As the more widely dispersed receptor, adenosine A2 is divided into two receptors on the basis of high- and low-affinity for adenosine, A2A and A2B [4]. Striatal neurons are where the adenosine A2A are highly enriched; however its lower levels can also be found in glial cells and neurons outside the striatum [5]. The adenosine A2B receptors are highly expressed in the gastrointestinal tract, bladder, lung, and on mast
cells. The most widely dispersed receptor is the A3 receptor which can be found in the kidney, testis, lung, mast cells, eosinophils, neutrophils, heart, and the brain cortex [4].

Adenosine A2A receptors are found to be concentrates in GABAergic medium-sized spiny neurons in the dopamine-rich regions of the brain. The protein translated in the adenosine A2A is carried by many other tissues such as blood vessels, endothelial, lymphoid cells, smooth muscle cells, and several neurons in sympathetic and parasympathetic systems [6]. Therefore, the dispersion of adenosine A2A is not limited to the medium spiny neurons in the basal ganglia. It stimulates the modulation of cAMP production and increases the level of adenylyl cyclase. This receptor is essential in giving the medium of vasodilation of coronary arteries which then supports the combination of new blood vessels and giving protection for tissues from indirect inflammatory damage [7]. The role of the A2A in the brain includes influencing the activity within the indirect pathway of the basal ganglia. The A2A has complicated actions because it colocalizes and is physically combined with other unrelated G-protein-coupled receptors. Therefore, it can form heterodimers such as dopamine D2/A2A, and D3/A2A, cannabinoid CB1/A2A, and glutamate mGluR5/A2A, as well as CB1/A2A/D2 heterotrimers [7].

The pathways which give signals used by the A2A receptor depend on the location of the cell and tissue, the specific G protein which couples it, and the signaling in the cell. The brain also carries the A2A receptor in which it plays an important role in regulating the glutamate and releasing the dopamine [8]. In the striatopallidal neurons, dopamine D2 receptors are colocalized with adenosine A2A receptors. Adenosine A2A receptor activity that mediates stimulation and D2 receptors that mediate inhibition in the striatopallidal pathway are balanced [9]. The adenosine A2A
likely affects motor activity by acting at different levels of the basal ganglia network. The basal ganglia comprise the striatum (putamen), the globus pallidus externa (GPe), the globus pallidus interna (GPi), substantia nigra pars compacta (SNC), substantia nigra reticulata (SNr), and the subthalamic nucleus (STN). The striatum is represented by medium-sized spiny projection neurons (MSNs), accounting for almost 95% of striatal neurons and using γ-aminobutyric acid (GABA) as neurotransmitter. The GABAergic spiny neurons give rise to the two main striatal efferent circuits: the striatonigral and the striatopallidal pathway. The neurons of the striatonigral (direct) pathway contain the neuropeptide substance P and dynorphin and mainly express D1 receptors; this pathway directly projects from the striatum to the GPi/SNr. The neurons of the striatopallidal (indirect) pathway containing the neuropeptide, enkephalin (ENK), predominantly express D2 receptors; this circuit connects the striatum with the GPi/SNr via synaptic connections in the GPe and STN in Figure 1. Dopamine modulates motor coordination and fine movements by facilitating the action of the direct pathway on stimulatory D1 receptors and by inhibiting indirect pathway function acting on inhibitory D2 receptors [10].

The adenosine A2A receptor has agonists and antagonists of which the roles are potentiating and inhibiting, respectively. The D2 receptor agonist has effects on motor activity, the releasing of neurotransmitter, and the expression of striatal of c-Fos, a factor of transcription which is used as neuronal activity’s indirect marker [11]. The adenosine A2A receptor has a key role in regulating the striatal dopaminergic neurotransmission which produces substances that are valuable to treat neurological disorders that are relevant with dopaminergic dysfunction. The topology of G-protein-coupled receptor is displayed in the structure of the adenosine A2A receptor. These receptors have a central core which consists of seven
transmembrane helices (7TM). Each of the TM is mainly α-helical and consists of 20–27 amino acids. Three intracellular (IL1, IL2, and IL3) and three extracellular (EL1, EL2, and EL3) loops connect each of the TM domain. A short helix TM8 runs parallel to the cytoplasmic surface of the membrane. The adenosine A2A receptor has differences in length and N-terminal extracellular domain function, their domain of C-terminal intracellular, and their loops of intracellular/extracellular. These differences are shown in Figure 2.

2. The role of adenosine A2A in Parkinson’s disease

Parkinson’s disease (PD) is a chronic neurodegenerative disorder in the brain, marked by motoric symptoms [12]. The motoric symptoms in PD are resting tremor, rigidity, bradykinesia, and postural disorder. Besides motoric symptoms, PD also has non-motoric symptoms such as depression, hallucination, sleeping disorder, and decreasing cognitive and sensory functions. The main pathological characteristic of PD is the loss of dopaminergic neurons in substantia nigra pars compacta, a region in the brain that controls all the body movement and forms the dopamine. The development of PD also includes the formation of Lewy body, a deposit of cytoplasmic, eosinophilic neuronal inclusions, composed of the presynaptic protein α-synuclein [13, 14].

The current therapy of PD is targeted at dopamine replacement, thereby decreasing the motor symptoms. It includes precursor of dopamine (levodopa), dopamine agonists [15, 16] monoamine oxidase type B (MAO-B) inhibitors [17], and catechol-O-methyltransferase (COMT) inhibitors [17, 18]. These agents produce undesirable side effects such as on-off effects, hallucinations, and dyskinesia. These effects get more severe as the treatment continued. The efficacy of these agents is also decreasing as the disease progressed [19].

Because of the undesirable side effects of dopamine replacement therapy, the non-dopaminergic therapy is continuously being explored. One of the approaches is selective adenosine A2A antagonist [20, 21]. Adenosine A2A receptors are found mainly in the striatum of rat [22, 23], which has similar distribution with the human brain [24, 25]. In the striatum, adenosine A2A receptors are colocalized with dopamine D2 receptors. These two receptors have opposite effect on motoric function [26]. The activation of adenosine A2A receptors will inhibit the signaling of dopamine D2 receptors, and conversely, the inhibition of signaling of adenosine A2A receptors will increase the activation of dopamine D2 receptors, therefore facilitating dopamine D2-mediated responses [11]. The inhibition of adenosine A2A receptors showed motoric improvement in animal models of PD [27–30]. This also has desirable effect on long-term levodopa treatment such as decreasing the dyskinesia and increasing the therapeutic effect on levodopa [31, 32].

3. Adenosine A2A receptor antagonist as a neuroprotective

For years, adenosine-dopamine interactions have been investigated in order to observe their relevance for treatment of central nervous system (CNS) disorders [33]. It is assumed that adenosine A1 receptors (A1Rs) play an important role in neuroprotection as their activation at the onset of neuronal injury has shown to reduce brain damage in adult animal model. Vice versa, their blockade aggravates the damage. In other hand, adenosine A2 receptors (A2Rs) are shown to be upregulated in harmful brain conditions, and their blockade shows brain neuroprotection in studied animals [34]. The blockade of A2Rs alleviates the long-term
burden of brain disorders in different neurodegenerative conditions, namely, ischemia, epilepsy, and Parkinson’s and Alzheimer’s disease, through its control on neuronal cell death [35].

A2A Rs have been shown to be viable in serving as alternative non-dopaminergic strategy of Parkinson’s disease treatment because of their limited distribution in the striatum and the intense interaction between adenosine and dopamine receptors in the brain. A2A Rs antagonists were shown to improve motor function in different animal models (primates and rodents), alone or co-administered with dopaminomimetic drugs, levodopa, or dopamine agonists [35]. Based on rigorous preclinical animal studies, istradefylline (KW6002) has shown its promising ability to increase motor activity in PD of the advanced stage in clinical phase IIB trial [36]. It became the first therapeutic agent developed to target A2A Rs, and other similar compounds will be available in near future [37].

The recent meta-analysis (n = 6) suggested that 20 mg of istradefylline improves unified Parkinson’s disease ranking scale (UPDRS) III. Meanwhile at 40 mg per day, istradefylline could alleviate off time and motor symptoms derived from Parkinson’s disease [38]. Phase 3 study (613 randomized patients), done by Isaacson et al. concluded that greater reduction from baseline in total hours off time/day were shown at all-time points for istradefylline 20 and 40 mg/day, compared to placebo. However, future development is needed as the study has not yet reached statistical significance [39].

In the case of Parkinson’s disease, microglia has been suggested to be the most likely cell type to be targeted by A2A Rs antagonists [40]. In vitro and in vivo studies showed that local neuroinflammation make glial cells (especially microglial cells) particularly sensitive to A2A R modulation [41]. Previous research done by Gao and Phillis is the first study to demonstrate nonselective A2A R antagonist action in reducing cerebral ischemic injury in the gerbil, following global forebrain ischemia [42]. After that, many studies have reported the neuroprotective of A2A R antagonists in different models of ischemia [43].

Alzheimer’s disease (AD) is a chronic neurodegenerative disorder that is indicated by the progressive loss of memory and other cognitive functions, leading to dementia [44, 45]. Adenosine can control and integrate cognition and memory [46]. Both A1 Rs and A2A Rs, mainly located in synapses, control the release of neurotransmitters which are involved in memory or other cognitive processes [34, 47]. Methylxanthine was discovered to act as nonselective adenosine receptors antagonist. Caffeine, the most famous methylxanthine found in common beverages, is the most widely consumed psychoactive drug. Maia and de Mendonca presented the first epidemiological data showing that the incidence of AD is inversely proportioned with coffee consumption [48]. Several other studies also show this inverse relationship [49–51]. Animal models also shown that caffeine intake may be beneficial for AD. In a study, a 6-month period of 0.3 g/L caffeine intake alleviated the cognitive deficits found in AD transgenic mice (APPsw). Furthermore, these mice culture neurons showed the reduced production of Aβ1-40 and Aβ1-42 peptides [52]. A2A Rs antagonists and/or caffeine prophylactic and long-term neuroprotective process are suggested to be based on inhibition of reactive oxygen species activity, tau pathology, and Aβ production by neuronal cells [53].

A2A Rs antagonist may also serve as antidepressants, as observed in animal model of antidepressants screening test done by El-Yacoubi et al. [54, 55]. In both tests, A2A Rs antagonists prolong escape-directed behavior. Additionally, potential role as antidepressants was also observed in attenuated behavioral despair displayed in both tests [55]. The relation between adenosine and depression in preclinical models was obtained from the genetic manipulation model of A2A R. Genetic depletion of A2A Rs resulted in antidepressant-like phenotype in animal models [55]. The
A2ARs blockade also relieves stress-induced early hippocampal modifications [56]. However, the effect of adenosine neuromodulation system in depression is complex, as it has the ability to modulate several other neurotransmission systems [35].

As addressed in previous paragraphs, A2AR emerges as potential target candidate in various disorders. This is majorly caused by its unique interaction with D2 receptors, a major psychoactive drug target. Important roles of A2AR were also observed in its robust neuroprotective activity, in which it mainly acts in the normalization of glutaminergic synapses, the control of mitochondria-induced apoptosis, and the control of neuroinflammation [35].

4. Current sources of the adenosine A2A antagonist

The treatment of PD currently focuses on symptom management with dopaminergic therapy, such as dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA) (in combination with peripheral decarboxylase inhibitors) and dopamine agonists [57]. Although L-DOPA is beneficial in patients with PD, with time, the span of the effect is shortened, the response becomes less probable, and involuntary muscle movements or, in a severe situation, dystonia can emerge [57]. These problems highlight the urgent medical need for an alternative mode of therapeutic intervention that can relieve the symptoms of the disorder while also allowing a decrease in the occurrence of side effects.

Among the non-dopaminergic therapies investigated for the treatment of PD, the adenosine A2A receptor antagonists show very convincingly for two main reasons: their selective and restricted localization in the basal ganglia circuitry and their interaction with dopaminergic receptors. In another word, inhibition of the interaction of adenosine with the A2A receptor may provide a potential treatment for PD.

![Figure 3. Adenosine A2A inhibitors.](image-url)
Many highly selective $A_{2A}$ antagonists, both xanthine and non-xanthine derivatives, have been created, and some of them are being investigated as treatment for subjects with PD in various stage of clinical trials (Figure 3) [7, 19, 58–61]. Caffeine as a xanthine derivative is developed as a lead compound for the design of antagonist of adenosine $A_{2A}$ receptor [62]. Experimental model using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism is known to be an evidence that caffeine have a protective effect in Parkinson's disease [36, 63]. Some $A_{2A}$ antagonists have progressed to clinical trials by various pharmaceutical companies including istradefylline [59], PBS-509, ST1535 and its metabolite ST4206, tozadenant, V81444, preladenant, and vipadenant [64]. Several studies of novel series of 2-aminoimidazo[4,5-b]pyridine-derivatives [65], arylidenopyrimidine [66], and bicyclic aminoquinazoline derivatives [67] as adenosine $A_{2A}$ antagonists are reported.

Various computational methods were used to study neuroprotective effect from adenosine $A_{2A}$ antagonists such as pharmacophore model [68], QSAR, molecular docking [69–71], and molecular dynamics [72, 73]. Orally bioavailable adenosine $A_{2A}$ receptor antagonists have been studied for its QSAR and pharmacokinetics properties [74].

The study of structure-kinetics relationship (SKR) is done as a complement to a SAR analysis at the adenosine $A_{2A}$ receptor. The series of 24 triazolotriazine derivatives showing a similar binding kinetics to the putative antagonist ZM241385 (4-(2-((7-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-yl)amino)ethyl)phenol) revealed minor affinity changes, although they varied substantially in their dissociation rates from the receptor [75].

**5. Future direction of drug discovery of Parkinson's disease**

Various studies have been conducted in the discovery of Parkinson's drugs against the target $A_{2A}$ receptors. The discovery of drugs assisted by computers has accelerated in obtaining lead compounds. Apparently, this method takes a lot of consideration before entering the preclinical and clinical phases. It is because this computational method is more able to describe the answer in preparing the next design. This method can also make various predictions of activities that are difficult to do in the absence of chemical compounds before they are synthesized. In silico prediction of various pharmacokinetic parameters and toxicity can also be done faster. All of these things can provide a better picture of getting a cure for Parkinson's disease.

**6. Conclusions**

$A_{2A}$ receptors emerge as potential target candidate in various disorders, caused by its unique interaction with D2 receptors, a major psychoactive drug target. Various studies have been conducted in the discovery of Parkinson's drugs against the target $A_{2A}$ receptors. In silico study brings a new approach of study with $A_{2A}$ receptors.

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Conflict of interest

The authors declare that they have no conflict of interest or involvement with any organization of affiliation.

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Chapter 4
Adrenergic Receptors as Pharmacological Targets for Neuroinflammation and Neurodegeneration in Parkinson’s Disease
Monika Sharma and Patrick M. Flood

Abstract
Inflammation is a key component of the dopaminergic neurodegeneration seen in progressive Parkinson’s disease (PD). The presence of activated glial cells, the participation of innate immune system, increased inflammatory molecules such as cytokines and chemokines, and increased oxidative stress and reactive oxygen species are the main neuroinflammatory characteristics present in progressive PD. Therapeutic targets which suppress pro-inflammatory responses by glial cells (mainly microglia) have been shown to be effective treatments for slowing or eliminating the progressive degeneration of neurons within the substantia nigra. In this chapter, we will detail a specific anti-inflammatory therapy using agonists to \( \beta_2 \)-adrenergic receptors that have been shown to be effective treatments for models of dopaminergic neurodegeneration and that have had efficacy in patients with progressive PD. We will also detail the possible molecular mechanisms of action of this therapeutic in stopping or reversing inflammation within the CNS.

Keywords: \( \beta_2 \)-adrenergic receptor, Parkinson’s disease, microglia, neuroinflammation

1. Introduction
There are a number of neurological disorders that fall under the umbrella of neurodegeneration, with the major ones including Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), spinal cord injury (SCI), and others. Currently, there are no generally effective treatments available to slow down or reverse the debilitating effects of these diseases, and the long-term effects of these diseases are the progressive degeneration and death of neurons. A majority of the neurodegenerative diseases are linked with inflammation in CNS [1], and the presence of activated glial cells, infiltration and activation of adaptive and innate immune cells, increased presence of inflammatory molecules such as cytokines and chemokines, and increased oxidative stress and reactive oxygen species (ROS) are the main neuroinflammatory characteristics present in lesions associated with...
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1. Introduction

There are a number of neurological disorders that fall under the umbrella of neurodegeneration, with the major ones including Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), spinal cord injury (SCI), and others. Currently, there are no generally effective treatments available to slow down or reverse the debilitating effects of these diseases, and the long-term effects of these diseases are the progressive degeneration and death of neurons. A majority of the neurodegenerative diseases are linked with inflammation in CNS [1], and the presence of activated glial cells, infiltration and activation of adaptive and innate immune cells, increased presence of inflammatory molecules such as cytokines and chemokines, and increased oxidative stress and reactive oxygen species (ROS) are the main neuroinflammatory characteristics present in lesions associated with
2. Parkinson’s disease: a chronic neurodegenerative and neuroinflammatory disease

Parkinson’s disease (PD) is a progressive neurodegenerative disorder which leads to impaired motor skills. The major pathological feature of PD is the degeneration of dopaminergic (DA) neurons which project from substantia nigra (SN) to the striatum in the midbrain (nigro-striatal pathway) [2]. Another neuropathological feature of PD is the cytoplasmic inclusion of misfolded α-synuclein protein in degenerating dopaminergic neurons called Lewy bodies [3]. The primary motor symptoms of PD, such as tremor, rigidity, and bradykinesia, are caused by inadequate formation and neurotransmission of dopamine within the nigro-striatal pathway [4, 5]. Dementia is reported in 28% of PD cases with the prevalence rising to 65% in those aged 85 years and above. Patients with PD also show non-motor-related symptoms such as olfactory deficits, depression, cognitive deficits, sleep disorders, and autonomic dysfunction [6]. The majority of PD cases are idiopathic Parkinson’s, and the disease mechanism that ultimately causes idiopathic PD is largely unknown. In the remainder of the cases of PD, about 10–15% of patients do have a family history and those patients are referred to as having the familial form of PD. For these patients, their PD appears to be caused by a mutation in one of a few selected genes (such as SNCA, Parkin, LRRK2, DJ-1, etc.) [7, 8]. Although the etiology of the idiopathic form of the disease remains elusive, there are some risk factors associated with the development of the disease. These risk factors include exposure to environmental toxins, severe cranial trauma, systemic or localized infections, and inherited genetic risk factors. These genetic and nongenetic risk factors have the potential to initiate neurodegeneration and subsequent chronic inflammation in the brain which eventually contributes to the pathophysiology of PD [9]. In addition, several cellular and molecular pathways such as oxidative stress [10], proteosomal dysfunction [11], excitotoxicity [12], and mitochondrial dysfunction [13] have also been identified which contributes to neuronal death.

The presence of activated glial cells, increased inflammatory molecules such as cytokines/chemokines, and increased oxidative stress and ROS are the main neuroinflammatory characteristics present in PD [14]. PD is now not only characterized as loss of DA-neurons and motor impairment, but also recognized to have an inflammatory component which plays a crucial role in the progression of the disease. Several inflammatory mediators such as TNF-α, IL-1β, ROS, and nitric oxide (NO), released from nonneuronal cells exacerbate the disease pathology [3, 15]. It has been suggested that α-synuclein released from dying neurons also activate the microglia via TLR2 activation [16]. Furthermore, the elevated levels of inflammatory cytokines such as TNF-α, IL-1β, and IL-6 have been reported in serum, cerebrospinal fluid (CSF), and striatum of PD patients [17]. The influx of peripheral macrophages has been reported in brains of patients with PD, but the role of these cells in disease pathology remains to be tested [18]. Additionally, activation and increased number of glial cells and infiltrating peripheral lymphocytes such as cytotoxic CD4+ and CD8+ cells in SN also support the role of adaptive immunity in
the etiology of the disease [8]. Overall, these studies and others suggest the contribution of the immune system in the pathophysiology of PD.

3. Microglial activation and neuroinflammation in PD

Microglia originate from erythromyeloid progenitors in the yolk sac which migrate and differentiate during development to form the central nervous system (CNS). Fully differentiated microglial cells are also considered to be the resident macrophages of the CNS [19], although some phenotypic and functional differences between microglia and macrophages have been found [20]. Growing evidence suggests that the activation of microglia in CNS plays an important role in the pathogenesis of PD. It is not well understood how microglia activation is either beneficial or detrimental to the neuron or how microglial activity is regulated. It has been found that microglial activation is required for neuronal survival by the removal of toxic substances through innate immunity [21]. On the other hand, it has been found that over-activated microglial cells are detrimental and neurotoxic [22]. Research studies of post-mortem brain tissue from patients with PD and related parkinsonian syndromes suggest the presence of activated microglia around degenerating DA-neurons in the SN [23] and these activated microglia are not only limited to the SN but also present in extended brain areas such as hippocampus, putamen, trans-entorhinal cortex, cingulate cortex, and temporal cortex [24]. Imaging of activated microglia in the striatum could be used as a biomarker for detecting neuroinflammation in neurodegenerative parkinsonian disorders [25]. The resting microglia switches to an activated microglia phenotype in response to pathogen invasion or release of toxic or inflammatory mediators and thereby promotes an inflammatory response [1]. Once activated, microglial cells produce a wide range of inflammatory mediators which serve to initiate an innate immune response or glial cell-propagated inflammation termed as neuroinflammation [26]. Also, the degenerating DA-neurons release many toxic factors that activate microglia and these degenerating neurons are vulnerable to inflammatory insult. Degenerating neurons will co-localize or attract an even larger population of microglia in the SN [27]. Collectively, these activated microglia and damaged neurons form a repetitive and vicious cycle that leads to chronic inflammation and continued extensive DA neurodegeneration over time, leading to the progression of PD [27]. These findings confirm neuroinflammation as a pivotal process in the progression of neurodegenerative disorders and the central role of microglia in this process [22]. Targeting neuroinflammatory pathways within microglia could be a significant step in the development of new therapeutics for neurodegenerative diseases, including PD.

4. Therapies targeting neurodegeneration/neuroinflammation in PD

Treatment for PD normally involves medications such as Levodopa to enhance the dopamine levels and deal with movement symptoms [28]. While none of our current treatments are able to stop the disease, medication and surgery can be helpful for managing the symptoms [29]. These treatments work well in patients initially, but they are also associated with unwanted side-effects and reduced efficacy over time [30]. On the other hand, many studies suggest that inflammatory mediators such as TNF, PGE₂, NO, free radicals, and other immune mediators play role in the pathogenesis of PD and degeneration of dopamine-producing neurons and that targeting these mediators can be an effective treatment for PD. This opens up the potential of using anti-inflammatory drugs as an effective and long-term
treatment in PD. These anti-inflammatory drugs can act by arresting the disease onset (primary prevention) or by interrupting or even reversing the disease progression (secondary prevention). Epidemiological and observational studies suggest that the use of anti-inflammatory drugs lower the risk of developing PD [31]. Observations which demonstrated that inflammation in SN plays a role in PD have led many investigators to initially consider the potential use of both steroidal and nonsteroidal anti-inflammatory drugs for the treatment of PD. Steroidal anti-inflammatory drugs (SAIDs), such as dexamethasone, have shown neuroprotective effects in LPS-induced neurotoxicity in the SN in LPS models of PD [32]. Nonsteroidal anti-inflammatory drugs (NSAIDs) have also been used as analgesics and antipyretics to suppress the adverse effects of inflammation [33]. The neuroprotective effects of Ibuprofen have been studied in PD pathogenesis and these studies demonstrate the protective effect on dopaminergic neurons against glutamate toxicity in vitro [34, 35]. Previously, we have established several therapies targeting neuroinflammation and neurodegeneration in an animal model of PD and these therapies include D-morphinan-related compounds [36], anti-inflammatory cytokines such as TGF-β (transforming growth factor-beta) [37] and IL-10 [38, 39], IKK (inhibitor of kappa B (IκB) kinase) inhibitors [40], NADPH (nicotinamide adenine dinucleotide phosphate) oxidase inhibitors [41], and β2-AR (beta 2-adrenergic receptor) agonists [42, 43].

We have conducted a number of experiments using different classes of anti-inflammatory compounds to determine their efficacy in preventing dopaminergic neurotoxicity by activated microglial cells both in vitro and in vivo. First, it was found that morphinan compounds and their stereoisomers (L-morphine and its D stereo enantiomers) can inhibit microglial activation and LPS- or MPP⁺-induced neurotoxicity in rat primary mesencephalic cultures. We and others observed that several dextrorotatory isomers of morphine compounds, including D-morphine, dextromethorphan, and sinomenine, showed neuroprotective effects against LPS and MPP+ (1-methyl-4-phenylpyridinium) which were mediated through the inhibition of microglial PHOX activity [36, 44, 45]. Furthermore, these studies also suggest that these morphinan compounds bind to the catalytic subunit of PHOX, inhibit its activity, and reduce the production of superoxide and other pro-inflammatory cytokines [44]. In another set of studies using a different anti-inflammatory approach, a specific inhibitor of IKK-β (IκB kinase-beta) protects dopaminergic neurons against LPS-induced neurotoxicity both in vitro and in vivo through inhibition of NF-κB activation, resulting in the decreased production of ROS and inflammatory cytokines [40]. We have also developed therapies targeting neuroinflammation in PD models by using anti-inflammatory cytokines such as IL-10 and TGF-β1, and found that treatment with IL-10 on rat mesencephalic neuron-glia culture protects against LPS-induced neurotoxicity via suppression of pro-inflammatory mediators and superoxide production [38]. Similarly, the neuroprotective effect of TGFβ1 is primarily due to its ability to inhibit ERK phosphorylation, the serine phosphorylation on p47phox, and the production of ROS from microglia during activation by LPS [37].

5. Adrenergic receptors

One of the most potent and successful therapeutic treatments for inflammation-mediated dopaminergic neurotoxicity is the use of long-acting agonists to the β2-AR. Adrenergic receptors (AR) are seven-transmembrane proteins that serve as adrenoreceptors for catecholamines such as norepinephrine and epinephrine on multiple cell types, and cells within the CNS that express AR include neurons, immune cells, and
astrocytes. Pharmacological classification of the adrenergic receptor was first introduced in 1948 and broadly classified as α and β adrenergic receptors [46] by Ahlquist. The classification was based on the order of potency and specificity of natural and synthetic agonist and blocking agents. The α-AR response corresponds to mainly excitatory response, while β-AR responses were correlated mainly with the inhibitory response. The α-AR response showed the order of potency: norepinephrine > epinephrine > isoproterenol and β-AR-mediated response exhibited order of potency: isoproterenol > epinephrine > norepinephrine [47, 48]. After the discovery of new drugs which have a high affinity to adrenergic receptors, these receptors were sub-classified. α-AR were subdivided into α1 and α2 adrenergic receptors [49]. Further studies subdivided β-AR into β1 and β2 which are normally present on immune cells, cardiac muscles, and airway smooth muscles, respectively [50]. A third β-AR, now called as β3-AR was identified on adipose tissues [51]. Tissue distribution, physiological effects, mechanism of action, and the major agonists/antagonists of ARs are summarized in Table 1. Pharmacological compounds that serve as short, long, and ultra-long-acting agonists for these receptors have now been developed, and they are normally thought to stimulate adrenergic receptors by four different mechanisms: (1) by direct receptor binding, the most common mechanism where drugs activate peripheral adrenergic receptors via direct binding to receptor and mimic the actions of endogenous agonists (NE, epinephrine), (2) by ameliorating NE release, where drugs act on sympathetic nerve terminals and results into NE release, (3) by inhibition of NE reuptake, where these drugs can cause NE to accumulate within synaptic gaps at sympathetic nerve terminals, (4) by blockade of NE inactivation where drugs inhibit the activity of monoamine oxidase (MAO) which inhibits the activity of monoamines such as NE and dopamine [52].


6.1 Structure

The β2-ARs belong to a diverse superfamily of human cell surface seven transmembrane receptors for hormones and neurotransmitters called G-protein-coupled

<table>
<thead>
<tr>
<th>Receptor Type</th>
<th>Tissue Distribution</th>
<th>Mechanism of Action</th>
<th>Agonist Potency</th>
<th>Physiological Effects</th>
<th>Agonist</th>
<th>Antagonist</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1</td>
<td>Vascular Smooth Muscles, Visceral smooth Muscles</td>
<td>Gq-protein coupled activates Phospholipase C, IP3, DAG</td>
<td>Epi ≥ NE &gt;&gt; Iso</td>
<td>Smooth muscle contractions, Glucagonogenesis, Vasoconstriction</td>
<td>Norepinephrine, Phenylephrine, Methoxamine</td>
<td>Doxazosin, Phenotolamine, Prazosin</td>
</tr>
<tr>
<td>α2</td>
<td>Pre-synaptic terminals, pancreas, platelets, Ciliary epithelium, Salivary Glands</td>
<td>Gi-protein coupled inhibits Adenyl cyclase</td>
<td>Epi ≥ NE &gt;&gt; Iso</td>
<td>Inhibits release of Neurotransmitter</td>
<td>Clonidine, Minoxidil</td>
<td>Yohimbine, Idoxan, Tolazoline</td>
</tr>
<tr>
<td>β1</td>
<td>Heart, Kidney, some pre-synaptic terminals</td>
<td>Gs-protein coupled activates Adenyl cyclase +PKA</td>
<td>Iso &gt; Epi ≥ NE</td>
<td>Increase heart rate and Renin secretion</td>
<td>Isoproterenol, Noradrenaline, Dobutamine</td>
<td>Propranolol, Metoprolol, Atenolol</td>
</tr>
<tr>
<td>β2</td>
<td>Visceral smooth muscles, Bronchioles, Liver, Skeletal Muscles</td>
<td>Gs-protein coupled activates Adenyl cyclase +PKA, Ca-channels</td>
<td>Iso &gt; Epi &gt;&gt; NE</td>
<td>Vasodilation, Bronchodilation, Inhibits insulin secretion</td>
<td>Isoproterenol, Salbutamol, Salmeterol, Albuterol, Formoterol, Terbutaline, Levibuterol</td>
<td>Propranolol, IC3-118,551, Nadolol, Butoxamine</td>
</tr>
<tr>
<td>β3</td>
<td>Adipose Tissue</td>
<td>Gs-protein coupled activates Adenyl cyclase +PKA</td>
<td>Iso ≥ NE &gt; Epi</td>
<td>Increase lipolysis</td>
<td>Isoproterenol, Armodraon, Soludraon</td>
<td>SRS9230A</td>
</tr>
</tbody>
</table>

NE: Norepinephrine, Epi: Epinephrine and Iso: Isoproterenol
receptors (GPCRs). GPCRs are divided into six classes on basis of sequence homology: class A (Rhodopsin-like), class B (Secretin receptor family), class C (Metabotropic glutamate), class D (Fungal mating pheromone receptor), class E (Cyclic AMP receptor), and class F (Frizzled/smoothed) [53]. GPCRs are one of the most extensively studied proteins for the development of pharmaceutical drugs and target for approximately 50% of the marketed pharmaceutical drugs [54]. The adrenergic receptor family belongs to the rhodopsin-like subfamily, the largest class of the GPCR. The β2-AR is an intron-less gene is present on the long arm of chromosome 5 (5q31) and encodes for 413 amino acid polypeptide of 46kD [55]. Similar to all GPCRs, β2-AR is composed of seven transmembrane spanning α-helices with an intracellular C-terminus and an extracellular N-terminus. The β2-AR was the first GPCR to be cloned [56] and the first GPCR structure to be solved [57]. The β2-AR has been studied extensively and also serves as a model system for investigating the regulation and signal transduction of GPCRs. The study of the 3D protein structure of this family of GPCRs took a giant leap forward when rhodopsin was first crystallized in 2000 and this crystalline structure has been used as an important template for modeling other GPCRs in this family [58]. The crystalline structure of human β2-AR was not solved until 2007, when a nonactive structure of β2-AR was identified [57]. Post-translational modifications such as glycosylation, palmitoylation, disulfide bond formation, and phosphorylation have now been found to affect receptor functions. Interestingly, β2-AR is glycosylated at amino acid 6, 15, and 187 which is important for the trafficking of the β2-AR from the endoplasmic reticulum to the plasma membrane [59]. Mutation in these sites also result in reduced expression of receptor on the cell membrane, suggesting a role for glycosylation in cell surface expression [60]. Conversely, the cysteine amino acid in the cytoplasmic tail at position 341 is palmitoylated, and is now found to be an important residue for the adequate coupling of the receptor to the Gα-protein [61]. Finally, β2-AR has disulfide bonds which are essential for agonist binding and also for maintaining their tertiary structure [62].

6.2 Localization

Adrenergic receptors are widely distributed on human body organs and regulate physiologic functions such as bronchodilation [63], vasodilation, glycogenolysis in the liver, and relaxation of uterine and bladder muscles [64]. The human β2-AR are widely expressed not only on airway smooth muscles, but also on the wide variety of cells such as epithelial cells, endothelial cells, brain cells, and immune cells including mast cells, macrophages, adaptive immune cells, and eosinophils [65]. The expressions of β1- and β2-AR have also been found on microglial cells, suggesting that microglia, the brain’s resident immune cell, is predominantly regulated by NE since NE is the predominant catecholamine in the CNS. Conversely, peripheral immune cells such as macrophages and T cells, which also express high levels of β1 and β-2 AR, are thought to be regulated primarily by epinephrine [66].

6.3 β2-AR activation and signaling pathways in inflammation

Activation of adrenergic receptors could result into both pro- and anti-inflammatory actions, depending on certain parameters such as the type of cell, duration of ligand exposure to the receptor, and type of the adrenergic receptor [67]. It is the diversity of the β2-AR that leads to the complexity of signaling mechanisms and to this duality of function. Activation of β2-AR by receptor agonists initiate intracellular signaling pathways that function either via G-proteins or through β-arrestins. Like other GPCR, β2-AR can activate either canonical (traditional)
or noncanonical (nontraditional) signal transduction pathway. In the canonical pathway, similar to a typical GPCR the β2-AR signals via a heterotrimeric G-protein complex, and when the receptor is coupled to inactive GDP-bound G-protein, it appears to have high affinity to the agonist or ligand. After ligand binding, the transmembrane domains of the receptor undergo conformational change with the exchange of GDP to GTP. Further, this conformational change reduces the affinity of the ligand to its receptor, increasing the possibility of retraction of ligand from the receptor, thereby preventing the over-activation of G-protein. This provides evidence that β2-AR appear to oscillate between an active and inactive form under normal conditions. After the exchange of GDP to GTP, the Gα-subunit dissociates from Gβγ-subunit which remains associated with plasma membrane and the Gα-subunit activates effector proteins. The downstream signaling of this process normally results in the production of intracellular second messengers which further activates the cAMP-PKA-mediated intracellular signaling pathway. The activated β2-AR binds with the α-subunit of the G-protein together with a guanosine triphosphate (GTP) molecule. Further, the receptor coupled with adenylate cyclase (AC) which catalyzes the conversion of ATP into cAMP (a second messenger for β2-AR) by hydrolysis of GTP into GDP. The cAMP activates and regulates protein kinase A (PKA) which further mediates the transcription of genes and degradation of cAMP by phosphodiesterase (PDE) leading to termination of signaling [68].

Earlier it was determined that β2-AR exhibits their inhibitory signals in immune cells via the canonical (PKA) signaling pathway. It has now been found that GPCR can also signal through a noncanonical pathway in addition to their classical signaling pathway [69]. Activation through the noncanonical signaling pathway is cell type dependent and G-protein independent, but rather the G-protein-coupled receptor kinases (GRKs) and β-arrestins are involved in activation of this noncanonical signaling pathway. Various types of GRKs phosphorylate specifically serine and threonine at C-terminal of the β2-AR which further determines whether receptors undergo desensitization or initiate noncanonical signaling [70]. For example, phosphorylation of receptor by GRK5/6 initiates β-arrestin-mediated noncanonical signaling, while phosphorylation by GRK2 leads to β-arrestin-mediated desensitization of the receptor [71]. During noncanonical signaling, β-arrestin2 couples β2-AR to MAPK signaling pathways which induces activation of transcription factors and allows their nuclear translocation. Activation of β2-AR with high agonist concentration can lead to sustained activation of ERK1/2 via β-arrestin2. This explains why β2-AR activation can either enhance or suppress the proliferation of immune cells and cytokine production particularly at a high concentration of agonists [67, 72]. Studies suggest that during inflammatory conditions immune cells can switch from canonical to the noncanonical pathway [67, 68]. Engagement of β2-AR receptors by agonists can result in immunomodulatory actions. Depending on the type of immune stimuli and timing of β2-AR activation relative to immune activation, β2-AR stimulation can positively or negatively regulate the response of immune activator [67, 73]. The initial data obtained in animal models of dopaminergic neurotoxicity suggests that the primary immunomodulatory mechanism of β2-AR activation that regulates CNS inflammation in microglial cells occurs through the noncanonical β-arrestin2 pathway of activation.

7. β2-agonists

β-agonists are a group of pharmaceutical compounds or sympathomimetic drugs that mimic the effects of endogenous catecholamines such as epinephrine, norepinephrine, and dopamine. These drugs do not comprise a similar structure to
catecholamines but still directly or indirectly activate the β2-adrenergic receptor. The first β-agonist was used around 5000 years ago in Chinese medicine where an ephedrine containing plant, Ma-huang, was used to treat respiratory problems [74]. Further research in the twentieth century has led to increased use of β-agonists for the treatment of respiratory diseases. The first β2-AR selective agonist, Salbutamol was synthesized by Glaxo in 1968 [75]. Later, the same team at Glaxo modified Salbutamol into Salmeterol with long-lasting effects and reduced side effects. Recently, they have synthesized β2-agonists with ultra-long-lasting effects such as Indacaterol [76]. After successful trials, these β2-agonists were approved by the US Food and Drug Administration (FDA) for the treatment of respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD). Since 1968, a number of companies have labored to develop β2-AR agonists, and some have now been commercialized for use in the treatment of COPD. A list of some of these agonists is given below and in Table 1.

7.1 Classification of β2-agonists

A pharmacogenetic study of β2-agonists has summarized the relationship between polymorphisms in the β2-adrenoreceptor (ADRB2) gene and the effects of select β2-agonists [77]. Two hypotheses aim to account for the differences in functioning and in vivo half-lives of these compounds: exosite/exoreceptor or plasmalemma diffusion microkinetics. Briefly, the exosite hypothesis focuses on the ability of the side-chain of these compounds to interact with a distinct site on the receptor such that it allows the active component to “swing back-and-forth” to activate the receptor. The plasmalemma diffusion microkinetic hypothesis suggests that high concentrations of agonists are achieved in close proximity to the receptor and allows for a longer duration of action [78]. Both of these hypotheses require further investigation and need to be studied within the CNS. Depending upon their mechanism and duration of action, all β2-agonists are grouped into three major classes: short-acting, long-acting, and ultra-long-acting β2-agonists.

7.1.1 Short-acting β-agonists (SABA)

These drugs are mostly hydrophilic in nature, access the active site of β-AR directly from the aqueous extracellular area and show the fast onset of action [79]. These SABAs bind to the receptor for short time; therefore, their duration of action is short. Some of the more common SABAs include Salbutamol (Ventolin), Albuterol (AccuNeb), Pirbuterol (Maxair), and Levalbuterol (Xopenex).

7.1.2 Long-acting β-agonists (LABA)

These drugs are a frontline treatment for COPD, and usually prescribed alone or in combination with inhaled corticosteroids. LABAs are lipophilic in nature and taken up by cell membrane as a reservoir, progressively seep out and interact with the active site of the receptor [79]. They diffuse in the plasma membrane, where they interact with the active site of the β2-AR which allows for the close proximity with the receptor and longer duration of action. The onset of action of these drugs is slower as compared to SABAs, but the duration of action is prolonged thereby, called as LABAs. The duration of action is also dependent on the concentration of the agonist. Salmeterol, Salmeterol with an inhaled corticosteroid, Formoterol, and Formoterol with an inhaled corticosteroid are commercially available LABAs and used in medication for asthma and COPD [80].
7.1.3 Ultra-long-acting β-agonists

These agonists are also lipophilic in nature and onset of action is similar to LABAs, but the duration of action lasts longer than LABAs. Vilanterol with an inhaled corticosteroid and Indacaterol are ultra-LABAs, approved by FDA for the treatment of COPD [81].

8. β2-adrenergic receptors agonists in neuroprotection

The majority of adrenergic neurons are present in brainstem locus coeruleus (LC) nuclei, which is a predominant site for the production of norepinephrine (NE) in the brain. LC neurons play a key role in the regulation of cognitive behavior such as attention, mood, and arousal [82]. These neurons also play role in the development of the brain, mainly the neocortex [83]. The degeneration of LC-neurons has been identified in patients with PD and AD [84]. Also, the classical “monoamine hypothesis of depression” says that the deficiency of NE is a culprit for the cognitive impairment [85]. NE/noradrenaline, the primary neurotransmitter released by the LC neurons targets the adrenergic receptors present on the microglia and astrocytes in the brain [86]. NE-activated ARs on glial cells stimulate the second messenger system and maintain the homeostasis in the brain. Activation of AR on glial cells elicits anti-inflammatory actions, inhibits neuroinflammation, and thereby limits the degeneration of neurons [87]. Moreover, drugs that stimulate the release of NE/NA have potential to reduced inflammation and amyloid pathology in a mouse model of AD [88]. According to Braak’s hypothesis, early stage of progression starts in LC before it spreads to SN [89]. Overall, these and many other studies suggest the role of the adrenergic signaling in neurodegeneration. Therefore, enhancing NE/NA signaling, transplanting noradrenergic neurons, or use of drugs that mimic the activity of NA/NE on glial cells have great potential to reverse or halt the progressive degeneration of neurons [90]. The endogenous agonist/ligand for β2-AR is norepinephrine which acts as a neurotrophic factor and can influence protein/DNA synthesis in developing adult brain [91, 92]. NE protects cholinergic and dopaminergic cultured neurons against oxidative stress and catechol moiety of NE plays role in neuroprotection [93, 94]. It suggests that a compound containing catechol moiety, such as β-agonists, can mimic the neuroprotective effects of NE. Treatment with NE stimulates the synthesis of BDNF in astrocytes and neuron in vitro and in vivo [95, 96] and these neuroprotective effects were reversed by the antagonist of α1, β1, and β2-AR [97].

The use of β2-agonists as an adjunct therapy to L-DOPA in PD was first described in 1994 [98]. Chai et al. showed that the β2-AR activation enhances hippocampal neurogenesis, ameliorates memory deficits, and increases dendritic branching and spine density in a mouse model of Alzheimer’s disease [99]. Recently, Mittal et al. have found that β2-AR activation regulates the gene expression of α-synuclein in various animal and in vitro models of PD. Salbutamol, a blood-brain-barrier-permeable β2-agonist, reduces expression of SNCA gene via histone-3-lysine-27 acetylation of its promoter and enhancer. They also analyzed the pharmacological history of 4 million Norwegians over 11 years and found that Salbutamol was also associated with reduced risk of developing PD [100]. In a mouse model of Down syndrome, Formoterol, a long-acting β2-AR agonist, causes significant improvement in synaptic density and cognitive functions [101]. Salmeterol (Sal) is an inhaled long-acting highly selective β2-AR agonist which is currently being used as the active ingredient in Advair® as a bronchodilator. Our previous studies and others have shown that Salmeterol has anti-inflammatory and DA-neuroprotective activities, even at very low doses. Pre-treatment with Salmeterol protects DA neurons against LPS- and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced toxicity in both in vitro and in vivo animal models of PD [42, 102]. The
mechanism how Salmeterol regulates the activation of microglia is described in **Figure 1**. Collectively, these studies suggest that β2-AR agonists not only protect neurons against degeneration, but also have anti-inflammatory effects, and therefore, hold significant promise for the treatments of a wide variety of neurodegenerative conditions including PD [43]. The clinical efficacy of β2-AR agonists have been examined in various neurological disorders and few of them are summarized in **Table 2**.

**Figure 1.**
Schematic of microglia-mediated neurotoxicity and inhibitory effects of Salmeterol on microglial activation.

<table>
<thead>
<tr>
<th>Disease Condition</th>
<th>Design</th>
<th>Doses</th>
<th>Drug</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinal Cord Injury</td>
<td>Randomized controlled</td>
<td>4mg twice/day for 1st week then 8mg twice/day for 15 weeks</td>
<td>Albuterol</td>
<td>[129]</td>
</tr>
<tr>
<td>Alzheimer’s Disease</td>
<td>Randomized controlled</td>
<td>20mg/2ml for 12 months</td>
<td>Formoterol</td>
<td>[130]</td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>Blinded controlled</td>
<td>4mg/day</td>
<td>Albuterol</td>
<td>[131]</td>
</tr>
<tr>
<td>Neuropathic pain</td>
<td>Controlled, double blinded</td>
<td>5mg twice/day for 28 days</td>
<td>Terbutaline</td>
<td>[132]</td>
</tr>
<tr>
<td>Memory and Cognition</td>
<td>Randomized controlled</td>
<td>4mg, single oral administration</td>
<td>Salbutamol</td>
<td>[133]</td>
</tr>
<tr>
<td>SMA</td>
<td>Uncontrolled</td>
<td>3-8mg/day for 6 months</td>
<td>Albuterol</td>
<td>[134]</td>
</tr>
<tr>
<td>ALS</td>
<td>Uncontrolled</td>
<td>60ug/day for 6 months</td>
<td>Clenbuterol</td>
<td>[135]</td>
</tr>
<tr>
<td>SBMA</td>
<td>Uncontrolled</td>
<td>20ug/day for 2 days, then 40ug/day</td>
<td>Clenbuterol</td>
<td>[136]</td>
</tr>
</tbody>
</table>

**SMA:** Spinal Muscular Atrophy, **SBMA:** Spinal and Bulbar Muscular Atrophy, **ALS:** Amyotrophic Lateral Sclerosis.

**Table 2.** Clinical trials using β2-agonist in neurological conditions.
9. β2-adrenergic receptors and neuroinflammation

Extensive previous investigations into the etiology of PD demonstrate a central role for the inflammatory microglial cell in the progression of PD. Thus, targeting neuroinflammation mediated by microglia may serve as a potential therapeutic benefit in the treatment of PD. Since traditional treatment for PD is aimed only at controlling the disease symptoms, the search for more effective neuroprotective therapies which target the cause of the disease is now receiving significant attention. Studies targeting neuroinflammation are aimed to promote the development of a novel therapeutic approach and aid in the drug discovery for neurodegenerative conditions such as PD.

One such anti-inflammatory approach that has been found to be effective in protection against dopaminergic neurodegeneration is accomplished by natural and therapeutic compounds that activate the β2-AR. Brain cells including neurons, microglia, and astrocytes as well as immune cells express a high density of β2-AR on their surface [66, 103]. Catecholamines such as epinephrine (adrenaline), norepinephrine (noradrenaline), and dopamine are the most abundant catecholamines found in the nervous system. As evidenced by many unrelated studies, catecholamines can modulate the immune response [87, 104]. Further studies have found that the endogenous agonist of β2-AR, norepinephrine (NE), controls microglial motility and functions during pathogenic conditions [105]. NE also protects cortical neurons against microglia-mediated inflammation, while decreased levels of NE enhance microglial activation [106]. One study showed that β2-AR negatively regulates NF-κB activation and stabilizes the NF-κB/IkBα complex via β-arrestin 2 in LPS activated murine macrophages [107]. Interestingly, activation of β2-AR in astrocytes modulates TNF-α-induced inflammatory gene expression in vitro and in vivo. In addition, an in vivo study demonstrated increased expression of β2-AR in glial cells in response to neuronal injury. This suggests that β2-AR may provide a therapeutic target for regulation of glial cell functioning and the inflammatory response in the brain [108]. Activation of β2-AR on astrocytes stimulates the release of trophic factors such as BDNF, bFGF, NGF-1, and TGF-β1 via canonical signaling, showing anti-apoptotic and neuroprotective effects in animal models of cerebral ischemia and excitotoxicity [109, 110]. It has also been shown that noradrenaline acting on β2-AR enhances the expression of anti-inflammatory and neurotrophic cytokine IL-10 in the brain. This suggests an endogenous ligand of β2-AR is neuroprotective during inflammatory conditions in CNS disease pathology [108, 111]. Both canonical and noncanonical signaling of β2-AR can selectively regulate the adaptive immune response [67], since β2-AR are expressed by naïve CD4+ T (T-helper (Th0)) and Th1 cells but absent on Th2 cells [112, 113]. Naïve CD4+ T-cell treated with a β2-AR agonist or NE suppresses the production of interferon (IFN)-γ and IL-2 and affects their differentiation [114]. Collectively, these studies and several others suggest the role of β2-AR in the regulation of immune response.

10. Molecular mechanism of inflammation in PD or molecular mediators of inflammation in PD

10.1 Effect of β2-AR agonists on NF-κB pathway

We have characterized and examined the effects of β2-AR agonists including Salbutamol, Salmeterol, Indacaterol, and Vilanterol on neuroinflammation in models of PD in vitro and in vivo. However, the short-acting agonists were neuroprotective and able to reduce inflammation in vitro at higher doses, but the long-acting
agonist showed beneficial effects at low concentration (10^{-9} M) in neurotoxicity and inflammatory models of PD. Salmeterol, a β2-AR agonist, can effectively serve as a therapeutic treatment for PD by inhibiting microglia-mediated inflammatory responses in vivo. We have found that Salmeterol functions to inhibit innate pro-inflammatory response in both murine macrophages and microglia through its inhibition of the NF-κB signaling pathways [42]. We have also investigated whether Salmeterol is specific to neuroinflammation in PD or if it can be used as a universal anti-inflammatory drug against other chronic inflammatory diseases. To test this, we used murine macrophages stimulated with LPS from Porphyromonas gingivalis (PgLPS), an oral pathogen as an in vitro model for the periodontal disease. We have found that Salmeterol shows similar anti-inflammatory effects on PgLPS-stimulated macrophages [115]. Additionally, Feng et al. have also shown neuroprotective effects of β-arrestin2 via endogenous opioid arrest in inflammatory microglial cells [116].

10.2 Effect of β2-AR agonists on MAPK pathway

The agonist-activated β2-AR stimulates MAPK signaling pathway via noncanonical and G-protein-independent pathway. Agonist-activated β2-AR reduces phosphorylation of ERK1/2 and p38 MAPK in macrophages stimulated with LPS. In contrast, β2-AR activation stimulates MAPK signaling and TNF-α, IL-12, and NO production in murine macrophages treated with PMA (phorbol 12-myristate-13-acetate) [73]. Similarly, our previous studies have shown that activation of β2-AR with the high concentration of agonist (up to 10^{-5} M) leads to sustained phosphorylation of ERK1/2 and enhanced production inflammatory mediators in murine microglia and macrophages [117]. High-dose treatment of β2-AR agonists on mixed neuroglia culture enhances neurotoxicity via NADPH oxidase activity in the ERK-dependent manner [118]. Like others, we have found that the low-doses of the β2-AR agonist Salmeterol reduces the MAPK activity, NF-κB activation and production of TNF-α in LPS-activated primary microglia [42]. We have also found that low-dose Salmeterol inhibits the phosphorylation of TAK1 (TGF-β-activated kinase1) which is an upstream regulator of NF-κB signaling in LPS-stimulated microglia. We have also found that Salmeterol increases the expression of β-arrestin2 and enhances the interaction between β-arrestin2 and TAB1 (TAK1-binding protein), reduced TAK1/TAB1 mediated activation of NFκB and expression of pro-inflammatory genes. Furthermore, silencing of β-arrestin2 abrogates the anti-inflammatory effects of Salmeterol in LPS-stimulated BV2 cells [119]. These studies suggest that the anti-inflammatory effects of Salmeterol work through the inhibition of pro-inflammatory pathways in microglial cells.

10.3 The β-arrestin-mediated biased effects of β2-AR agonist

Previous findings show that high dose Salmeterol enhances the expression of IL-1β and IL-6 mRNA and protein in unstimulated human monocytes and murine macrophages. These effects were β-arrestin2-dependent but PKA and NF-κB independent, while treatment with ERK1/2 and p38 MAPK inhibitor could reverse this effect [117]. This finding and several others suggest Salmeterol or other long-acting agonist have β-arrestin “biased” signaling of β2-AR. These agonists activate receptors via β-arrestin signaling with a much greater extent than their effect on G-protein-dependent signaling [120]. Our studies suggest that a very low concentration of Salmeterol does not enhance cAMP signaling and its downstream mediators, while it activates the β-arrestin2-mediated signaling events [42]. β-arrestin2 has been shown as a novel regulator of IκB stability via the direct interaction of β-arrestin2 and IκB in HEK293 cells [121]. In addition, β-arrestin2 negatively regulates the activation of NF-κB via direct binding with IκBα [122]. One study showed
that overexpression of β-arrestin2 significantly reduces L-DOPA-induced dyskinesia in animal models of PD [123]. Collectively, these studies suggest that β2-AR agonists can be used therapeutically not only to inhibit chronic inflammation and progressive degeneration of neurons, but also to treat some of the most debilitating neurologic symptoms in PD.

### 10.4 cAMP/PKA/CREB pathway induced by β2-AR

After binding with an agonist or endogenous ligand, β2-AR normally activates the classical cAMP-dependent signaling pathway. The downstream effect of the cAMP/PKA pathway is the phosphorylation and nuclear translocation of the CREB transcription factor which further enhances the expression of cAMP-inducible genes [79]. Activation of CREB via this pathway regulates the synthesis of proteins which are mandatory for neuronal homeostasis [124]. The classical signaling of β2-AR also increases the activity of PGC-1α (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha), which is a key regulator of mitochondrial biogenesis and ROS metabolism [125]. Activation of β2-AR also elevated the release of neurotrophic factors via cAMP/PKA/CREB pathway and provides neuroprotective benefits against degeneration [126]. An endogenous agonist of β2-AR (NE) affects immune cell functions, production of cytokines, and antibody secretion [112]. β2-AR agonists have anti-inflammatory activity and inhibit release of pro-inflammatory mediators via cAMP/PKA/CREB pathway and also by alternate cAMP-dependent pathway (cAMP/Epac1/2) [42, 127, 128]. We have also found that pro-inflammatory effects of high-dose of Salmeterol are through cAMP/Epac pathway, while the anti-inflammatory effects of low-dose of Salmeterol are independent on cAMP and Epac activation [42, 118].

### 11. From bench to bedside: challenges in translation to the clinic

The β2-AR agonists discussed above are FDA-approved for the treatment of respiratory diseases such as asthma and COPD, but none of these β2-AR agonists are specifically developed for PD. Although, Mittal et al. have found in a Norwegian population that using Salbutamol, a SABA, lower the risk of developing PD whereas the use of Propranolol, a β2-AR antagonist (commonly used to treat hypertension and certain other forms of heart disease) was associated with increased risk of PD [100]. Furthermore, this risk of developing PD was dependent on the duration of Salbutamol intake in those patients. In the patient population who used Salbutamol for at least 6 months, it was expected that 43 would develop PD, but only 23 patients were ultimately diagnosed with the disease (rate ratio 0.66). On the other hand, in the cohort who used Salbutamol for 2 months or less, there was no decreased risk of developing PD in this population [100]. In contrast, patients on Propranolol (which is also used as therapeutic for tremors in PD) for at least 1 year showed a significantly increased risk of developing PD compared to patients not on propranolol (rate ratio 2.2). Therefore, it is clear that patients on long-term Salbutamol (a β2-AR agonist) had significantly decreased the risk of developing PD, while patients on long-term propranolol (a β2-AR antagonist) therapy had significantly higher rates of PD, suggesting that β2-AR inhibition is a highly significant risk factor in developing PD. When we compared the effectiveness of Salbutamol to Salmeterol (a more lipophilic drug) in animal models of PD, Salmeterol was much more effective both *in vitro* and *in vivo* in dopaminergic neuroprotection [42]. More importantly, we found that animals given Salmeterol treatment well before the appearance of symptoms in a long-term model of PD showed little evidence of dopaminergic neurodegeneration
compared to untreated animals. Taken together, this data suggests that administration of β2-AR agonists may have a profound preventative effect on the development of PD. Since the blood-brain-barrier penetration is a major obstacle in the development of therapeutics targeting CNS disorders, it will be important to consider the importance of lipophilic properties, concentration within the CNS, as well as the specificity, half-life and safety in using β2-AR agonists in older patients before and after the initial appearance of symptoms associated with PD. Consequently, these drugs require further investigation in a large cohort study to assess their utility as a potential therapeutic for PD and other neurodegenerative diseases.

12. Conclusion

Natural or synthetic activation or inhibition of the β2-AR can have profound effects on the development and progression of Parkinson's disease, a chronic neurodegenerative disorder which involves both neuroinflammatory and cellular mechanisms in dopaminergic neurotoxicity. It is now clear that the therapeutic use of β2-AR agonists can both inhibit the cause of neurodegeneration and activate a mechanism that can enhance recovery of patients with this disease, and serves as an important new therapeutic approach to the treatment of chronic neurodegenerative disorders.

Conflict of interest

Authors declare no “conflict of interest.”

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Section 2
Neuroprotection to the CNS
Section 2

Neuroprotection to the CNS
Chapter 5
Protecting the Aging Retina
Shen Nian and Amy C. Y. Lo

Abstract
Aging retina, notably the aging macula, is prone to develop degenerative diseases, such as age-related macular degeneration (AMD), the leading cause of visual loss in individuals aged 65 or above in developed countries. However, current treatments are very limited. Since degeneration, dysfunction, and death of retinal neurons are demonstrated in the pathogenesis of AMD, neuroprotective strategies could serve as a possible way to treat AMD. In this chapter, we will briefly introduce risk factors, pathophysiology, affected neurons, classification, clinical manifestation, and current treatments of AMD. Finally, neuroprotection in both AMD animal models and patients will be discussed.

Keywords: neuroprotection, degeneration, photoreceptor, age-related macular degeneration, vision loss

1. Introduction
Retina, which forms the innermost layer of the eyeball, is considered as the end organ of the central nervous system. Macula, located in the central and posterior part of retina, possesses the highest concentration of photoreceptors and, therefore, is responsible for central vision and high-resolution visual acuity (VA). The fovea is a tiny pit in the center of the macula and in charge of the central, sharpest vision. Unfortunately, the macula is more prone to experience degenerative changes with age, such as age-related macular degeneration (AMD), leading to visual impairment.

Due to the increase of life expectancy worldwide, the size of aging population will become larger in the coming decades [1]. According to "World Population Prospects: The 2017 Revision" released by the United Nations, the number of persons aged 60 or above will more than double, from 962 million in 2017 to 2.1 billion by 2050. The number of people aged 80 or above is predicted to triple by 2050, from 137 million in 2017 to 425 million by 2050 [2]. In mainland of China, people aged above 65 represented 11.4% of the total population in 2017 [3]. As a consequence, the increasing prevalence of AMD will be foreseen globally in the future.

In 2015, it was estimated that AMD was the fourth leading cause of blindness and the third most common cause of moderate to severe visual impairment globally [4]. The meta-analysis performed by Wong and collaborators has estimated that the number of persons with AMD will increase from 196 million in 2020 to 288 million in 2040 worldwide [5]. A similar trend is observed in the projected number of individuals affected by AMD in China, rising from 31.23 million in 2020 to 55.19 million in 2050 [6]. In addition, the prevalence of late AMD did not show significant difference among Asian, European, and North American studies, whereas the number of individuals with early AMD was more in European and North American studies than in Asian studies [7].
Chapter 5

Protecting the Aging Retina

Shen Nian and Amy C.Y. Lo

Abstract

Aging retina, notably the aging macula, is prone to develop degenerative diseases, such as age-related macular degeneration (AMD), the leading cause of visual loss in individuals aged 65 or above in developed countries. However, current treatments are very limited. Since degeneration, dysfunction, and death of retinal neurons are demonstrated in the pathogenesis of AMD, neuroprotective strategies could serve as a possible way to treat AMD. In this chapter, we will briefly introduce risk factors, pathophysiology, affected neurons, classification, clinical manifestation, and current treatments of AMD. Finally, neuroprotection in both AMD animal models and patients will be discussed.

Keywords: neuroprotection, degeneration, photoreceptor, age-related macular degeneration, vision loss

1. Introduction

Retina, which forms the innermost layer of the eyeball, is considered as the end organ of the central nervous system. Macula, located in the central and posterior part of retina, possesses the highest concentration of photoreceptors and, therefore, is responsible for central vision and high-resolution visual acuity (VA). The fovea is a tiny pit in the center of the macula and in charge of the central, sharpest vision. Unfortunately, the macula is more prone to experience degenerative changes with age, such as age-related macular degeneration (AMD), leading to visual impairment.

Due to the increase of life expectancy worldwide, the size of aging population will become larger in the coming decades [1]. According to “World Population Prospects: The 2017 Revision” released by the United Nations, the number of persons aged 60 or above will more than double, from 962 million in 2017 to 2.1 billion by 2050. The number of people aged 80 or above is predicted to triple by 2050, from 137 million in 2017 to 425 million by 2050 [2]. In mainland of China, people aged above 65 represented 11.4% of the total population in 2017 [3]. As a consequence, the increasing prevalence of AMD will be foreseen globally in the future.

In 2015, it was estimated that AMD was the fourth leading cause of blindness and the third most common cause of moderate to severe visual impairment globally [4]. The meta-analysis performed by Wong and collaborators has estimated that the number of persons with AMD will increase from 196 million in 2020 to 288 million in 2040 worldwide [5]. A similar trend is observed in the projected number of individuals affected by AMD in China, rising from 31.23 million in 2020 to 55.19 million in 2050 [6]. In addition, the prevalence of late AMD did not show significant difference among Asian, European, and North American studies, whereas the number of individuals with early AMD was more in European and North American studies than in Asian studies [7].
2. Anatomy and function of the retina

The eye is composed of three layers, which are the inner retina layer, middle vascular choroid layer, and outer fibrous sclera layer, respectively. Retina, the innermost layer of the eye, consists of two parts: the inner transparent neurosensory retina and outer pigmented epithelial layer—the retinal pigment epithelium (RPE). There is a potential space between neural retina and RPE, called subretinal space. In the neural retina, the neural cell bodies are situated in three layers (Figure 1), including the outer nuclear layer (ONL) occupied with nuclei of photoreceptors; the inner nuclear layer (INL) filled with nuclei of horizontal, bipolar, and most of the amacrine cells; as well as ganglion cell layer (GCL) containing nuclei of retinal ganglion cells and the rest of displaced amacrine cells. Additionally, axons and dendrites of these retinal neurons constitute two synaptic layers: the inner plexiform layer (IPL) and outer plexiform layer (OPL) [8]. The RPE cells form a continuous polarized cell monolayer with its apical surface adjacent to the outer segment apices of the photoreceptors and its basal aspect lying on supportive substrate Bruch’s membrane.

Retinal neurons are mainly distributed in three layers: ONL with nuclei of photoreceptors; INL with nuclei of horizontal, bipolar, and most of the amacrine cells; and GCL with nuclei of retinal ganglion cells and the rest of displaced amacrine cells. Additionally, axons and dendrites of these retinal neurons constitute two synaptic layers, including IPL and OPL [8].

Photoreceptors are divided into two types: rods, which are dominated in the peripheral retina and responsible for dim light vision and detecting movement and contrast, and cones, which are dominated in the macula, especially the fovea (only cones), and are responsible for bright light vision and sensing color vision.

Figure 1. Retinal layers of the human eye.
and resolution. Outer segments of the photoreceptor are generated by the cell body, and the distal parts of outer segments are phagocytosed by RPE every day. There are abundant mitochondria in inner segments to supply energy for these cells with high metabolic rate. Bruch’s membrane, where the RPE cells are tightly attached, consists of five layers including, from inner to outer, the RPE basal lamina, inner collagenous zone, elastic layer, outer collagenous zone, and basement membrane of the choriocapillaris [9].

In brief, the function of the retina is to convert the external light signals into electrical impulses by photoreceptors. The impulses, which are partly integrated locally by horizontal cells and amacrine cells, are then processed by bipolar cells and sent to ganglion cells which further transmit them to the brain. Moreover, RPE is crucial for maintaining the microenvironment of neural retina by exchanging nutrients and wastes between neural retina and choroid, secreting numerous growth factors, phagocytosing shed photoreceptor outer segments (POS), absorbing light and converting all-trans-retinal into 11-cis-retinal [10]. Any defects in any of these functions may result in retinal degeneration, deficits of visual function, and eventually loss of sight.

3. Current knowledge of AMD

3.1 Classification and clinical manifestations

A variety of classification systems are employed for both clinical and basic research of AMD. However, the most commonly used classification is the one defined by the Age-Related Eye Disease Study (AREDS) in terms of the characteristics of drusen (yellow deposits in the macula), abnormal hypo- or hyperpigmentation in the first eye, and how it affected the fellow eye. Based on the above criteria, AMD is classified into 4 categories: (1) no clinical manifestation of AMD if there was no drusen or only non-extensive, small drusen (<63 μm in diameter) in both eyes; (2) mild AMD classified by extensive small drusen, non-extensive intermediate drusen (63–124 μm in diameter), or abnormalities of pigments in at least one eye; (3) intermediate AMD characterized with large drusen (>124 μm in diameter), extensive intermediate drusen, or noncentral geographic atrophy (GA) in at least one eye; (4) advanced AMD defined as central GA or choroidal neovascularization (CNV) resulting in VA less than 20/32 [11–13].

There are two clinical types of advanced AMD: a non-exudative or atrophic (dry) form, accounting for 90% of AMD, and an exudative (wet) form, accounting for only 10% of AMD. The atrophic form is characterized by progressive degeneration of RPE cells and photoreceptors in the macula, affecting central vision to varying degrees over months or years [14, 15]. The exudative form is associated with CNV in the submacular area and subsequent retinal hemorrhage due to leakage of these newly formed fragile blood vessels, leading to severe central vision loss within a very short period of time [15].

At the early stage of AMD, patients are normally asymptomatic and may be diagnosed by the presence of round, yellowish drusen through the routine ophthalmological examinations. Patients with CNV usually suffer from sudden loss of vision, describing unexpected deterioration of central visual field, distorted straight line (metamorphopsia), and/or a dark area in the central visual field (scotoma). On fundus examination, macular edema and hemorrhage are observed and fluorescein angiography shows leakage. In non-exudative AMD, it takes years for patients to develop visual loss gradually, and fundus examination shows a well-defined RPE atrophic area with depigmentation (Figure 2) [15].
A number of risk factors are thought to be related to the pathogenesis of AMD, such as increasing age, smoking, family history of AMD, overweight or obesity, cataract and cataract surgery, eye exposure to the sunlight, etc. Of these factors, age is the strongest risk factor, with around 30% of cases older than 85 years diagnosed with AMD in US [17, 18]. The risk of developing AMD was reported to be more than triple in patients above 75 years than patients aged between 65 and 74 years [18, 19]. In populations of European ancestry, the projected prevalence of late AMD was 0.08% at age 50, 0.33% at age 60, 1.38% at age 70, 5.60% at age 80, and 20.10% at age 90, respectively [20]. Similarly, the estimated prevalence of any AMD increased from 2.44% in persons at the age of 45–49 years to 18.98% in persons at the age of 85–89 years [6].

Ethnicity and family history are also strongly related with AMD prevalence. The highest rate was reported in Caucasians, followed by Hispanics and Asians, while African Americans showed the lowest morbidity rate [21]. Increased risk of AMD was observed in individuals with positive family history, approximately three to six times higher than it was for those from the general population [22]. Furthermore, genetic studies have revealed 34 loci linked to AMD until now, of which complement factor H (CFH) on 1q31.3, age-related maculopathy susceptibility 2 (ARMS2) on 10q26, and CFB/C2 on 6q21.3 were the most discussed genes [15, 23, 24].

The strong relationship between cigarette smoking and development of AMD has been widely accepted [17, 25, 26]. Several studies revealed that current smoking was strongly associated with the progression of AMD [27]. Moreover, past smoking was reported to be a risk factor to develop AMD as well, especially in the rural areas [28]. There was an increased risk for individuals with high intake of several types of fat, such as omega-6 polyunsaturated fatty acids and saturated fats, whereas diets rich in monounsaturated fatty acids might reduce the prevalence of AMD [29]. Inadequate uptake of antioxidants also resulted in AMD, and numerous studies have suggested that antioxidant supplementation, including vitamins, lutein, zeaxanthin, beta-carotene, zinc, etc., helped to halt the progression of AMD to some extent [12, 30, 31].

The effects of sunlight exposure on the occurrence of AMD are controversial. A study performed by Khan and colleagues demonstrated that no significant association between light exposure and AMD was observed when comparing patients at the end stage of AMD with spouse controls [32]. However, other studies suggested that damages induced by either ultraviolet (UV) or visible sunlight might lead to...
AMD [33–35]. There was conflicting evidence regarding the role of cataract surgery in the progression of AMD [36]. Earlier, a large population-based research indicated a stronger relationship between cataract surgery with the development of end-stage AMD, especially neovascular AMD, in older patients [36, 37]. On the contrary, several recent clinical studies failed to demonstrate similar results [36, 38].

In addition to the risk factors mentioned above, other relevant factors included hypertension, cardiovascular diseases, iris color, hyperlipidemia, diabetes, alcohol consumption, and so on [22]. According to a systematic review of 18 prospective and cross-sectional studies and 6 case-control studies engaging 113,780 individuals, advancing age, cigarette smoking, previous cataract surgery, and family history of AMD were considered as the strong risk factors of AMD, while hypertension, cardiovascular disease, overweight or obesity, and elevated plasma fibrinogen were regarded as the moderate risk factors [17].

3.3 Pathophysiology

AMD is regarded as a complicated multifactorial disease, although the exact pathogenesis still remains poorly understood. It is generally recognized that impairment of aged RPE cell functions served an important role in the progression of non-exudative AMD. The aged RPE cells were less efficient in phagocytosing and degrading POS, resulting in the progressive accumulation of lipofuscin consisting of phagosomal and lysosomal constituents in the cytoplasm. The cytotoxic elements in lipofuscin, such as bisretinoid fluorophore, were able to generate reactive oxygen species leading to the damage of DNA, lipids, and proteins [39].

Another age-dependent change was the formation of focal extracellular yellow deposits known as drusen, the hallmark of AMD, between the RPE and Bruch’s membrane. Accumulation of drusen relied on the changes in the permeability of Bruch’s membrane due to the decline of RPE cell functions with aging [40]. In addition, it has been estimated that breakdown of choriocapillaris, which was next to RPE and Bruch’s membrane, resulted in insufficient elimination of extracellular wastes causing drusen [41]. According to their shape and size, drusen are separated into small (<125 μm), round hard drusen with well-defined borders, and relatively large, (125–250 μm) soft drusen with poorly defined borders. Soft drusen, especially with depigmentation or pigment abnormalities, was considered to be an indication to give rise to severe vision loss at the late stage.

The mechanism of drusen causing adjacent RPE and photoreceptors’ damage was not only dependent on the structural disturbance of RPE and photoreceptor by them, but also on the indirect effects through stimulation of local inflammation and immune system [42, 43]. Components in complement pathway and inflammatory processes were observed in drusen and aged RPE cells that are closely related to drusen [42]. It was demonstrated that in AMD, genetic variation of factor H gene (HP1/CFH) induced the abnormal activities of factor H (the inhibitor of complement cascade) in drusen, leading to the activation of complement pathway and subsequent inflammation in subretinal tissues [44]. Furthermore, in AMD patients, decreased CFH level was reported in smokers than nonsmokers, suggesting the activation of complement cascade might contribute to the significantly higher risk of AMD progression in cases with smoking [45].

DICER1, the ribonucleic acid (RNA)-cleaving enzyme, was decreased in RPE cells in cases with non-exudative AMD. A group of researchers in the University of Kentucky suggested that the lower level of DICER1 led to the lower rate of Alu RNA degradation in RPE cells. Therefore, accumulation of Alu RNA in cytoplasm resulted in RPE cytotoxicity and consequent degeneration via activating NLRP3 inflamasome [46, 47].
Neuroprotection

In exudative AMD, local nonspecific inflammation stimulated the upregulation of angiogenic factors, such as vascular endothelial growth factor (VEGF), and/or downregulation of anti-angiogenic factors, such as pigment epithelium-derived factor (PEDF), causing CNV development in the avascular outer 1/3 retina. The new vessels are fragile, thus macular edema and hemorrhage occur due to the leakage of these new blood vessels, which finally resulted in the fibrovascular scars in the macula [41, 48].

3.4 Affected neurons

Due to the dysfunction of aged RPE cells, especially the failure of POS phagocytosis, photoreceptor loss occurs subsequently. Although the most remarkable clinical and pathological injuries are present in RPE and its underlying Bruch’s membrane, it is the structural and functional disruptions, even the death of photoreceptors, via either atrophic or neovascular process, that take responsibilities for the visual impairment in AMD. Furthermore, the condition of photoreceptors directly reflects the significance of lesions in RPE/Bruch’s membrane complex.

It is crucial to identify which type of photoreceptors are most severely damaged in AMD, not only for the potential therapeutic strategies targeting the most affected cells, but also for investigation of mechanism of these pathological changes. The rate of rod and cone degeneration is representative in different situations affecting photoreceptors. In aging retina without age-related maculopathy, the number of cells in the cone-dominated fovea remained stable, while the number of rods in the parafovea was reduced by 30% [49]. In both non-exudative and exudative forms of AMD, photoreceptors were lost. In addition, more rod loss was observed than that of cones; gradually, only degenerated cones were left; finally, all photoreceptors might die [50]. The pathological changes mentioned above were consistent with the functional research exhibiting that scotopic sensitivity decreased more than photopic sensitivity in cases with AMD [51]. Maeda and colleagues revealed the apoptosis of photoreceptors after RPE damage caused by intravitreal injection of ornithine in rats, indicating the important role of RPE cells in maintaining photoreceptor integrity [52]. In AMD patients, apoptotic photoreceptors and RPE cells (TUNEL positive) were observed as well. Most of TUNEL-positive photoreceptors were rods and located at the edge of RPE atrophy. Moreover, Fas was upregulated in apoptotic photoreceptors, indicating Fas/FasL might be involved in the apoptosis process [53]. Studies performed by Kim and collaborators demonstrated that significant reduction of photoreceptors was shown in the areas where RPE cells were totally lost in GA, and where disciform scar formed in wet AMD [54, 55]. In terms of disciform scar, thickness of the scar was closely associated with photoreceptor loss, which means that the thicker the disciform scar, the less photoreceptors survived [56].

In addition to photoreceptors, other retinal neurons are also affected. Joshua et al. first reported that TUNEL-positive cells were detected in the inner side of INL, indicating that these cells might be amacrine cells [53]. In cases with GA, retinal ganglion cells were significantly decreased by 30.7% compared to age-matched control. However, cell nuclei in INL were not significantly different [54]. In wet form of AMD, a decrease of ganglion cells and increase of cells in INL were observed, but there was no significant difference [55].

3.5 Current treatments

Several treatments have been adopted for AMD management. However, current therapeutics can only slow the progression of the disease, trying to delay the onset of vision loss as much as possible.
To date, there are no approved drugs for the dry form of AMD. Therefore, much effort has been made to reduce risk factors. Among various risk factors, oxidative stress induced by inflammation, light exposure, and so on is considered as one of the most important risk factors for the occurrence and progression of AMD. Numerous studies have been conducted to evaluate the relationship between antioxidant nutrition supplementation and AMD. In the Age-Related Eye Disease Study (AREDS), it was demonstrated that oral dietary supplementation containing vitamin C (500 mg/day) and E (400 IU/day), zinc oxide (80 mg/day), cupric oxide (2 mg/day), and beta-carotene (15 mg/day) can decrease the risk of development of AMD from intermediate stage to advanced stage [11]. Since beta-carotene was observed to increase the risk of lung cancer in cigarette smokers, and 80 mg/day zinc is out of tolerance for individuals, there were elimination of beta-carotene, decreased dose of zinc (25 mg/day), and adding of lutein and zeaxanthin in the AREDS2 formula [12, 57, 58]. A 10% reduction in developing to advanced AMD was present in patients treated with AREDS2 formula containing lutein and zeaxanthin [59]. In addition, healthy diet rich in fish, green leafy vegetables, and nuts together with healthy lifestyle are strongly recommended to reduce AMD risk factors [15].

For the wet form of AMD, therapies mainly focus on halting the progression of CNV. Thermal laser photocoagulation is the first treatment to stop the progression of CNV successfully, but with no significant vision improvement and high recurrence of CNV. Photodynamic therapy with verteporfin can selectively damage the CNV tissue without additional injuries of neighboring tissue, but this therapy has no effects on visual improvement either. In terms of upregulation of VEGF in the development of CNV, intravitreal injection of anti-VEGF drugs has been widely used by ophthalmologists as a standard treatment. Anti-VEGF drugs (Pegaptanib sodium, Ranibizumab, and Bevacizumab) for exudative AMD have demonstrated exciting results: the vision in the majority of patients remained stable for 1 year, of which 40% of patients had visual improvement. Surgical intervention to remove CNV and submacular hemorrhage did not improve VA, which is the result of recurrence of CNV [15, 60].

4. Neuroprotection in AMD experimental studies and clinical approaches

Neuroprotection comprises a large number of therapeutic interventions to improve survival of neurons by modifying the structure and function of neurons, and/or their microenvironment. Initially, neuroprotective therapies are focused on central nervous system diseases including stroke, Alzheimer’s disease, Parkinson’s disease, etc. Since retina is regarded as the end part of central nervous system, a series of neuroprotective strategies has been applied to prevent vision loss by protecting retinal neurons. Furthermore, tremendous neuroprotective strategies are under investigation in both experimental and clinical research.

4.1 Studies in animal models

Neurotrophic factors, belonging to the family of growth factors, have the ability to promote survival of retinal neurons. Ciliary neurotrophic factor (CNTF) is one of the most extensively studied neurotrophic factors for neural retina protection. La Vail and collaborators first reported that intraocular injection of CNTF obviously prevented photoreceptor death from light-induced damage in Sprague Dawley rats [61]. Subsequently, intravitreal injection of adenoviral vector containing CNTF cDNA in rd1 mice, a naturally occurring mouse model for
retinal degenerative diseases, demonstrated the reduction of photoreceptor loss, conservation of ONL thickness, and increase of photoreceptor segments’ length. Moreover, the amplitudes of a-wave and b-wave in electroretinogram (ERG) were significantly increased compared with those of the control group, suggesting the preservation of retinal functions [62, 63]. Later, long-term protective effects of photoreceptors were shown using adeno-associated virus to deliver CNTF to the retina [64]. In order to sustainably deliver neurotrophic factors, encapsulated human RPE cells secreting CNTF were transplanted into the vitreous of rcd1 dog (a dog model of retinal degeneration). A significant increase of ONL thickness was observed in the treated eye as a result of continuous release of CNTF at the nanogram level [65]. No adverse effects were exhibited in the retina of transplanted eyes during the whole experiment period (7 weeks). In addition, CNTF was proved to protect loss of cone outer segments, an early sign of cone degeneration, indicating that CNTF could not only slow or halt progression of degeneration but also might reverse degeneration [66].

Placental growth factor (PIGF), one of the members of vascular endothelial growth factor family, was believed to prevent neuronal injury in the brain. In the retina, the role of PIGF was exhibited quite differently in in vitro and in vivo studies. Blue light-induced murine photoreceptor cell death was significantly attenuated after the treatment of PIGF by suppressing caspase-3/7 activity through the mitogen-activated protein kinase (MEK) and phosphoinositide 3-kinase (PI3K) pathway. Anti-PIGF antibody eliminated these protective effects [67]. However, in the light-induced retina-damaged mouse model, PIGF induced decreased ONL thickness and dysfunction of retina. Anti-PIGF antibody diminished neuroretinal injury and disruption of RPE cell-cell junctions after exposure to the white light for 3 h [68]. The opposite effects of PIGF and its antibody in mice were later found to be due to the hyperpermeability of RPE induced by PIGF, leading to the breakdown of retina-blood barrier and subsequent damages [68].

Ursodeoxycholic acid (UDCA) and its taurine-conjugated derivative taouroursodeoxycholic acid (TUDCA) were first found in the bile acid of hibernating bears. They have been used for liver detoxification, dissolution of gallstone and kidney stone, suppression of convulsions, and visual improvement in traditional Chinese medicine for a very long time. According to the theory of modern medicine, UDCA and TUDCA exhibit neuroprotective effects through prevention of cell apoptosis [69]. TUDCA treatment significantly preserved the number and structure of photoreceptors and retinal functions in different murine models of photoreceptor degeneration, including rd10 mice, rd1 mice, BALB/c mice, Bardet-Biedl syndrome type 1 mice, and transgenic P23H rats [69]. Furthermore, TUDCA manifested greater protective effects in cones [70]. In vitro studies using photoreceptor 661 W cells revealed that reduced endoplasmic reticulum (ER) stress and improved trafficking of cyclic nucleotide-gated channels in cones contributed to neuroprotective effects of TUDCA [71]. In addition, TUDCA improved phagocytosis of POS in H2O2-treated RPE cells via activating Mer tyrosine kinase receptor (MerTK), which indirectly protected photoreceptors [72].

Endogenous and exogenous progesterone have been certified to have neuroprotective effects in brain and retina for several decades. A broad range of studies have been conducted in either light-damaged or genetic murine models of retinal degeneration, demonstrating improvement in photoreceptor survival, decreased gliosis, and reduced retinal dysfunction after administration of progesterone or synthetic progestins [69]. A group of researchers in Spain revealed that rd1 mice orally administered with progesterone (100 mg/kg body weight) at postnatal day 7 (P7) exhibited significantly decreased number of apoptotic cells in ONL in the far peripheral retina and increased amplitude of ERG b-wave at P15, but no
significant change was observed at P17. There was also a transient reduced gliosis in the treated rd1 mice [73]. Similar results were observed with oral administration of synthetic progestin, known as the FDA-approved Norgestrel, showing reduction of photoreceptor death by 70 and 75% in light-damaged mouse model and rd10 mice, respectively [74]. The rescue effects were achieved by increasing production of basic fibroblast growth factor and its downstream pro-survival reactive oxygen species [74, 75].

Crystallins, critical family members of small heat shock proteins, have been identified to have novel functions in both retina and RPE as in the lens, such as anti-apoptosis and anti-inflammation. αB-Crystallin is secreted in exosomes released from apical surface of polarized RPE cells and accumulates in the matrix among photoreceptors, and therefore may protect neighboring RPE and photoreceptors [76]. RPE cells in αB-crystallin knockout mice exhibited high susceptibility to oxidative stress and endoplasmic reticulum stress compared to the RPE cells from wild-type mice. Furthermore, RPE cells overexpressing αB-crystallin were more resistant to apoptosis, indicating the protective effects of αB-crystallin [77]. In the mouse model of AMD induced by sodium iodate (NaIO3), absence of αB-crystallin accelerated RPE apoptosis with subsequent death of photoreceptors through upregulation of AKT phosphorylation and expression of peroxisome proliferator-activator receptor-γ, suggesting αB-crystallin, especially the small peptide called mini cry, may play an important role in the protection of retinal degeneration [77]. In order to prolong the life of mini cry in the vitreous, free mini cry was fused to form an elastin-like polypeptide (ELP), which could be detected in the vitreous for up to 2 weeks. One intravitreal injection of ELP-linked peptide prevented RPE cells from apoptosis, inhibited activation of caspase-3 activation, and protected neural retina for up to 1 month after NaIO3 challenge [78].

NF-E2-related factor 2 (Nrf2) is a transcription factor that regulates antioxidant responses in many tissues and cell types, providing protection against oxidative stress. Under the oxidative stress, Nrf2 is translocated from cytoplasm to nucleus, and subsequently binds to the corresponding sites to activate transcription of a wide range of antioxidant genes. In the central nervous system, Nrf2 was proved to slow the neurodegeneration by means of antioxidative stress and neuroinflammation [79]. In the mice undergoing optic nerve crush (an animal model of glaucoma), retinal ganglion cells were significantly decreased than in the wild-type mice. With the treatment of Nrf2 activator, retinal ganglion cell loss was decreased by upregulating gene expression of antioxidant and phase II detoxifying enzymes [80]. After retinal ischemia-reperfusion injury, Nrf2 knockout mice showed greater loss of retinal ganglion cells when compared with the wild-type mice. Moreover, after ischemia-reperfusion injury, Nrf2 activator increased survival of retinal ganglion cells in wild-type mice, but not Nrf2 knockout mice, indicating the neuroprotective effects of Nrf2 [81]. An in vitro study has demonstrated that siRNA knockdown of Nrf2 led to significant increase of reactive oxygen species and cell death after blue light exposure in murine photoreceptor cells, suggesting that Nrf2 could be used to protect photoreceptors in AMD and other retinal degeneration [82].

There is no direct evidence that dopamine protects retinal neurons through anti-inflammation, apoptosis, and oxidative stress. However, decreased expression of dopamine may play a negative role in neuron survival in the brain [83]. Thus, dopamine receptor agonists may achieve the goal to promote retinal neuron survival and function by restoring the lost dopamine resulting from certain diseases. Dopamine receptor agonist pramipexole was shown to ameliorate structural and functional injuries in the light-damaged mice, exhibiting decreased photoreceptor death, damage of photoreceptor outer and inner segments, TUNEL-positive cells in ONL, and preservation of a-wave and b-wave in ERG [84]. Additionally, pramipexole
inhibited ARPE-19 cell (an immortalized RPE cell line) death after \( \text{H}_2\text{O}_2 \) treatment, suggesting its antioxidative effects [84].

Regular general physical exercise is not only a part of healthy life style, but also a rehabilitation strategy showing neuroprotective effects in numerous diseases. Recently, exercise has been proven to be neuroprotective in animal models of retinal degeneration [85–87]. Wild-type BALB/c mice were forced to exercise for 5 days/week for 2 weeks before being exposed to bright light. Exercised mice showed greater improved amplitude of ERG b-wave and photoreceptor nuclei than the mice without exercise after light exposure [85]. In order to exclude the impact of stress caused by forced exercise, voluntary wheel running was adopted in rd10 mice, which also demonstrated protection of VA, and the number of cones and total photoreceptors [86]. No matter if it was involuntary or voluntary exercise, either one benefited damaged photoreceptors in animal models of retinal degeneration through increased expression of brain-derived neurotrophic factor (BDNF) and activation of tropomyosin-related kinase B (TrkB) signaling pathway [85, 86].

Electrical neurostimulation has developed rapidly in recent years, covering a range of neurological diseases, such as neurostimulation for epilepsy, spinal cord stimulation for chronic pain, brain stimulation for Parkinson’s disease, and so on [88]. Electrical neurostimulation in vision research has also made a great improvement, from transcorneal, subretinal, to whole-eye electrical stimulation. Transcorneal electrical stimulation (TES) was performed in SD rats before or after exposure to the intense light for 14 days. Both the stimulation before and after light exposure slowed the progression of photoreceptor degeneration. Furthermore, TES after light exposure exhibited a longer and better protective effect. The neuroprotection effects may result from anti-apoptosis (upregulation of Bcl-2 and downregulation of Bax) and increased expression of neurotrophic factors (CNTF and BDNF) [89]. Subretinal electrical stimulation (SES) in the eye of Royal College of Surgeons (RCS) rats, a commonly used model of retinal degeneration, significantly preserved amplitudes of b-wave and oscillatory potential, and implicit times of a-wave and b-wave, suggesting the preservation of not only photoreceptors, but also signal transmission in the retina [90]. Although the whole-eye electrical stimulation did not provide protection for rod structure in P23H rats, b-wave amplitudes and rod sensitivity were significantly increased [91].

Although the neuroprotective strategies mentioned above have acquired promising results in animal models, damaged human retina can be only partially rescued. Miller and colleagues have investigated the underlying reasons using many animal models. There are three types of cell death including caspase-mediated apoptosis, autophagy-mediated cell death, and necrosis which is regulated by receptor-interacting protein kinases (RIPK). In the animal model of retinal detachment, elevated expression and phosphorylation of RIPK together with activation of caspases were observed. When either RIPK or caspases were inhibited, no obvious rescue effects were demonstrated. However, inhibition of both RIPK and caspases resulted in significant protective effects. Similar results were found in the animal model of retinal degeneration which experienced both photoreceptor and RPE cell death. In this animal model, the dominant type of cell death in photoreceptors was apoptosis, while necrotic RPE cells were mainly exhibited. Therefore, therapies that block both RIPK and caspase pathways may provide more satisfactory neuroprotection [92].

4.2 Current medicine/neuroprotective agents in clinical trials

Neurotech Pharmaceuticals developed an intraocular drug delivery system using encapsulated cell technology, called NT-501, to consistently release CNTF in the vitreous for more than 2 years [93]. A double-masked, randomized,
sham-controlled, phase II study enrolling 51 GA patients who were randomly divided into 3 groups, high-dose NT-501 implant, low-dose NT-501 implant, and sham control group, demonstrated promising outcomes after a 2-year evaluation [94]. Another phase II study using intraocular implant secreting CNTF in GA patients has also been completed. In this multicenter, double-masked, randomized, sham-controlled, 1-year, dose-ranging phase II study, VA stabilization, defined as loss of less than 15 letters, was observed in the high-dose group (96.3%) versus low-dose group (83.3%) and sham surgery (75%). All the patients with best corrected VA ≥20/63 in the high-dose group lost less than 15 letters, while it was only 55.6% in the combined group of patients treated with low-dose implant and sham surgery. Additionally, increase of retinal thickness was consistent with the stabilization of VA [95].

Brimonidine, an α-2 agonist, is usually used to treat glaucoma patients in ophthalmology. Since brimonidine has been reported to protect neuroretinal cells in murine models, the effects of this drug on dry AMD patients were under evaluation [96]. A randomized, double-masked, sham-controlled, phase II study involved 119 patients with bilateral GA who were randomly divided into 3 groups including 200 µg treatment, 400 µg treatment, and sham control group. The efficacy and safety of brimonidine on biodegradable implant were evaluated after intravitreal transplantation [97]. But the results were not reliable, so another multicenter study is currently performed with larger samples (311 eyes receiving either treatment of 400 µg brimonidine on biodegradable implant or sham treatment) and longer evaluation period (up to 24 months) [98].

A retrospective analysis was performed to analyze the association between intake of L-DOPA and incidence of AMD. The results showed that the onset of AMD in individuals prescribed with L-DOPA was 8 years later when compared with those without the uptake of L-DOPA. The protective effects of L-DOPA might be through GPR143 (the only known L-DOPA receptor) pathway [99, 100].

There are few studies about exercise as a clinical intervention in AMD, and the published papers are observational research showing the correlation between exercise and prevalence of AMD. A study lasting for 15 years demonstrated that active lifestyle with physical exercise 3 times per week or more was associated with reduced risk to develop wet AMD [101]. In another cohort study, an inverse relation was observed between vigorous exercise (≥3 times/week) and occurrence of intermediate AMD in women, not in men [102]. In addition, low physical exercise was related to the formation of drusen larger than 63 µm [103].

Electrical neurostimulation has already been applied to patients using less invasive approaches, such as transcorneal or whole-eye electrical stimulation, to protect the structure and function of retinal neurons. In a clinical trial conducted by Anastassiou and colleagues, 22 patients with dry AMD received transpalpebral electrical stimulation twice a day for 5 days. Most of the patients demonstrated the improvement in VA and contrast sensitivity at 4 weeks after the treatment; however, only contrast sensitivity was significantly different when compared with sham control group [104]. Similarly, microcurrent stimulation (150 µA for 35 min) once a week for 3 months was applied to both dry and wet AMD. Significantly increased VA was demonstrated in dry AMD, but not in wet AMD. Moreover, the number of patients showing increased VA was twice as those exhibiting deterioration [105].

5. Conclusions

AMD is believed to have stronger relationship with age. With increase of aging population worldwide, more individuals are suffering from visual damage,
resulting in poor quality of life for the aged people and elevated cost of medical care. In AMD, it is not only the degeneration of RPE cells, but also the neural retina degeneration, especially photoreceptors, that leads to the visual impairment. Therefore, neuroprotection can be one of the therapeutic strategies to slow, halt, or even reverse the progression of retina degeneration. Although the neuroprotective interventions that are currently investigated in both animal models and patients demonstrate promising results, it is of importance to identify the long-term efficacy and safety of these interventions. Only after that, the therapeutics will be provided to patients to help them maintain vision and further improve the quality of life.

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Conflict of interest

The authors declare no conflict of interest.

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Protecting the Aging Retina

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Neuroprotection


Neuroprotection is a strategy to prevent or delay the progression of chronic neurodegenerative diseases, acute neurological disorders, or even mental disorders. The major aim of this book is to focus on different approaches to achieve neuroprotection. In this book, there are chapters discussing imidazoline ligands and opioid ligands in Alzheimer’s disease, the beneficial effects of adenosine A2A receptor antagonist, adrenergic receptor agonists and antagonists modulating microglial responses, and different approaches to achieve neuroprotection against aging-associated macular degeneration. This book will give insights to scientists in the field to stimulate their research, medical professionals to review their clinical practices, and others who would like to learn more about different neuroprotective approaches.