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Chapter

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 β2-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: β2-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

these neurodegenerative disorders. Recent approaches found to be effective in the treatment of Parkinson's disease involve the use of anti-inflammatory agents and cytokines such as agonists to the β2-adrenergic receptors (β2-AR) to inhibit neuroinflammation and the progression of dopaminergic neurodegeneration. In this chapter, we will address the current understanding of therapeutic approaches targeting neuroinflammation linked with PD and the use of β2-AR agonists as an effective treatment for PD.

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, PGE_2, 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 neuro-protective 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 (IkappaB 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 produc-tion 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 mesence-phalic neuron-glia culture protects against LPS-induced neurotoxicity via suppres-sion 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 p47^{phox}, 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

Receptor Type	Tissue Distribution	Mechanism of Action	Agonist Potency	Physiological Effects	Agonist	Antagonist
α1	Vascular Smooth Muscles, Visceral smooth Muscles	Gq-protein coupled activates Phospholipase C, IP3+DAG	Epi ≥ NE >> Iso	Smooth muscle contractions, Gluconeogenesis, Vasoconstriction	Norepinephrine, Phenylephrine, Methoxamine	Doxazosin, Phentolamine, Prazosin
α2	Pre-synaptic terminals, pancreas, platelets, Ciliary epithelium, Salivary Glands	Gi-protein coupled inhibits Adenyl cyclase	Epi ≥ NE >>Iso	Inhibits release of Neurotransmitter	Clonidine, Monoxidine	Yohimbine, Idazoxan, Tolazoline
β1	Heart, Kidney, some pre-synaptic terminals	Gs-protein coupled activates Adenyl cyclase +PKA	Iso > Epi ≥ NE	Increase heart rate and Renin secretion	Isoproterenol, Norepinephrine, Dobutamine	Propranolol, Metoprolol, Atenolol
β2	Visceral smooth muscles, Bronchioles, Liver, Skeletal Muscles	Gs-protein coupled activates Adenyl cyclase +PKA, Ca-channels	Iso > Epi >> NE	Vasodilation, Bronchodilation, Inhibits insulin secretion	Isoproterenol, Salbutamol, Salmeterol, Albuterol, Formoterol, Terbutaline, Levalbuterol	Propranolol, ICI-118,551, Nadolol, Butoxamine
β3	Adipose Tissue	Gs-protein coupled activates Adenyl cyclase +PKA	Iso = NE > Epi	Increase lipolysis	Isoproterenol, Amibegron, Solabegron	SR59230A

NE: Norepinephrine, **Epi**: Epinephrine and **Iso**: Isoproterenol

Table 1.
Characteristics of adrenergic receptors.

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. General properties of β2-adrenergic receptors: a G-protein-coupled receptor

6.1 Structure

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

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/smoothened) [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, pamitoylation, 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 results 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 G_s-protein [61]. Finally, β2-ARs have 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 G_α-subunit dissociates from $G_{\beta\gamma}$-subunit which remains associated with plasma membrane and the G_α-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**.
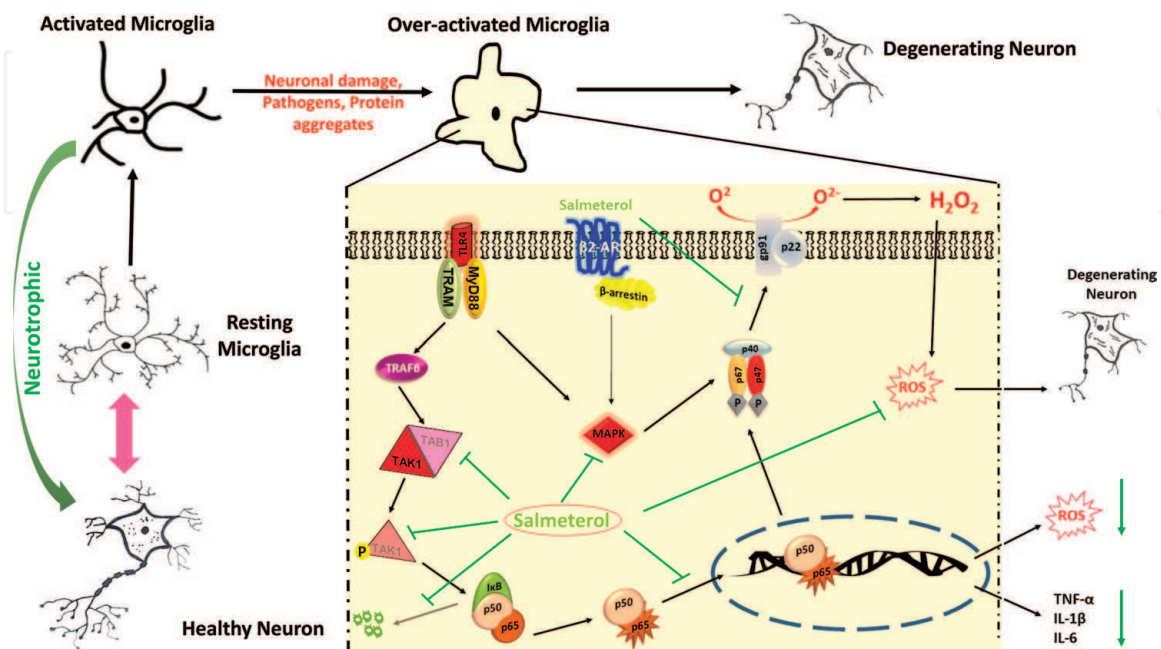

Figure 1.
Schematic of microglia-mediated neurotoxicity and inhibitory effects of Salmeterol on microglial activation.

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

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/IκBα 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* (*Pg*LPS), an oral pathogen as an *in vitro* model for the periodontal disease. We have found that Salmeterol shows similar anti-inflammatory effects on *Pg*LPS-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."

Author details

Monika Sharma[1] and Patrick M. Flood[2,3,4*]

1 Department of Medical Microbiology and Immunology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

2 Department of Dentistry, Neuroscience and Mental Health Institute, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

3 Department of Medical Microbiology and Immunology, Neuroscience and Mental Health Institute, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

4 School of Dentistry, Katz Group Centre for Pharmacy and Health Research, University of Alberta, Edmonton, Alberta, Canada

*Address all correspondence to: pflood@ualberta.ca

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