Serotonin 1A Receptors on Astrocytes as a Potential Target for the Treatment of Parkinson’s Disease

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Abstract: Astrocytes are the most abundant neuron-supporting glial cells in the central nervous system. The neuroprotective role of astrocytes has been demonstrated in various neurological disorders such as amyotrophic lateral sclerosis, spinal cord injury, stroke and Parkinson’s disease (PD). Astrocyte dysfunction or loss-of-astrocytes increases the susceptibility of neurons to cell death, while astrocyte transplantation in animal studies has therapeutic advantage. We reported recently that stimulation of serotonin 1A (5-HT₁A) receptors on astrocytes promoted astrocyte proliferation and upregulated antioxidative molecules to act as a neuroprotec-tant in parkinsonian mice. PD is a progressive neurodegenerative disease with motor symptoms such as tremor, bradykinesia, rigidity and postural instability, and with non-motor symptoms such as orthostatic hypotension and constipation based on peripheral neurodegeneration. Although dopaminergic therapy for managing the motor disability associated with PD is being assessed at present, the main challenge remains the development of neuroprotective or disease-modifying treatments. Therefore, it is desirable to find treatments that can reduce the progression of dopaminergic cell death. In this article, we summarize first the neuroprotective properties of astrocytes targeting certain molecules related to PD. Next, we review neuroprotective effects induced by stimulation of 5-HT₁A receptors on astrocytes. The review discusses new promising therapeutic strategies based on neuroprotection against oxidative stress and prevention of dopaminergic neurodegeneration.

Keywords: 5-HT₁A receptor, S100β, astrocyte, Parkinson’s disease, neuroprotection, dopaminergic neuron.

1. INTRODUCTION

Parkinson’s disease (PD) is a progressive neurodegenerative disease with motor symptoms such as tremor, bradykinesia, rigidity and postural instability that are based on selective loss of nigrostriatal dopaminergic neurons, and with non-motor symptoms such as orthostatic hypotension and constipation caused by peripheral neurodegeneration. The onset of motor features in PD is caused when dopamine (DA) terminal or DA content in the striatum is reduced below 50% or 60-70%, respectively [1, 2]. Although various treatments for PD are being assessed at present, the major is dopaminergic therapy for managing motor disability. Presently, some drugs such as D3 agonist pramipexole have reported their neuroprotective effects in parkinsonian models [3-5]. However, no conventional treatment has been demonstrated to slow or stop PD progression. Therefore, it is desirable to develop neuroprotective treatments that can prevent the dopaminergic neurodegeneration.

Astrocytes are abundant neuron-supporting glial cells and prone to improve dopaminergic neuronal survival by production of antioxidants, release of neurotrophic factors and uptake of potentially neurotoxic molecules, such as excess amount of glutamate in the synaptic space and extracellular α-synuclein aggregates [6-8]. Astrocytes have stronger antioxidative property than neurons [9] and can protect neurons against oxidative stress. Indeed, astrocyte-derived antioxidants reduce oxidative stress level in the surrounding neurons [10, 11]. Dysfunction of astrocytes is involved in increased susceptibility of neuronal cells, and astrocyte transplantation demonstrates therapeutic effects in several neurological disorders [12-17]. Taken together with previous reports, increasing the number of healthy
Astrocytes containing various antioxidative molecules could be a potential therapeutic approach to prevent the degenerative process of PD. We have recently shown that stimulation of serotonin 1A (5-HT1A) receptors on astrocytes promotes astrocyte proliferation and upregulates antioxidative molecules to act as a neuroprotectant in PD model mice [18]. In this article, we review the neuroprotective role of astrocytes in the brain and neuroprotective approaches targeting astrocytes for PD models, especially stimulation of 5-HT1A receptors on astrocytes. The review allows formulating a promising therapeutic strategy of neuroprotection against oxidative stress and progressive dopaminergic neurodegeneration.

2. NEUROPROTECTIVE PROPERTIES OF ASTROCYTES IN PARKINSON’S DISEASE

2.1. Role of Antioxidant Supply

2.1.1. Glutathione

Glutathione (GSH) is the most potent intrinsic antioxidant against reactive oxygen species (ROS). GSH is a tripeptide comprising the amino acids glutamate, cysteine and glycine. GSH is generated via a two-step reaction catalyzed by γ-glutamyl cysteine ligase (GCL) and GSH synthetase. Because intracellular glutamate and glycine are relatively sufficient, cysteine is the rate-limiting precursor for GSH synthesis. Since extracellular cysteine is readily auto-oxidized to cystine, cystine transport mechanisms are essential to supply a GSH substrate cysteine to cells. Cystine uptake is mediated by cystine/glutamate exchange transporter (xCT), which is expressed primarily on astrocytes, but not on neurons [19-21]. Astrocytes take up cystine via xCT, reduce it to cysteine to synthesize GSH, and consequently release it through the transporter multidrug resistance protein 1 (MRP1). Cysteine is generated from the extracellular thiol/disulfide exchange reaction of GSH and cystine, or generated by a peptidase from the dipeptide cysteinylglycine (CysGly), and then taken up by neighboring neurons for GSH synthesis [22-24]. Therefore, GSH synthesis in neurons is dependent on cystine uptake via xCT and GSH synthesis in astrocytes. Astrocytes are more resistant to oxidative stress than neurons, and GCL expression is upregulated following exposure of astrocytes, but not neurons, to oxidative stress [42]. In this regard, a previous study demonstrated that the conditioned medium of cultured astrocytes (glia conditioned medium: GCM) obtained from parkin-null mice had less neuroprotectives and contained lower levels of GSH than those from wild-type animals [43]. Furthermore, we demonstrated that zonisamide, a novel antiparkinsonian agent used in Japan, increased the expression of xCT and GSH levels in the activated striatal astrocytes and showed neuroprotective effects against dopaminergic neurodegeneration in parkinsonian mice [44]. Zonisamide (1,2-benzisoxazole-3-methanesulfonamide) was originally synthesized as an antiepileptic agent in Japan, and used clinically not only in Japan but also in South Korea, the United States and Europe. In addition, it was reported that the combination of zonisamide and levodopa improved motor impairments in PD patients [45-47]. Since 2009, zonisamide has been used as a novel antiparkinsonian agent in Japan. We reported for the first time that treatment with zonisamide upregulated xCT expression and GSH synthesis in astrocytes and prevented dopaminergic

active quinones, such as DA quinones or DOPA quinones, are produced by spontaneous oxidation of free cytosolic DA outside the synaptic vesicle in dopaminergic neurons [29-32]. The generated DA quinones can covalently react with the sulfhydryl residues on functionally important proteins in the pathogenesis of PD, e.g., tyrosine hydroxylase (TH), DA transporter (DAT) and parkin to form quinoproteins, and cause the dysfunction of these proteins [33-35]. Therefore, the pathogenic effects of DA quinone have been focused as dopaminergic neuron-specific oxidative stress. In our previous study, it is reported that repeated treatment with levodopa increased DA turnover and quinoprotein formation specifically in the striatum of parkinsonian model mice [36, 37]. The sulfhydryl groups of free cysteine in GSH and thiol reagents compete with the sulfhydryl group on cysteine in functional proteins to prevent the formation of quinoprotein [29, 33, 34, 38-40]. Therefore, GSH acts as an antioxidant by quenching not only general ROS but also DA quinones [41]. Thus, GSH can be regarded as an important neuroprotectant especially in dopaminergic neurons. However, it is difficult to maintain an effective concentration of GSH against quinone toxicity in the brain, because the small peptides can be resolved easily before crossing the blood-brain barrier. As mentioned above, GSH synthesis in neurons is dependent on cystine uptake via xCT and GSH synthesis in astrocytes. Astrocytes are more resistant to oxidative stress than neurons, and GCL expression is upregulated following exposure of astrocytes, but not neurons, to oxidative stress [42]. In this regard, a previous study demonstrated that the conditioned medium of cultured astrocytes (glia conditioned medium: GCM) obtained from parkin-null mice had less neuroprotectives and contained lower levels of GSH than those from wild-type animals [43]. Furthermore, we demonstrated that zonisamide, a novel antiparkinsonian agent used in Japan, increased the expression of xCT and GSH levels in the activated striatal astrocytes and showed neuroprotective effects against dopaminergic neurodegeneration in parkinsonian mice [44]. Zonisamide (1,2-benzisoxazole-3-methanesulfonamide) was originally synthesized as an antiepileptic agent in Japan, and used clinically not only in Japan but also in South Korea, the United States and Europe. In addition, it was reported that the combination of zonisamide and levodopa improved motor impairments in PD patients [45-47]. Since 2009, zonisamide has been used as a novel antiparkinsonian agent in Japan. We reported for the first time that treatment with zonisamide upregulated xCT expression and GSH synthesis in astrocytes and prevented dopaminergic
neurodegeneration in parkinsonian mice [44]. Taken together with these observations, the cystine uptake system and consequent GSH synthesis in astrocytes could be targets for dopaminergic neuroprotection in PD therapy.

2.1.2. Metallothionein

Metallothioneins (MTs) are low molecular weight and cysteine-rich proteins with antioxidative, anti-apoptotic, and anti-inflammatory properties. MTs bind to metals such as zinc, copper, and cadmium to function in metal homeostasis and detoxification [48]. MTs provide neuroprotection by regulating zinc-mediated transcriptional activation of genes involved in growth, proliferation, and differentiation. In the mammalian tissue, MT family comprises four isoforms: MT-1, MT-2, MT-3, and MT-4. MT-3 is a predominantly brain-specific isoform, which is expressed in neurons and stimulated glial cells [49]. On the other hand, the two abundant isoforms, MT-1 and -2 (MT-1/-2), are expressed in most organs including the brain. MT-1/-2 isoforms have radical-scavenging property based on their abundant thiol groups, which form metal-thiolate clusters [48, 50, 51]. Therefore, MT-1/-2 isoforms play an important role in the regulation of metal homeostasis in the brain and neuroprotection in various pathological and inflammatory states [48, 52]. We reported previously that MT-1 quenched DA semiquinones in vitro, and that MT-1/-2 protected dopaminergic neurons against DA quinone toxicity both in vitro and in vivo [53], suggesting that MTs can bind DA quinones by the sulphydryl groups of their rich cysteines. MTs also inhibit Charnoly body (CB) formation, which is a pleomorphic, multi-lamellar, electron-dense stack of degenerated membranes formed in the most vulnerable cells due to compromised mitochondrial bioenergetics in severe malnutrition and free radical overproduction by their antioxidative actions [54-56]. Furthermore, MTs attenuate peroxynitrite-induced oxidative and nitritative stress to reduce α-synuclein index and 8-hydroxy-2'-deoxyguanosine accumulation to provide mitochondrial protection and prevent CB formation involved in Lewy body pathogenesis and various other neurodegenerative α-synucleinopathies [57-59]. Although MT-1/-2 can protect neurons directly, they are not mainly expressed in neurons in the normal or injured state [60, 61]. There are a number of evidences demonstrating that MT-1/-2 are produced primarily by astrocytes [62-73], and that extracellular MTs secreted from astrocytes mediate neuronal survival and axonal regeneration [60]. Michael et al. [64] reported upregulation of MT expression in reactive astrocytes of

![Role of antioxidant supply by astrocytes.](image)
the substantia nigra in PD patients, suggesting a neuroprotective role for dopaminergic neurons. Chung et al. [74] also reported that MT-1/-2 expression is specifically upregulated in astrocytes in response to neuronal injury. In our previous study, MT-1/-2 were produced by astrocytes in response to excess DA exposure, and extracellular MTs protected dopaminergic neurons against DA-induced neurotoxicity [75]. In addition, MTs modulate zinc availability for the zinc-binding enzymes H2O2 through oxidation of its Cys106-sulfenic acid to protect neurons against oxidative stress [85, 86]. Kitamura et al. [87] screened DJ-1-binding compounds and demonstrated neuroprotective effects of compound-23, N-[4-(8-methyl(4-hydroimidazo[1,2-a]pyridin-2-yl)phenyl)](3,4,5-trimethoxyphenyl)carboxamide, against ROS-induced neurotoxicity in parkinsonian and ischemic animal models. DJ-1 is mainly expressed in astrocytes in the human brain and acts as a sensor of oxidative stress [88]. Reactive astrocytes enhance DJ-1 expression in the case of oxidative stress and release DJ-1 protein extracellularly to protect neurons [89]. Several studies demonstrated that DJ-1 deficiency or mutation in astrocytes impairs astrocyte-mediated neuroprotection in parkinsonian models [90-92]. Clements et al. [93] reported that Nrf2 protein without intact DJ-1 is unstable and its basal or inducible levels of transcriptional responses are decreased. These observations suggest that DJ-1 stabilizes antioxidant transcriptional regulation of Nrf2. As described above, because Nrf2 is a key molecule in antioxidative pathway in living cells, modulation of DJ-1 expression in astrocytes could be a suitable target to secure neuroprotection. Furthermore, DJ-1 is identified as a critical molecule for mitochondrial function [94, 95]. In the presence of oxidative environment, DJ-1 plays an important role to maintain mitochondrial function as well as PINK1/parkin pathway [95].

2.1.4. DJ-1

DJ-1 (PARK7) gene deletions and point mutations have been identified as one of the causes of early-onset autosomal recessive PD (AR-PD) [84]. DJ-1 has various functions related to PD pathogenesis. DJ-1 scavenges H2O2 through oxidation of its Cys106-sulfenic acid to protect neurons against oxidative stress [85, 86]. Kitamura et al. [87] screened DJ-1-binding compounds and demonstrated neuroprotective effects of compound-23, N-[4-(8-methyl(4-hydroimidazo[1,2-a]pyridin-2-yl)phenyl)](3,4,5-trimethoxyphenyl)carboxamide, against ROS-induced neurotoxicity in parkinsonian and ischemic animal models. DJ-1 is mainly expressed in astrocytes in the human brain and acts as a sensor of oxidative stress [88]. Reactive astrocytes enhance DJ-1 expression in the case of oxidative stress and release DJ-1 protein extracellularly to protect neurons [89]. Several studies demonstrated that DJ-1 deficiency or mutation in astrocytes impairs astrocyte-mediated neuroprotection in parkinsonian models [90-92]. Clements et al. [93] reported that Nrf2 protein without intact DJ-1 is unstable and its basal or inducible levels of transcriptional responses are decreased. These observations suggest that DJ-1 stabilizes antioxidant transcriptional regulation of Nrf2. As described above, because Nrf2 is a key molecule in antioxidative pathway in living cells, modulation of DJ-1 expression in astrocytes could be a suitable target to secure neuroprotection. Furthermore, DJ-1 is identified as a critical molecule for mitochondrial function [94, 95]. In the presence of oxidative environment, DJ-1 plays an important role to maintain mitochondrial function as well as PINK1/parkin pathway [95].

2.1.5. Parkin

Parkin (PARK2) is one of the genes responsible for AR-PD and exerts E3 ubiquitin ligase [96, 97]. Parkin forms a complex with PINK1 and DJ-1, and promotes degradation of unfolded or misfolded proteins [98]. Recently, parkin and PINK1 have been identified as essential proteins for the removal of damaged mitochondria through autophagy (mitophagy) [99-101]. Therefore, parkin deficiency induces aberrant ubiquitination and compromised mitochondrial integrity, leading to neuronal dysfunction and degeneration. LaVoie et al. [34] demonstrated that DA quinone covalently modifies parkin protein in living dopaminergic cells to inactivate ubiquitin ligase function of parkin. Together,
these findings indicate that dopaminergic neurons are more vulnerable because DA quinone can induce mitochondrial dysfunction. Constitutive parkin expression is especially higher in neurons than astrocytes. However, astrocytes but not hippocampal neurons increase parkin expression during unfolded protein stress [102]. It is demonstrated that knock-down of parkin causes impairment of GSH synthesis in astrocytes and shows neurodegenerative pathogenesis of parkin-linked AR-PD [43]. Therefore, it is suggested that parkin dysfunction in astrocytes produces oxidative stress environment in the brain. Furthermore, it was reported recently that parkin mutation in the fly results in severe motor impairment and shortened life span, and that these phenotypes are rescued by up-regulation of metal-responsive transcription factor (MTF-1) [103]. MTF-1 regulates transcription of its target genes by binding to metal response elements. MTF-1 is also known to promote the gene expression of MT in response to oxidative stress and inflammation [104, 105]. Indeed, MT overexpression in a parkin mutant background could improve the motor impairment of parkin mutants [103]. These suggest that modulating parkin expression in astrocytes could be a useful therapeutic strategy in PD.

### 2.2. Release of Neurotrophic Factors

Neurotrophic factors are secreted proteins, which trigger survival pathways upon binding to their target receptors to prevent neuronal loss. Astrocytes secrete neurotrophic factors, such as glial cell line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and basic fibroblast growth factor (bFGF). Novel neurotrophic factors have been identified, such as cerebral dopamine neurotrophic factor (CDNF) and its homolog mesencephalic astrocyte-derived neurotrophic factor (MANF). In this section, we will focus on some molecules that are closely related to dopaminergic neurons.

#### 2.2.1. GDNF

GDNF is a member of the transforming growth factor-β superfamily [106]. In 1993, Lin et al. [107] identified GDNF as a potent neurotrophic factor, which enhances survival of mesencephalic dopaminergic neurons. Earlier studies described GDNF to specifically promote the survival of primary cultured mesencephalic DA neurons from rat embryos and a potent and selective stimulator of DA uptake and neurite outgrowth in TH-positive DA neurons [107-109].

#### 2.2.2. bFGF

Basic FGF that was initially identified as a mitogen with angiogenic properties, is produced mainly by astrocytes and promotes the growth and survival of dopaminergic neurons [110-113]. Engele and Bohn [114] reported that mesencephalic astrocytes provide neurotrophic bFGF for central dopaminergic neurons. Recently, it has been revealed that upregulation of bFGF level in endogenous astrocytes of the substantia nigra enhances dopaminergic differentiation of transplanted stem cells in parkinsonian mice [115]. Interestingly, mesencephalic neurons show enhanced growth and survival when they are cultured with astrocytes. The bFGF from astrocytes might be partly involved in such neurotrophic effects of astrocytes on dopaminergic neurons.

#### 2.2.3. CDNF and MANF

CDNF [116] and MANF [117] are novel but evolutionarily conserved neurotrophic factors. Both factors are found in vertebrates, while a single homolog is present in the invertebrates [118, 119]. These two proteins contain eight conserved cysteine residues, which determine the protein conformation. CDNF and MANF consist of two domains; an N-terminal saposin-like domain interacting with lipids or membranes, and an unfolded C-terminal domain related to protection against endoplasmic reticulum stress. MANF, which is identified in the culture medium of rat type-1 astrocyte ventral mesencephalic cell line 1 (VMCL1), provides protection for embryonic mesencephalic DA neurons in vitro [117]. MANF and CDNF are the most potent factors that can provide protection and enhance the repair of dopaminergic neurons in parkinsonian model rats [120, 121].

### 2.3. Clearance of Potentially Neurotoxic Molecules

#### 2.3.1. Aggregated α-Synuclein

α-Synuclein that consists of 140 amino acids is mainly found in the presynaptic nerve ends under normal physiological conditions. This protein is reported to be involved in modulating synaptic vesicle function in cooperation with synapsin III [122]. The aggregated and insoluble fibrillar form of α-synuclein constitutes a major component of Lewy bodies or Lewy neurites in neurodegenerative diseases, such as PD, dementia with Lewy bodies and multiple system atrophy [123, 124]. Although it is still controversial whether Lewy bodies initiate neurodegeneration or damaged neurons form Lewy bodies for self-defense, it is generally accepted that the oligomeric and fibrillar α-synuclein species are
 responsible for the toxicity, and oligomeric α-synuclein is more toxic than the larger aggregated forms. α-Synuclein is a cytosolic protein, but it can be released from neuronal cells into the surrounding extracellular space via exocytosis [125]. Although the structure of the released α-synuclein is unknown, several studies report that extracellular α-synuclein contains aggregated forms and that misfolding and aggregation facilitate the release of α-synuclein from neuronal cells [126]. The uptake of extracellular α-synuclein occurs via endocytosis in neurons and glial cells [127]. α-Synuclein is degraded in the lysosome of astrocytes, suggesting that astrocytes exert dopaminergic neuroprotection by clearing excess extracellular toxic α-synuclein [128]. Indeed, Lee and colleagues [129] demonstrated that α-synuclein can be propagated from neuron to neuron and from neurons to astrocytes [6]. All the major brain cell types, neurons and astrocytes, are able to clean extracellular α-synuclein aggregates by internalization and degradation [128].

2.3.2. Damaged Mitochondria

As mentioned above, damaged mitochondria are removed through endocytosis to maintain the quality of mitochondria [99-101]. Such maintenance is important not only for proper bioenergetic function, but also to prevent the release of ROS followed by cell death. Mitochondria degradation is generally thought as a cell-autonomous process. Recently, it is reported that retinal ganglion cell axons of mice shed mitochondria at the optic nerve head, and that these mitochondria are internalized and degraded by surrounding astrocytes [130]. The authors also demonstrated structurally similar accumulations of degrading mitochondria along neurites in superficial layers of the cerebral cortex. While it is unclear whether dopaminergic neurons also use this transcellular endocytosis as a primary mitochondrial quality control mechanism, the degradation of neuron-derived mitochondria in astrocytes is a notable phenomenon as a target for neuroprotection.

3. SEROTONIN 1A (5-HT1A) RECEPTORS ON ASTROCYTES AS A NOVEL TARGET OF NEUROPROTECTION

3.1. Pharmacological Function of 5-HT1A Receptors

The 5-HT1A receptor, which is one of 14 subtypes (5-HT1A/B/D/E/F, 5-HT2A/B/C, 5-HT3, 5-HT4, 5-HT5A/B, 5-HT6 and 5-HT7), is a G-protein-coupled receptor with a 7-transmembrane-spanning structure. The 5-HT1A receptor is a key mediator of serotonergic signaling in the CNS. The serotonergic system plays an important role in various physiological and behavioral functions such as mood- and anxiety-related behavior, cognitive function, food intake, sexual behavior, sleep and body temperature. Therefore, considerable attention has been focused on the 5-HT1A receptors. The 5-HT1A receptors function both at pre- and postsynaptic (autoreceptor) and postsynaptic (heteroreceptor) sites. Activation of 5-HT1A autoreceptors on the cell bodies of serotonergic neurons in the raphe nucleus exerts inhibitory feedback in response to local release of serotonin. The 5-HT1A heteroreceptors are expressed in limbic regions such as prefrontal cortex, hippocampus, lateral septum, and amygdala, as well as in thalamus, hypothalamus and basal ganglia [131, 132]. Activation of 5-HT1A heteroreceptors reduces postsynaptic neuronal firing and excitation. To date, 5-HT1A receptors have been considered as a target for the action of antidepressants [133, 134]. Recently, several studies reported new insights into the therapeutic function of 5-HT1A receptors in treating schizophrenia and Parkinson’s disease [135, 136]. Stimulation of 5-HT1A receptors improves cognitive deficits in patients with schizophrenia possibly by normalization of lactate metabolism in the prefrontal cortex [137]. Furthermore, 5-HT1A agonists are expected to improve not only psychiatric symptoms such as anxiety and depression, but also L-DOPA-induced side effects of dyskinesia [138-142]. These pharmacological actions are mainly mediated by neuronal 5-HT1A receptors. On the other hand, we have demonstrated the expression of 5-HT1A receptors in striatal astrocytes, and stimulation of the receptors exerts neuroprotection against dopaminergic neurotoxicity [18].

3.2. Stimulation of 5-HT1A Receptors Enhances Astrocyte Proliferation via S100β Secretion

Astrocyte proliferation is promoted by S100β protein, which is released by astrocytes and affects in the autocrine fashion [143-146]. S100β is a small EF-hand Ca2+-binding protein. This protein is expressed in various cell types such as astrocytes, oligodendrocytes, renal epithelial cells, and neural progenitor cells. The highest level of S100β expression is found in the cytoplasm of astrocytes [145, 146], and astrocytes secret the protein to the extracellular space. Extracellular S100β has various functions and affects on not only astrocytes but also neurons. S100β has opposite effects on neurons depending on its concentration. Nanomolar levels of S100β protect neurons from stress-induced apoptosis [147], stimulate neurite outgrowth and microtubule associated protein2 (MAP2) expression [148, 149] and modulate long-term neuronal plasticity [150]
(Fig. 2). On the other hand, exposure to micromolar levels of S100β increases β-amyloid neurotoxicity [151] and causes neuronal apoptosis [152]. In addition, extracellular S100β at low dose enhances glutamate uptake activity of astrocytes, which plays essential roles in regulating extracellular levels of glutamate [153-155] (Fig. 2). Tramontina et al. [154, 155] provided direct evidence for the stimulatory effect of extracellular S100β on glutamate uptake into astrocytes by addition of S100β protein, and that anti-S100β antibody decreased glutamate uptake without affecting cell integrity or viability. It is also reported that epicatechin gallate, which is an abundant polyphenol in green tea, induces glutamate uptake and S100β secretion in astroglial C6 cells [153]. These results suggest that S100β secretion by epicatechin gallate may be associated with the improvement of glutamate uptake. Other studies demonstrated that extracellular S100β could protect hippocampal neurons against glutamate-induced damage [147, 156]. These findings reinforce the importance of astrocytes in the neuroprotective role of S100β against excitotoxic damage. Astrocytes seem to secrete S100β to the extracellular space to reduce the excitotoxicity (Fig. 2).

Extracellular S100β has autocrine effects that promote astrocyte proliferation [145, 146] (Fig. 2). It is previously reported that stimulation of astrocytic 5-HT_{1A} receptors results in the release of S100β protein from astrocytes [143, 144]. Recent studies from our laboratory also demonstrated the significant increase in S100β levels in media of striatal astrocytes treated with a full 5-HT_{1A} agonist (R)-(−)-8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH-DPAT) [18]. Since stimulation of 5-HT_{1A} receptors on astrocytes induced the release of S100β protein from astrocytes [144] and extracellular S100β enhanced astrocyte proliferation [145, 146], it is expected that 5-HT_{1A} agonists could promote astrocyte proliferation. In this regard, a previous study demonstrated that buspirone, a partial 5-HT_{1A} agonist, increased the number of cultured astrocytes [157]. In a more recent study, we demonstrated that 8-OH-DPAT (1-10 nM) promoted astrocyte proliferation via secretion of S100β [18]. The enhancement of astrocyte proliferation was also observed in the striatum of mice after repeated administration of 8-OH-DPAT (0.05, 0.1 mg/kg, i.p. for 7 days). However, relatively high doses of 8-OH-DPAT (>10 nM) on astroglial C6 cells or 8-OH-DPAT administration (0.5 mg/kg for 7 days) in mice had no effects on S100β secretion or astrocyte proliferation [18]. As stated above, S100β can induce astrocyte proliferation at low-to-medium dose, but not at high dose [146]. Although the mechanism in lack of promotive effects of 8-OH-DPAT at higher dose is obscure, these previous reports suggest that there is effective window of doses to induce astrocyte proliferation by stimulation of 5-HT_{1A} receptors.

3.3. 5-HT_{1A} Agonist Produces Nrf2 Activation

Nrf2 is normally sequestered in the cytoplasm and degraded in the proteosome through its regulatory protein Keap1, but can translocate into the nucleus and consequently activate various antioxidant molecules upon exposure to oxidative stress [79, 158]. Therefore, Nrf2 activation has recently received attention as a new therapeutic strategy to protect against dopaminergic neurotoxicity induced by oxidative stress. We demonstrated that Nrf2 in astrocytes is activated after excess amount of DA exposure as dopaminergic oxidative stress [75]. We also recently reported that 5-HT_{1A} agonist 8-OH-DPAT produced Nrf2 activation in astrocytes [18]. Expression of nuclear Nrf2 was significantly increased in these astrocytes 6 h after 8-OH-DPAT exposure, and this up-regulation was suppressed by the 5-HT_{1A} antagonist. Furthermore, we have also demonstrated that 8-OH-DPAT can increase the binding activity of Nrf2 to ARE of MT-1 promoter region followed by up-regulation of MTs in astrocytes. These finding suggest that 8-OH-DPAT induces nuclear translocation of Nrf2 followed by binding to ARE prior to MT induction. Further studies are needed to determine the mechanism of 8-OH-DPAT-induced Nrf2 nuclear translocation in astrocytes.

3.4. Stimulation of Astrocytes 5-HT_{1A} Receptors Protects Dopaminergic Neurons

As mentioned above, stimulation of 5-HT_{1A} receptors on astrocytes promotes astrocyte proliferation and Nrf2 activation, suggesting up-regulation of antioxidative pathways in astrocytes. Therefore, it is expected that 5-HT_{1A} agonists can protect against oxidative stress-induced dopaminergic neurodegeneration. Indeed, we have demonstrated that stimulation of 5-HT_{1A} receptors on astrocytes by 8-OH-DPAT could protect dopaminergic neurons by using primary cultured neuron-astrocyte mixed cells and parkinsonian mice [18]. The conditioned media from 8-OH-DPAT-treated striatal astrocytes conferred significantly higher protection against dopaminergic neurotoxicity, compared with media from astrocytes free of 8-OH-DPAT. In addition, such neuroprotection was completely blocked by 5-HT_{1A} antagonist WAY100635. The levels of nuclear Nrf2 expression and MT-1 secreted from astrocytes were increased after 8-OH-DPAT treatment, and these
effects were also inhibited by the 5-HT1A antagonist. Furthermore, the neuroprotection was cancelled in the presence of anti-MT-1/-2 antibody. These results suggest that stimulation of 5-HT1A receptors on striatal astrocytes can upregulate and secrete MTs by astrocytes to protect dopaminergic neurons against neurodegeneration. In another series of studies, we also demonstrated that 8-OH-DPAT administrations (0.1 mg/kg for 7 days) prevented gradual degeneration of dopaminergic neurons in parkinsonian mice induced by intrastriatal injection of 6-hydroxydopamine [18]. Several experiments have also demonstrated the protective effects of 5-HT1A agonists against neuronal damage [147, 159, 160]. For example, 5-HT1A agonist, Bay x 3702, protected neurons against glutamate- or staurosporine-induced neurotoxicity by secretion of S100β from striatal astrocytes [147]. Another 5-HT1A agonist, BAY 639044, ameliorated MPTP toxicity in mice and macaque models of PD [159]. In these reports, the presumed mechanism of neuroprotection by 5-HT1A agonists relates to a reduction in glutamate release and subsequent excitotoxicity. In another experiment, 8-OH-DPAT and (R)-5-fluoro-8-hydroxy-2-(dipropylamino)-tetralin (R-UH-301) attenuated NMDA- and MPP⁺-induced apoptotic cell death in striatal and mesencephalic cell cultures [160]. The authors interpreted that the mechanism of neuroprotection of 5-HT1A agonists was attenuation of NMDA-induced Ca²⁺ influx and prevention of apoptotic cascade in neurons (Table 1).

In these days, various approaches using 5-HT1A agonists target mainly motor fluctuation in PD [138-142, 161-167] (Table 1). Eltoprazine, a selective 5-HT1A and 5-HT1B agonist, is currently being tested in clinical trials for motor complications derived from dopaminergic therapy in PD [140, 141]. The dopamine D₂ and 5-HT1A dual-agonist, N-propynlororphin-11-yl 5-(1,2-dithiolan-3-yl)pentanoate, significantly reduced L-dopa-induced dyskinesia in parkinsonian rats [142]. In addition, previous studies demonstrated that 8-OH-DPAT also reduced dyskinesia and improved movement in parkinsonian animals [138, 139]. Almost all 5-HT1A agonists are thought to affect on neurons, and it is unclear whether these drugs affect on 5-HT1A receptors on astrocytes. Nevertheless, the treatment with 5-HT1A agonists is useful for L-dopa-induced side effects in PD (Fig. 3). On the other hand, neuroprotective or disease-modifying treatments are clinically required to prevent or delay disease progression in PD. Based on the neuroprotective property of astrocytes, many researchers have focused to astrocytes as a target for therapy in neurodegenerative diseases. Some recent studies have demonstrated that astrocyte transplantation produces neuronal repair in amyotrophic lateral

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**Fig. (2).** Function of extracellular S100β. S100β has an effect on neurons depending on its concentration. Nanomolar levels of S100β protect neurons from stress-induced apoptosis, stimulate neurite outgrowth and microtubule associated protein2 (MAP2) expression. Extracellular S100β exerts autocrine effects that promote astrocyte proliferation and glutamate uptake activity of astrocytes.
### Table 1. Summary of the effects of 5-HT_{1A} agonists on models or patients of Parkinson's disease

| 5-HT_{1A} agonist | Effectiveness | Targeted Cell | Reference |
|-------------------|---------------|---------------|-----------|
| Bay x 3702        | neuroprotection | astrocyte  | [147]    |
| BAY 639044        | neuroprotection | neuron     | [159]    |
| R-UH-301          | neuroprotection | neuron     | [160]    |
| 8-OH-DPAT         | neuroprotection | astrocyte  | [18]     |
| eltoprazine       | dyskinesia     | neuron      | [167]    |
| N-propylnoraporphin-11-y1,5-(1,2-dithiolan-3-yl)pentanoate | dyskinesia | neuron | [142]    |
| 8-OH-DPAT         | dyskinesia     | unknown    | [138]    |
|                    | improvement of motor fluctuation | unknown | [139]    |
|                    | anxiolytic effects | neuron | [162]    |
|                    | antidepressant-like effects | neuron | [163]    |
| F15599            | dyskinesia     | neuron      | [164]    |
| F13714            | dyskinesia     | neuron      | [165]    |
| F13640            | dyskinesia     | unknown    | [166]    |
|                    | anxiolytic effects | unknown | [166]    |
|                    | antidepressant-like effects | unknown | [166]    |
| buspirone         | dyskinesia     | neuron      | [161]    |

**Fig. (3).** Serotonin 1A (5-HT_{1A}) receptors on astrocytes as a novel target of neuroprotection. Stimulation of 5-HT_{1A} receptors on astrocytes promotes S100β secretion followed by astrocyte proliferation and Nrf2 activation to up-regulate expression of antioxidative molecules, such as glutathione (GSH) and metallothionein (MT). Therefore, the strategy of increasing the number of intrinsically healthy astrocytes through stimulation of 5-HT_{1A} receptors on astrocytes is conceivable and could help design new therapies that provide neuroprotection against oxidative stress and neurodegenerative disorders.
sclerosis, spinal cord injury, stroke and PD [12-17]. Therefore, the strategy of increasing the number of intrinsically healthy astrocytes through stimulation of 5-HT_{1A} receptors on astrocytes is conceivable and could help design new therapies that provide neuroprotection against oxidative stress and neurodegenerative disorders including PD.

At the present time, there is no validated imaging technology to detect neurodegeneration in PD patients. The clinical diagnosis of PD is based on the observation of motor symptoms, including tremor, bradykinesia, rigidity and postural instability, and 123I-metaiodobenzylguanidine (MIBG) cardiac scintigraphy. However, pathological process leading to PD can begin decades before developing these symptoms. One of the prerequisites is development of reliable diagnostic and prognostic biomarkers of early PD. Furthermore, objective reliable biomarkers to measure the progression of the disease are required to gauge the effectiveness of neuroprotective therapeutic approaches.

CONCLUSION

Astrocytes have various neuroprotective properties such as production of antioxidative or neurotrophic molecules and clearance of neurodegeneration-inducible molecules. Therefore, increasing the number of healthy astrocytes, which possess these neuroprotective ability, is a potentially viable therapeutic approach to prevent the degenerative process of PD. Stimulation of 5-HT_{1A} receptors on astrocytes promotes not only astrocyte proliferation via secretion of S100β but also Nrf2 activation leading to upregulation of antioxidative molecules, thus acting as a neuroprotectant in neurodegenerative disease. Development of pharmacological agents that can specifically target 5-HT_{1A} receptors on astrocytes could provide a promising therapeutic strategy of neuroprotection against progressive dopaminergic neurodegeneration.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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