Astrocytes protect dopaminergic neurons against aminochrome neurotoxicity

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Abstract

Astrocytes protect neurons by modulating neuronal function and survival. Astrocytes support neurons in several ways. They provide energy through the astrocyte-neuron lactate shuttle, protect neurons from excitotoxicity, and internalize neuronal lipid droplets to degrade fatty acids for neuronal metabolic and synaptic support, as well as by their high capacity for glutamate uptake and the conversion of glutamate to glutamine. A recent reported astrocyte system for protection of dopamine neurons against the neurotoxic products of dopamine, such as aminochrome and other *o*-quinones, were generated under neuromelanin synthesis by oxidizing dopamine catechol structure. Astrocytes secrete glutathione transferase M2-2 through exosomes that transport this enzyme into dopaminergic neurons to protect these neurons against aminochrome neurotoxicity. The role of this new astrocyte protective mechanism in Parkinson´s disease is discussed.

Key Words: aminochrome; astrocytes; dopamine; dopaminergic neurons; exosomes; glutathione transferase M2-2; neuroprotection; Parkinson's disease

Introduction

The loss of dopaminergic neurons containing neuromelanin from the nigrostriatal system is one of the most important events in the generation of motor symptoms in Parkinson's disease. The question is why these neuromelanin-loaded neurons degenerate in Parkinson's disease and not in healthy older adults who keep these neurons intact until death. For some time it has been argued that astrocytes play a fundamental role in the protection of neurons in general. Neuromelanin synthesis involves the generation of ortho-quinones that can be neurotoxic. Therefore, the objective of this review is to analyze the protective mechanisms that prevent the loss of dopaminergic neurons loaded with neuromelanin in healthy older adults and the role of astrocytes.

Search Strategy and Selection Criteria

Studies cited in this review published from 2000 to 2020 were searched on the PubMed database using the following keywords: astrocytes neuroprotection, dopamine neurons degeneration, neuromelanin synthesis, dopamine derived ortho-quinones, aminochrome, alpha-synyclein, mechanism involved in dopaminergic neurons degeneration, neuroprotective mechanisms against aminochrome-induced neurotoxicity, endogenous neurotoxins in dopaminergic neurons.

Metabolic Coupling between Astrocytes and Neurons

Astrocyte-neuron lactate shuttle

Astrocytes protect neurons by modulating neuronal function and survival. Astrocytes support the energy requirement of neurons via the astrocyte-neuron lactate shuttle, where lactate is a precursor of glucose in gluconeogenesis (Tarczyluk et al., 2013). Lactate transporters play an important role for preservation of myelin and axon function, functional recovery following nerve injuries, motor end-plate function, and the progress of diabetic peripheral neuropathy. Brain functions and energy metabolism are also dependent on lactate interactions between neurons and glia cells (Jha et al., 2020). Recently, a study of proteins involved in the astrocyte-neuron lactate shuttle (Ldha, Atp1a2, Gys1, Ldhb, Mct1, and Pfkfb3) revealed that Pfkfb3 and Atp1a2 genes, expressing fructose-2,6-biphosphatase 3/6-phosphofructo-2-kinase and the Na^+/K^+ transporting ATPase, respectively, were predominantly expressed by astrocytes (Margineanu et al., 2020). The most extensively supported idea of brain energy metabolism is the lactate shuttle between the astrocyte and the neuron. However, there is evidence that astrocyte and neuronal plasma membrane receptors, such as potassium ATP-channel, and GLUT2, perform a crucial role in brain glucose metabolism (Welcome and Mastorakis, 2018). Thus, the astrocyte-neuron lactate shuttle during activation, neurotransmission, and memory consolidation is an issue of controversy (Dienelet, 2019; **Figure 1**).

The close relationship between neurons and astrocytes can also be illustrated during an acute increase in interstitial K^+ where neuronal activity couples neuron energy demand to astrocytes metabolism. An increase in K^+ induces inhibition of mitochondrial respiration, activation of aerobic glycolysis, and the release of lactate. The glucose transporter GLUT1 in astrocytes is modulated by extracellular K^+ that regulates the efflux and influx modes of this transporter. The regulation of astrocytes GLUT1 by K^+ explains how astrocytes can preserve their glucose pool in the face of robust glycolysis stimulation. The effect of increased K^+ on astrocytes results in release of lactate after activation of aerobic glycolysis, and inhibition of mitochondrial respiration (Fernández-Moncada, 2021).

Lipid degradation

Another important interrelation between astrocytes and neurons concerns lipid degradation. Lipid droplets accumulate fatty acids as energy rich reservoirs. Astrocytes internalize neuronal lipid

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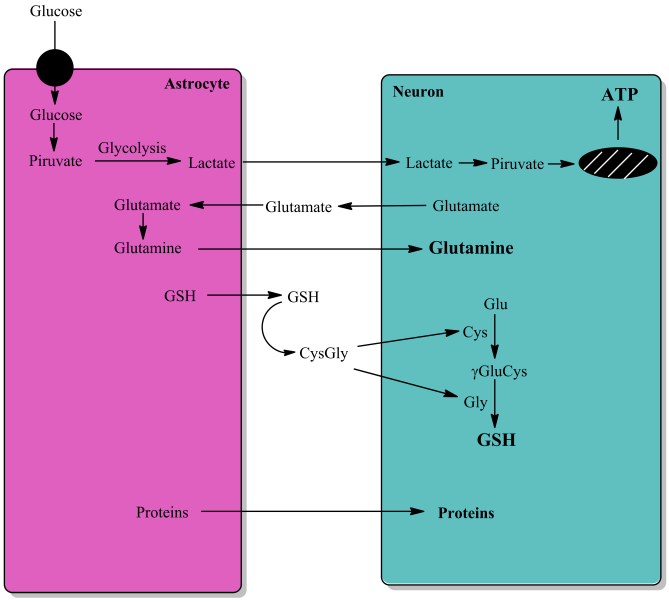

Figure 1 | **Astrocyte-neuron interactions.**
The interaction between astrocytes and neurons is very important for neuron function and survival. Astrocytes convert glucose to lactate that it is transported to neuron with aim to generate energy (ATP). Astrocyte take up glutamate released by neurons to convert it to glutamine by astrocytic expressed glutamine synthetase, and neurons take up glutamine. Astrocytes secrete GSH that it is converted to Cys-Gly, which is converted to cysteine and glycine. Neurons take up both cysteine and glycine that are used in the synthesis of GSH. Astrocytes also secret a large number of proteins, including GSTM2, that neurons take up. Unpublished data. ATP: Adenosine triphosphate; Cys: L-cysteine; CysGly: L-cysteinyl-glycine; GSH: reduced tripeptide composed of L-glutamine, L-cysteine, and glycine; γGluCys: gamma-glutamyl-cysteine.

droplets to eliminate lipids and degrade fatty acids to give neuronal metabolic and synaptic support. Apolipoprotein E reduces lipid droplets internalization into astrocytes, and reduces fatty acid oxidation, resulting in lipid accumulation in astrocytes (Qi, 2021). In this way, neurotoxic fatty acids generated in excessive active neurons are transported by apolipoprotein E-positive lipid particles into astrocytes as lipid droplets, where mitochondrial β-oxidation of the fatty acids deposited in lipid droplets induce the expression of lipid detoxification genes (Ioannou et al., 2019). Astrocytes expressing apolipoprotein E4 accumulate more and smaller lipid droplets in comparison to astrocytes expressing apolipoprotein E3. Astrocytes expressing apolipoprotein E4 present a diminished uptake of palmitate, decreased oxidation of palmitate and oleate, higher dioxygen consumption during fatty acid oxidation, more accumulation of lipid droplets-derived metabolites because of incomplete oxidation, and are more sensitive to carnitine palmitoyltransferase-1 inhibition (Farmer et al., 2019).

Medium-sized side chain fatty acids generated from dietary triglycerides penetrate the blood-brain barrier and can be used as an alternative energy source or activate related signaling functions in astrocytes. Decanoic acid, a saturated medium-sized side chain fatty acid and a ligand of Gαs protein-coupled receptors, is a signaling molecule in energy metabolism in astrocytes. Decanoic acid induces the synthesis of cAMP and the release of lactate, as well as the synthesis of GABA and its release from astrocytes, modulating neighboring neurons (Lee et al., 2018).

Glutamate-glutamine shuttle

Astrocytes have higher capacity for glutamate uptake than neurons after synaptic release of glutamate from glutamatergic neurons. Astrocytic glutamine synthetase catalyzes the formation of glutamine from glutamate taken up by astrocytes. Neurons, in turn, take up glutamine synthetized in astrocytes, which can be a mechanism to replenishment glutamate lost in oxidative metabolism (Schousboe, 2020). A protective mechanism that involves astrocyte-neuron coupling has been proposed: Astrocyte activation is required for memantine protective effects against okadaic acid, a strong PP2A and PP1 inhibitor, which induces cognitive decline. Memantine enhanced the liberation of S100B protein and reduced glutamate levels in the hippocampus and in the cerebrospinal fluid (Torrez et al., 2019).

Astrocytes are functionally and metabolically coupled to neurons by the uptake and recycling of neurotransmitters, which interconnects the function and metabolism of astrocytes and neurons. Astrocytes also secrete metabolites, such as lactate, glutamate, glutamine, adenosine triphosphate, D-serine, and regulate plasticity by pre- and postsynaptic mechanisms and synaptic activity (Mayorquin et al., 2018).

Neuroprotection

Astrocytes express Kir6.1, a principal pore unit of the K-ATP channel that is not found in neurons. In the presence of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), the Kir6.1 knockout mouse exhibited a lower concentration of striatal dopamine, increased dopamine neuron degeneration in substantia nigra compacta, and increased severe motor dysfunction, in comparison with control mice. Astrocytic Kir6.1 knockout also impaired mitophagy, which resulted in the accumulation of injured mitochondria, neuro inflammation and generation of free radicals in astrocytes. These results suggest that the astrocytic Kir6.1/K-ATP channel is neuroprotective to dopaminergic neurons (Hu et al., 2019). In addition, astrocytes protect neurons from excitotoxicity by activating the G protein-coupled receptor 30, an estrogen membrane receptor (Wang et al., 2020).

A study focused on the effect of Gq and Gi/o G protein-coupled receptors on astrocytes Ca^{2+}-based activity, on neuronal electrical activity, and gliotransmitter release, revealed that Gi/o G protein-coupled receptor activation induced cellular activation in astrocytes and cellular inhibition in neurons, while Gq G protein-coupled receptors activation induced neuronal and astrocytic activation. Transmitter liberated from glia cells enhances neurons excitability when astrocyte activation was exerted by either Gi/o or Gq protein-mediated signaling (Durkee, 2019).

Neurotransmission and neuronal activity require energy that it is produced by mitochondrial glucose oxidation and oxidative phosphorylation, which generates reactive oxygen species under normal conditions due to the leakage of dioxygen from the electron transport chain. The redox-regulated Nrf2 transcription factor activates and induces antioxidant genes transcription in astrocytes that stimulates glutathione synthesis in neighboring neurons. The enzyme fructose-2, 6-bisphosphatase-3/6-phosphofructo-2-kinase, a crucial regulator enzyme of glycolysis, is continuously degraded by the proteasomal system in neurons, which explains its low activity in neurons. Neurons stimulate glucose oxidation through the pentose-phosphate pathway to generate NADPH, which is required for reduction of oxidized glutathione (Bolaños, 2016). Co-culture of astrocytes and neurons showed that neuronal glutathione synthesis is induced by direct activation of Nrf2 in astrocytes, and that the inhibition of astrocytic Nrf2 function inhibited neuron-induced glutathione synthesis (McGann and Mandel, 2018).

Fibroblast growth factor-1 activates astrocytes to stimulate nerve growth factor secretion. Nerve growth factor induces apoptosis in motor neurons that express p75 neurotrophin receptor by inducing formation of nitric oxide (NO) and peroxynitrite. Fibroblast growth factor-1 stimulates NO production in astrocytes by increasing the expression of inducible NO synthase. However, fibroblast growth factor-1 also stimulates Nrf2 that increases glutathione synthesis in astrocytes, thereby reducing NO-dependent neurotoxicity in motor neurons (Vargas et al., 2006).

It has been reported that the glutathione antioxidant system under synaptic activity is coupled to the N-methyl-D-aspartic acid receptor. The control of neuronal oxidative stress and apoptosis is mediated by changing the synthesis, recycling, and utilization of glutathione to promote detoxification of reactive oxygen species. This mechanism is of importance in developing brain where the disruption of N-methyl-D-aspartic acid receptor-dependent transcriptional control of glutathione synthesis can lead to neurodegeneration (Baxter et al., 2015).

The metabolic coupling between astrocytes and neurons performs an important role in the prevention of oxidative stress in the brain, where astrocytes support the survival of neurons by secreting glutathione that can be taken up by neurons. This coupling between astrocytes and neurons is unidirectional, because neurons are not able to secrete glutathione to protect astrocytes. The high rate of oxidative metabolism of glucose in the brain that leaks electrons from the electron transport chain, reducing dioxygen to superoxide requires permanent secretion of glutathione by astrocytes to

maintain established levels of thiols in the neurons (Wang and Cynader, 2000). However, astrocytes are not always neuroprotective. It has been suggested that reactive astrocytes can be divided in neuroprotective phenotype (A1) and neurotoxic phenotype (A2), where astrocytes A2 has been reported to be linked to Parkinson's disease (Li et al., 2020).

Protein secretion

The metabolic coupling between neurons and astrocytes also involves astrocyte secretion of a large number of proteins such as superoxide dismutase [Cu-Zn], ceruloplasmin chain 1, antioxidant protein 2, 1-cys peroxiredoxin, apolipoprotein E chain 1, heat shock protein HSP90-alpha and beta, insulin-like growth factor binding protein 2, L-lactate dehydrogenase M and H chain, malate dehydrogenase cytoplasmic, metalloproteinase inhibitor 1 and 2 chain1, thioredoxin peroxidase 1 and 2, glutathione transferase class-mu, creatine kinase B chain, phosphoglycerate kinase 1, and carboxypeptidase H chain 1, among other proteins. Interestingly, most proteins secreted from astrocytes can also be detected in cerebrospinal fluid (Lafon-Cazal et al., 2003).

Parkinson's Disease

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease after Alzheimer's disease. PD has been recognized for the development of motor symptoms that are evident after 60–70% degeneration of the dopaminergic nigrostriatal neurons. However, non-motor symptoms have been observed in PD patients including olfactory dysfunction, sleep dysfunctions, and autonomic dysfunctions (Braak et al., 2003; Schrag et al., 2015; Roos et al., 2019; Wang et al., 2019; De Rui et al., 2020). Numerous mechanisms linked in the degeneration of dopamine neurons that contain neuromelanin have been reported, such as mitochondrial damage and dysfunction, protein degradation disruption of lysosomal and proteasomal systems, alpha-synuclein (SNCA) harmful oligomers formation, endoplasmic reticulum (ER) stress, neuro inflammation, and oxidative stress (Martinez-Vicente and Vila, 2013; Taylor et al, 2013; Ross et al., 2015; Zaltieri et al., 2015; Mercado et al., 2016; Moors et al., 2016; Ganguly et al., 2018; Fields et al., 2019; Jankovic et al., 2020). However, the identity of the neurotoxin that triggers these mechanisms in idiopathic PD is still not known.

A remarkable characteristic of idiopathic PD is the extremely slow rate of neurodegeneration and disease progression after diagnosis, taking years before motor symptoms develop. The possible link of exogenous neurotoxins and environmental factors in the degeneration of the nigrostriatal neurons seems to be excluded, because drug addicts that used synthetic drugs containing near pure MPTP developed a severe Parkinsonism in just 3 days (Williams, 1986). These results also discount the prion-like hypothesis based on the propagation of SNCA aggregates through Lewy bodies from different regions until the propagation affect the nigrostriatal neurons. Evidently, this propagation will not be as slow as the extremely slow rate of neurodegeneration and progression observed in PD (Volpicelli-Daley and Brundin, 2018). The particularly slow rate of the neurodegenerative process and progression in PD suggest that the degeneration of dopaminergic neurons containing neuromelanin must be induced by an endogenous neurotoxin, which triggers the degeneration of a single neuron. In this focused mode of degeneration, the accumulation of dead neurons induced by an endogenous neurotoxin will take years, which is observed in PD.

The endogenous neurotoxin that can be involved in the degeneration of dopamine neurons containing neuromelanin must be generated inside of these neurons in order to not induce a propagative and rapid degeneration. Possible endogenous neurotoxins that are generated inside dopaminergic neurons include neurotoxic oligomers of SNCA, 3,4-dihydroxyphenylacetaldehyde and o-quinones generated during dopamine oxidation to neuromelanin.

Alpha-synuclein

SNCA form fibril aggregates that generate large deposits in Lewy bodies, which are observed in postmortem tissues of PD brains. The possible role of SNCA aggregation and the deposition of fibrils in Lewy bodies in the loss of dopamine neurons containing neuromelanin is controversial because macrophages remove and degrade all the remains of dead neurons. Therefore, the Lewy bodies observed in postmortem tissue are the neurons and glia that have survived the degenerative process in PD, suggesting a protective role of Lewy bodies. Another observation supporting the idea that SNCA aggregation to fibrils is not involved in the degeneration of dopamine neurons containing neuromelanin in idiopathic PD is the absence of Lewy bodies in Parkinson's disease associated to parkin and LRRK2 mutations (Mori et al., 1998; Giassonet al., 2006; Rajput et al., 2006; Gaig et al., 2007; Ling 2013). It has been proposed that SNCA aggregation to oligomers is involved in the neurodegeneration of the nigrostriatal neurons, since these oligomers induce mitochondrial damage and impairment, synaptic dysfunction, ER stress, oxidative stress, and autophagy dysregulation when the gene is mutated (Fields et al., 2019; Du et el., 2020). In the familial form of PD mutations of the synuclein gene, such as A53T, induces the generation of neurotoxic oligomers of SNCA, however the question is the origin of these neurotoxic oligomers in the idiopathic form of PD. Dopamine oxidation has been suggested to induce the generation of neurotoxic oligomers in idiopathic PD. A study on the role of dopamine concentration and SNCA expression in aged mice revealed that only the combination of dopamine and SNCA caused motor impairment and progressive neurodegeneration of the substantia nigra (Mor et al., 2019).

3,4-Dihydroxyphenylacetaldehyde

3,4-Dihydroxyphenylacetaldehyde is generated during dopamine degradation. Monoamine oxidase catalyzes the oxidative deamination of dopamine to 3,4-dihydroxyphenylacetaldehyde, which is converted to 3,4-dihydroxyphenylacetic acid ammonia, and hydrogen peroxide by aldehyde dehydrogenase (ADH)-1. The catechol structure of 3,4-dihydroxyphenylacetaldehyde suggests that it can be oxidize to an o-quinone by reducing dioxygen to superoxide. It has been reported 3,4-dihydroxyphenylacetaldehyde is harmful by triggering oxidative stress and SNCA aggregation (Goldstein, 2020). The catechol structure of 3,4-dihydroxyphenylacetaldehyde suggests that it can be oxidized to an o-quinone by reducing dioxygen to superoxide. It has also been reported that 3,4-dihydroxyphenylacetaldehyde can induce covalent bonds with proteins such as L-aromatic-amino-acid decarboxylase and tyrosine hydroxylase (Goldstein, 2020; Yang et al., 2020).

A study of postmortem tissue from substantia nigra pars compacta of PD patients showed that ADH1 expression was decreased, suggesting that 3,4-dihydroxyphenylacetaldehyde accumulates in these patients (Grünblatt et al., 2004). Another investigation also reported a decrease in ADH1 expression in Parkinson's disease patients (Liu et al., 2014). However, we must remember that the postmortem tissue of Parkinson's disease patients with an average age of seventy-seven years contain neurons and glia cells that have survived the degenerative process induced by PD, suggesting that low expression of ADH1 is not linked in the loss of dopamine neurons.

The results from investigations with mice deficient of ADH have been contradictory. One study that used the ADH1A1 null mouse demonstrated significantly increased dopamine levels. However, no negative effect on substantia nigra dopaminergic neurons growth and development was observed, because the expression level of dopamine transporter, tyrosine hydroxylase, or vesicular monoamine transporter-2 were unchanged (Anderson et al., 2011). Another study with mice null for both ADH1A1 and ADH2 showed different results since significant degeneration of tyrosine hydroxylase immunopositively neurons in the substantia nigra was observed, accompanied with deficits in motor performance and an increase in 3,4-dihydroxyphenylacetaldehyde and 4-hydroxynonenal (Wey et al., 2012).

Based on the observed low expression of ADH1 in postmortem samples of PD patient neurons that survived the degenerative process over many years, and the lack of effect of ADH1A1 null mouse on substantia nigra dopaminergic neurons growth and development, it seems plausible that 3,4-dihydroxyphenylacetaldehyde accumulation is not involved to the loss of dopaminergic neurons of the nigrostriatal system in PD.

Ortho-quinones generated during neuromelanin synthesis

Neuromelanin formation requires dopamine oxidation to transient o-quinones such as dopamine o-quinone (DAoQ), aminochrome (AM), and 5,6-indolequinone (5,6-IQ) in a successive oxidative event (Segura-Aguilar, 2021a). Neuromelanin synthesis is a natural and inoffensive metabolic route, since this pigment increase when the life span increase in healthy aged individuals, which have undamaged dopamine neurons containing neuromelanin in substantia nigra postmortem tissue (Cassidy et al., 2019). However, under special circumstances these o-quinones can be harmful inside dopaminergic neurons, resulting in their loss in PD.

DAoQ is unstable only at pH higher than 2.0 and therefore, the amino group of this o-quinone undergo cyclization instantly to form AM at physiological pH (0.15 s^{-1}, Herrera et al., 2017). DAoQ generates harmful adducts with mitochondrial proteins, parkin, dopamine transporter, and tyrosine hydroxylase (Xu et al., 1998; Whitehead et al., 2001; LaVoie et al., 2005; Van Laar et al., 2009). DAoQ binds to many mitochondrial proteins when this *o*-quinone is formed in the presence of isolated mitochondria, but only a few proteins form adducts with DAoQ when dopamine is oxidized within a cell. Due to the high reactivity of DAoQ, the question remains whether the adducts that formed inside the cell are DAoQ or AM adducts. 5,6-IQ is also high reactive, and it polymerizes instantly to neuromelanin by autocondensation and the polymerization rate increases when AM concentration and pH rise. The neuromelanin structure is composed by 5,6-IQ units assembled by van der Waals interactions. It has been reported that 5,6-IQ reacts with glutathione and NADH and forms adducts with SNCA triggering the generation of oligomers (Bisaglia et al., 2007, 2010; Pezzella et al., 2007). 5,6-IQ forms adduct with Nurr1 and stimulates Nurr1 activity such as the transcription of genes for dopamine homeostasis (Bruning et al., 2019). AM is the less unstable o-quinone generated during dopamine oxidation to neuromelanin. This was illustrated by AM NMR spectra being stable 40 min before the appearance of 5,6-IQ NMR spectra (Bisaglia et al., 2007). AM is harmful by binding to proteins such as SNCA or when it is one-electron reduced by flavoenzymes that catalyze one-electron reductions of quinones (Herrera et al., 2017). In addition, AM has several other harmful effects in the brain; it induces mitochondrial damage, SNCA aggregates into harmful oligomers, and triggers proteasomal and lysosomal impairment. It also induces ER stress, neuro inflammation, oxidative stress, disruption of cytoskeleton architecture, and progressive neuronal dysfunction, as well as decreases synaptic dopamine release while increasing GABA release, inhibits axonal transport of monoaminergic vesicles to synaptic terminals, and induces cell shrinkage (Herrera et al., 2017; **Figure 2**).

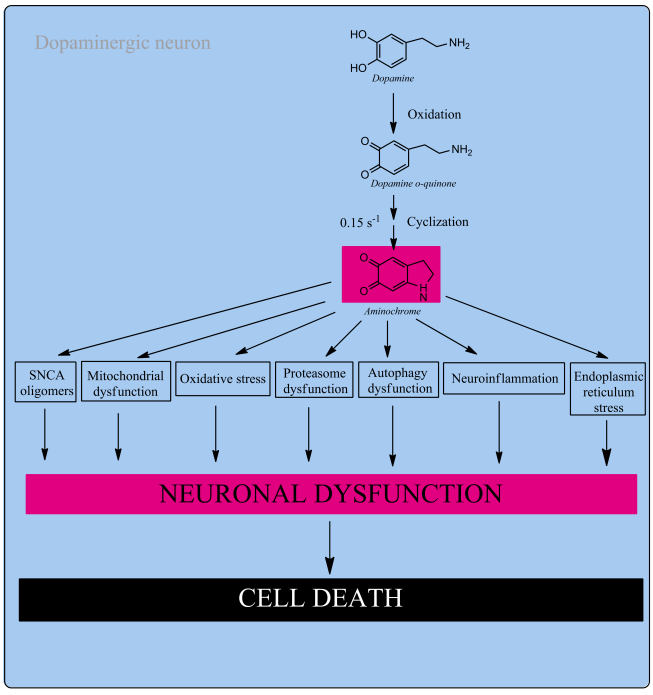

Figure 2 | **Aminochrome neurotoxicity.**
Neuromelanin synthesis in dopaminergic neurons require the formation of DAoQ that is stable at pH lower than 2.0 that instantly cyclizes to AM. AM rearranges to 5,6-IQ, the precursor of neuromelanin, but when the neuroprotective capacity of DT-diaphorase is surpassed aminochrome is neurotoxic. AM is harmful by triggering the formation of SNCA harmful oligomers, mitochondrial damage, oxidative stress, proteasomal impairment, autophagy impairment, neuro inflammation and ER stress. AM induces a progressive and slow neuronal dysfunction decreasing dopamine release and increase GABA release, dramatic morphological changes such as cell shrinkage. Unpublished data. 5,6-IQ: 5,6-Indolequinone; AM: aminochrome; DAoQ: dopamine o-quinone; ER: endoplasmic reticulum; GABA: γ-aminobutyric acid; SNCA: alpha-synuclein.

As we mentioned earlier, neuromelanin synthesis is a normal and harmless process that requires the generation of *o*-quinones that can be harmful under certain circumstances. The explanation for this paradox is the existence of two enzymes that inhibit the neurotoxic effects of AM.

(a) DT-diaphorase: DT-diaphorase inhibits the harmful effects of AM by reducing it with two electrons to leukoaminochrome. One-electron transferring flavoenzymes reduce AM to leukoaminochrome *o*-semiquinone radical, which is very unstable and instantly autoxidizes to AM in the presence of dioxygen (Segura-Aguilar et al., 1998). One-electron reduction of AM induces a redox cycling between AM and leukoaminochrome *o*-semiquinone radical that reduces dioxygen to superoxide and oxidizes NADH to NAD+ in the cytosol. DT-diaphorase also prevents the generation of AM-adducts with proteins, such as a- and b-tubulin, and actin (Paris et al., 2010). DT-diaphorase is a protecting enzyme against AM neurotoxicity by preventing (i) AM-dependent cell death (Lozano et al., 2010); (ii) mitochondrial damage (Herrera et al., 2017); (iii) lysosome impairment (Herrera et al., 2017); autophagy impairment (Herrera et al., 2017); (iv) generation of harmful SNCA oligomers (Herrera et al., 2017); (v) proteasome impairment (Zafar et al., 2006); (vi) aggregation of alpha- and beta-, actin, and impairment of the cytoskeleton architecture (Herrera et al., 2017). Dopaminergic neurons and astrocytes have constitutive expression of DT-diaphorase to prevent AM harmful effects (Herrera et al., 2017).

(b) GSTM2- GSTM2 catalyzes glutathione conjugation of AM and its precursor DAoQ to 4-S-glutathionyl-5,6-dihydroxyindoline and 5-glutathionyldopamine, respectively (Herrera et al., 2017). Dioxygen, superoxide and hydrogen peroxide are not able to oxidize 4-S-glutathionyl-5,6-dihydroxyindoline. 5-Glutathionyldopamine is degraded to 5-cysteinyldopamine as the final product, found in the human neuromelanin and cerebrospinal fluid (Segura-Aguilar, 2021b). GSTM2 prevents AM-dependent (i) cell death in astrocytes; (ii) autophagy dysfunction; (iii) mitochondria damage; (iv) generation of SNCA harmful oligomers; and (v) AM-induced lysosomal dysfunction (Huenchuguala et al., 2017; Segura-aguilar, 2021b).

AM is the less unstable and most studied *o*-quinone during neuromelanin synthesis and the most suitable endogenous neurotoxin to be used for preclinical model of Parkinson's disease because (i) AM is generated inside of dopamine neurons containing neuromelanin degenerated during Parkinson's disease; (ii) there is two enzymes that prevents its neurotoxic effects, explaining why healthy aged individuals have undamaged dopamine neurons containing neuromelanin. However, AM under certain circumstances is neurotoxic when the protective enzymes capacity is surpassed; (iii) AM neurotoxicity is not expansive and affects single neurons exposed to AM neurotoxicity; and (iv) AM triggers all the reported neurodegenerative mechanisms such as mitochondrial damage, the formation of harmful oligomers of SNCA, dysfunction of proteasomal and lysosomal systems, ER stress, neuro inflammation, and free radical formation (Herrera et al., 2017; Segura-Aguilar, 2021a).

Astrocytes Protect Dopaminergic Neurons against Aminochrome Neurotoxicity

A new astrocyte neuroprotective mechanism has been reported where astrocytes secrete GSTM2 that prevents AM-dependent neurotoxicity in dopaminergic neurons. These neurons do not express GSTM2 but astrocytes constitutively express this enzyme. However, astrocytes secrete GSTM2 that can be internalized by dopaminergic neurons, thereby protecting dopaminergic neurons against the harmful effects of AM (Segura-Aguilar et al., 2021b). The secretion of GSTM2 from astrocytes is mediated by exosomal release, explaining dopamine neuron ability to internalize GSTM2 into the cytosol to mediate protection against AM (Valdes et al., 2021).

This new astrocyte protective mechanism against AM neurotoxicity in dopamine neurons during dopamine oxidation to neuromelanin would be very important to prevent loss of dopaminergic neurons in substantia nigra when DT-diaphorase protective capacity is surpassed. It is important to remember that GSH conjugate of DAoQ and AM inhibit the participation of these *o*-quinones in neurotoxic redox cycling reactions. 5-Glutathionyldopamine, the GSH conjugate of DAoQ, is degraded to 5-cysteinyldopamine that has been observed in cerebrospinal fluids and in human neuromelanin. This is the final product eliminated from dopaminergic neurons. 4-S-Glutathionyl-5,6-dihydroxyindoline, the GSH conjugate of AM, is resistant to biological oxidizing agents preventing its participation in neurotoxic reactions that require oxidative activation (Segura-Aguilar, 2021b; **Figure 3**).

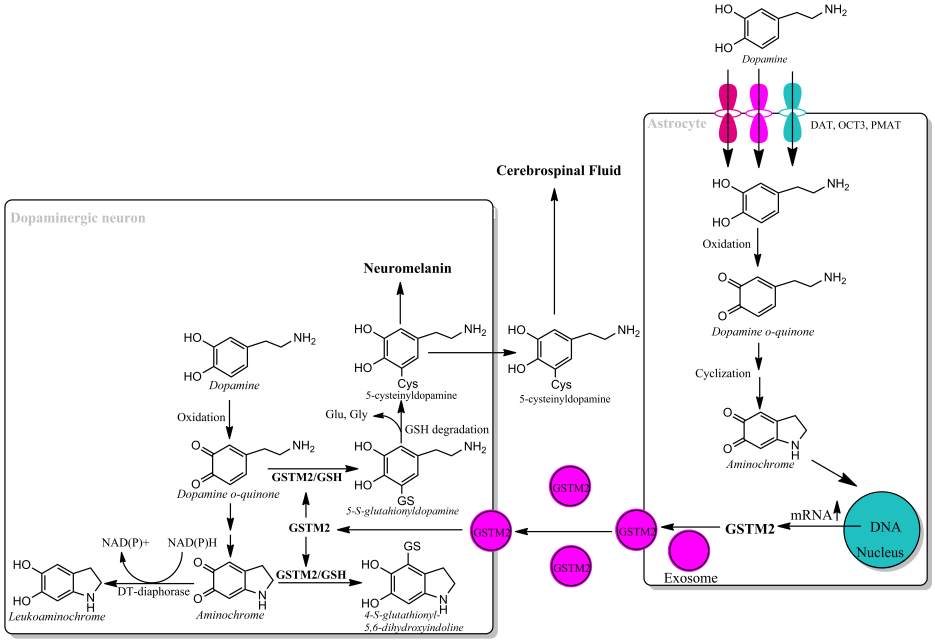

Figure 3 | Astrocyte protects dopaminergic neurons against aminochrome neurotoxicity. Astrocytes take up dopamine after neurotransmission through dopamine transporter (DAT), organic cation transporter-3 (OCT3) and plasma membrane transporter (PMAT) into cytosol where dopamine autoxidizes at physiological pH to DAoQ. DAoQ is very unstable and instantly cyclizes to AM at rate of 0.15 s^{-1}. AM induces an increase in mRNA expression of GSTM2 and exosomes mediate intercellular transport GSTM2 from astrocytes into dopamine neurons. GSTM2 conjugates DAoQ with GSH to 5-glutathionyldopamine that is degraded to 5-cysteinyldopamine, which will be deposited in the neuromelanin or be excreted into cerebrospinal fluid. GSTM2 also catalyze GSH conjugation of AM to 4-S-glutathionyl-5,6-dihydroxyindoline, which it is not able to oxidize in the presence of dioxygen, hydrogen peroxide and superoxide, and it is unable to participate in neurotoxic redox cycling reactions. Unpublished data. AM: Aminochrome; DAoQ: dopamine o-quinone; GSH: glutathione; GSTM2: glutathione transferase M2-2.

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