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The role of α-synuclein in brain lipid metabolism: a downstream impact on brain inflammatory response

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Abstract α-Synuclein (Snca) is an abundant small cytosolic protein (140 amino acids) that is expressed in the brain, although its physiological role is poorly defined. Consistent with its ubiquitous distribution in the brain, we and others have established a role for Snca in brain lipid metabolism and downstream events such as neuroinflammation. In astrocytes, Snca is important for fatty acid uptake and trafficking, where its deletion decreases 16:0 and 20:4n-6 uptake and alters targeting to specific lipid pools. Although Snca has no impact on 22:6n-3 uptake into astrocytes, it is important for its targeting to lipid pools. Similar results for fatty acid uptake from the plasma are seen in studies using whole mice coupled with steady-state kinetic modeling. We demonstrate in gene-ablated mice a significant reduction in the incorporation rate of 20:4n-6 into brain phospholipid pools due to reduced recycling of 20:4n-6 through the ER-localized long-chain acyl-CoA synthetases (Acsl). This reduction results in a compensatory increase in the incorporation rate of 22:6n-3 into brain phospholipids. Snca is also important for brain and astrocyte cholesterol metabolism, where its deletion results in an elevation of cholesterol and cholesteryl esters. This increase may be due to the interaction of Snca with membrane-bound enzymes involved in lipid metabolism such as Acsl. Snca is critical in modulating brain prostanoid formation and microglial activities. In the absence of Snca, microglia are basally activated and demonstrate increased proinflammatory cytokine secretion. Thus, Snca, through its modulation of brain lipid metabolism, has a critical role in brain inflammatory responses.

Keywords Acyl-CoA synthetase · α-Synuclein · Arachidonic acid · Cholesterol · Cholesteryl esters · Docosahexaenoic acid · Fatty acid metabolism · Palmitic acid · Triacylglycerol

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Introduction

α-Synuclein (Snca)

Snca is a 140-amino acid, cytosolic protein that is highly expressed in the central nervous system, where it is very conserved among vertebrates [1, 2]. Depending upon the analysis, Snca accounts for between 0.1% and 1% of neuronal cytosolic protein [3, 4]. It has three distinct domains: (1) an amphipathic N-terminal domain; (2) an internal hydrophobic domain, which comprises the non-amyloid component of Alzheimer's disease plaques (NAC); and (3) a C-terminal region rich in glutamate and aspartate residues [5, 6]. The carboxy terminal region is phosphorylated by Src

family members as well as G-protein coupled receptor kinases [7–13]. Snca is phosphorylated on Ser 129 [12, 13] and Tyr 125 [7–9], which are in the unordered tail region of the proteins, while Ser 87 is phosphorylated to a lesser extent [12], which is in the C helix of the protein. The physiological function of Snca phosphorylation is controversial but may be important in the interaction of Snca with proteins, such as long-chain acyl-CoA synthetases (Acsl), and with other membrane-associated proteins.

Snca is a very interesting cytosolic protein because in its native state the protein is a random coil, but undergoes a significant change in its secondary structure by increasing its α-helical content upon binding to membranes [14–16]. Recent evidence indicates that two helices are formed via a hinge region upon binding to vesicles and that upon binding the carboxy terminus remains in a highly unordered state [16]. Snca also binds to small phospholipid vesicles [14, 17], to brain vesicles [18] and is associated with the distal pool of synaptic vesicles [19, 20], suggesting that its membrane interaction is highly significant.

Although Snca is localized in presynaptic terminals of neurons [1, 21–23], it is also found in other locations in neurons [24, 25]. More importantly, Snca is also found in astrocytes [26, 27], in microglia [28, 29], and in oligodendroglia [24, 30], indicating that it is ubiquitously distributed in all the major cell types found in the brain. In addition, Snca is enriched in the microsomal fraction isolated from intact brain [31, 32] and is co-localized with endoplasmic reticulum (ER) found in synaptosomal preparations [4, 33, 34]. However, it is not found associated with synaptic vesicles [4].Therefore, although this wide distribution and ER localization of Snca is not consistent with a physiological role limited to synaptic function, it suggests a much broader physiological function for Snca in the brain.

Similar to its wide distribution, Snca may have many potential roles in the nervous system, including regulation of synaptic vesicle mobilization [19, 20, 35], chaperone activity [36, 37], modulation of dopamine transporter [38, 39] and dopamine biosynthesis [40, 41], regulation of inflammatory response [28, 29, 42], and regulation of lipid metabolism [27, 32, 43–49]. The role in brain fatty acid metabolism may be based in part on the structural similarity between Snca and class A2 apolipoproteins [14, 33], and it has some sequence homology to fatty acid binding proteins (FABP) [31]. However, Snca has a vast number of putative roles in the central nervous system. In dopaminergic neurons, Snca appears to have a role in the Rab1-mediated vesicular trafficking between the Golgi and ER [50]. Consistent with this role, Snca interacts with cysteine-string protein-α (CSP-α), which is involved in SNARE complex assembly [51]. In CSP-α knockout mice, Snca functionally substitutes for CSP-α activity through its downstream binding with phospholipids. Collectively, these studies demonstrate an emerging role for Snca in protein–membrane interactions that are localized in the ER–Golgi complex. For all essential purposes, Snca appears to be involved as an adapter protein, permitting the interaction of membrane-associated proteins with the various membranes [32, 38, 39, 50, 51]. Such a role for Snca is consistent with the diverse functions attributed to Snca in a variety of systems.

SNCA and fatty acid metabolism

There are a number of studies that strongly suggest that Snca has a role in brain lipid metabolism. As previously noted, Snca has structural similarities to class A2 apolipoproteins [14, 33] and some sequence similarity to FABP [31]. The link to a role in lipid metabolism is strengthened by the observation that it is found extensively in microsomes [31, 32], a location associated with complex lipid metabolism.

Does Snca function as an FABP?

The notion that Snca is functionally equivalent to FABP is tenuous at best. Although the direct binding of fatty acids to Snca remains controversial [31, 43, 52], our most recent studies demonstrate that Snca binds both monomeric 20:4n-6 and 22:6n-3 with a K_d around 1–4 μM [32, 44]. This binding affinity is two orders of magnitude less than that for FABP [32, 44, 53, 54], suggesting Snca does not function in the same manner as FABP [55–59]. In addition, FABP have a diverse and broad impact on tissue lipid metabolism both when expressed in cells [56–58, 60–63] and when removed from tissues via gene-ablation [55, 59, 61]. These changes include a dramatic increase in total phospholipid mass and individual phospholipid class mass in the presence of FABP including an increase in plasmalogen mass [55, 58, 59]. While fatty acid uptake is generally increased in the presence of FABP [56, 57, 60–63], there is also a concomitant alteration in the phospholipid acyl chain composition [55, 58, 59]. In Snca-ablated mice, there are no changes in brain individual phospholipid class mass, including plasmalogens, other than a 20% reduction in cardiolipin mass [45, 64]. Consistent with whole brain, no change in total phospholipid and individual phospholipid class mass was observed in astrocytes [43]. Similarly, in astrocytes [43] and in whole brain [44, 45], the only change seen with acyl chain composition is a consistent reduction in docosahexaenoic acid levels. In cardiolipin, we have observed an increase in saturated fatty acids, leading to a reduction in the free volume of the membrane and an increase in the rotational correlation time

of diphenylhexatriene [64]. However, this change in cardiolipin acyl chain composition is the only change we have observed that involves multiple fatty acids, and it is important to note that cardiolipin makes up less than 2% of the total brain phospholipid. Thus, unlike the dramatic changes seen in steady-state lipid mass upon expression of FABP in cells or its deletion in animals, Snca gene-ablation results in only minor changes in phospholipid mass that is restricted to cardiolipin. Based upon this inconsistency with what is observed for known FABP functions, as well as the very poor binding of monomeric fatty acids and the lack of a similar tertiary structure, we feel that Snca, while having a role in brain lipid metabolism, is not a protein comparable to FABP.

Snca and brain fatty acid uptake

Although we have postulated that Snca is not an FABP [27, 32, 43–45], we have strong evidence indicating that it has a critical role in brain fatty acid uptake and metabolism [27, 32, 43–45, 64]. In astrocytes lacking Snca, 16:0 and 20:4n-6 uptake is depressed, while 22:6n-3 uptake is unaffected [27]. In intact mice, a similar result is seen for brain fatty acid uptake from plasma as measured using steady-state kinetic modeling [32, 43, 44]. In addition, the absence of Snca disrupts the normal trafficking of these three fatty acids to specific lipid pools through an unknown mechanism [27], although it is consistent with our proposed modulation of an ER-localized Acsl (Fig. 1). Similar to astrocytes, Snca KO mice have reduced brain 16:0 uptake (45%) and 20:4n-6 (12%) uptake in the absence of any change in brain 22:6n-3 uptake [32, 43, 44]. Thus, fatty acid uptake into the intact brain is significantly depressed in the absence of Snca. This reduction in uptake is fatty acid dependent, and, more importantly, the changes we have observed in astrocytes occur in the whole brain. This validates the use of astrocytes to study the mechanisms underlying the impact of Snca on fatty acid uptake and trafficking.

Snca and brain fatty acid metabolism

In our laboratory, we use a well-characterized steady-state radiotracer kinetic model to measure the incorporation rate and turnover of fatty acids in individual brain phospholipid pools [65]. In Snca-deficient mice, 16:0 metabolism is significantly altered with an increased rate of incorporation into phosphatidylcholine (PtdCho) accompanied by reductions in incorporation rate into the other major phospholipids [43]. This increase targeting to PtdCho is consistent with the known tonic inhibition of PLD2 by Snca [47, 48, 66]. Hence, in the absence of Snca, the increase turnover of PtdCho 16:0 would be expected. While in the absence of Snca, there is only a minor reduction in the uptake of 20:4n-6 from the plasma, and there is a large and significant reduction in brain 20:4n-6 metabolism. The incorporation rate for 20:4n-6 into brain phospholipids is reduced over 50%, accounted for by a reduction in 20:4n-6-CoA formation through ER-localized Acsl activity [32]. Importantly, addition of physiologically relevant concentrations (3.15–6.3 nM) of wild type, but not mutant forms of Snca, completely restores 20:4n-6-CoA formation in microsomes isolated from Snca KO mice [32]. Unlike wt Snca, mutant forms of Snca (A30P, E46K, and A53T) fail to modulate acyl-CoA synthetase activities, indicating that expression of these forms may function like the null. The incorporation rate of 16:0 and 20:4n-6 into cardiolipin, a mitochondrial-specific phospholipid, is also reduced (50%) in these mice, which is highly suggestive of reduced cardiolipin synthesis when considering the significant reduction in cardiolipin mass [45, 64]. Although there is no net increase in 22:6n-3 fatty acid uptake into the whole brain, the incorporation rate for 22:6n-3 into brain phospholipids is increased over 50% in KO mice, even though there is no increase in ER-localized Acsl activity [44]. Because 20:4n-6 and 22:6n-3 are the major polyunsaturated fatty acids in the brain, the increase in 22:6n-3 incorporation into brain phospholipids is considered to be compensatory for the reduction in rate of 20:4n-6 incorporation into these lipid pools [44]. These results are consistent with the selective modulation by Snca of specific ER-localized Acsl involved in 20:4n-6-CoA formation, but not in 22:6n-3-CoA formation.

When the intact mouse is subjected to a neurotraumatic event, such as ischemia, there is a net increase in the amount of prostanoids formed in Snca-deficient mice [42]. This demonstrates the physiological relevance of the reduced recycling of 20:4n-6 in these mice. Under conditions where 20:4n-6 release is accelerated, such as ischemia and neuroinflammation, the reduction in recycling of 20:4n-6 into the 20:4n-6-CoA pool will result in elevated free 20:4n-6 mass, which can be utilized for downstream prostanoid biosynthesis. More importantly, the mutant forms of Snca fail to restore this activity, suggesting that with these mutations, the brain is subjected to a much greater proinflammatory response. Hence, the reduction in 20:4n-6 recycling has a physiological significance and demonstrates the importance of Snca in brain prostanoid production.

Snca and lipid-mediated signal transduction

Snca also directly affects lipid-mediated signal transduction through its interactions with phospholipases C and D. Studies in vitro demonstrate that Snca stimulates PLCβ activity by enhancing the G-protein coupled interaction with the enzyme [49] and that it directly inhibits the

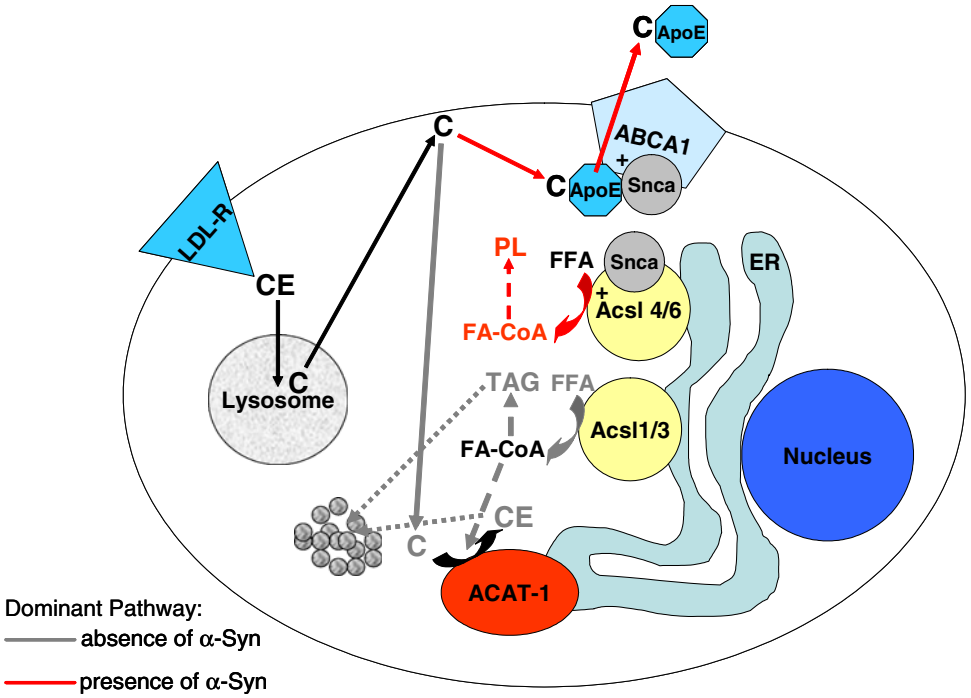

Fig. 1 Model of α-synuclein (Snca) regulation of brain lipid metabolism. We propose that Snca interacts with Acsl 6 in the ER in a manner that increases enzyme activity maintaining normal 20:4n-6-CoA pool size and targeting of fatty acids to the brain phospholipids. In the absence of Snca, Acsl 6 activity is reduced resulting in elevated free fatty acids (FFA) [45], which are used by Acsl 1 or 3 to maintain acyl-CoA pools destined for incorporation into neutral lipids. This scheme accounts for our observed increase in unesterifed fatty acids (FFA), triglycerides (TAG), cholesterol (C), and cholesteryl esters (CE) in Snca deficiency [27, 45] and is consistent with the reported trafficking of fatty acids by Acsl [87, 88, 123]. In addition, the increase in astrocyte cholesterol levels observed in the absence of Snca [27] may result from a reduction in Snca-dependent cholesterol export. The subsequent increase in astrocyte intracellular cholesterol drives the esterification of this cholesterol to form cholesteryl esters

activity of PLD2 [47, 48, 66]. In yeast, Snca overexpression inhibits PLD activity [67], consistent with the observations in the mammalian system. In Snca KO mouse brains, incorporation and turnover of 16:0 in phosphatidylcholine are increased [43], consistent with the observed tonic inhibition of PLD2 by Snca. Overexpression of Snca in *Drosophila* downregulates expression of both phospholipase A_2 and long-chain Acsl [68]. Collectively, these findings and our own demonstrate that Snca impacts lipid-mediated signal transduction, including 20:4n-6 release. Equally important is the positive impact that Snca, but not its mutant forms, has on modulating brain Acsl activities, thereby facilitating brain uptake of 20:4n-6 and its metabolism in the brain [32]. The modulation of brain 20:4n-6 metabolism by Snca is critical because release of 20:4n-6 during signal transduction is crucial for proper CNS function [69–74] and pathophysiological responses in the brain [75–78].

Acyl-CoA synthetases (Acsl)

Acsl are critical enzymes that activate fatty acids to their acyl-CoA moieties, which is an essential step for incorporation of fatty acids into more complex lipids such as phospholipids and triacylglycerols. In the brain, four different Acsl are expressed, Acsl 3 and 6, and to a much lesser extent Acsl 1 and 4 [32, 79–84]. Acsl 6 has two alternatively spliced variants: one (v.1) has selectivity for 20:4n-6 and the other (v.2) has selectivity for 22:6n-3 [84]. Because Snca stimulates 20:4n-6-CoA formation [32], but not 22:6n-3-CoA formation, we hypothesize that it modulates the activity of Acsl 6v.1. Brains also express fatty acid transport protein-4, which is a protein that has combined fatty acid uptake and Acsl activities [85, 86]. Because fatty acid transport proteins (FATP) are associated with the plasma membrane, these bifunctional proteins are thought to be involved in initial fatty acid uptake and acyl-CoA formation. However, ER-localized Acsl are involved in complex lipid biosynthesis and more importantly acyl chain recycling, which is reduced for 20:4n-6 by Snca deficiency [32].

Acsl are involved in targeting fatty acids for incorporation into specific lipid pools, and this observation, coupled with the observed modulation of specific Acsl by Snca, forms the foundation for our overall theory on modulation of brain fatty acid metabolism and targeting by

Snca. For instance, Acsl 6 targets fatty acids for esterification into phospholipid pools [87], Acsl 1, 3, and 4, all of which are inhibited by triacsin C, target fatty acids to triacylglycerols [88, 89]. Expression of Acsl in cells increases fatty acid uptake, presumably via increased acyl-CoA formation and subsequent incorporation into lipid pools. In PC-12 cells, Acsl 6 expression increases 20:4n-6 and 22:6n-3 uptake nearly 50%, targeting these fatty acids to phospholipid pools [87], which functionally results in increased neurite outgrowth [90]. Based upon these other studies, our observed increase in triacylglycerols in KO astrocytes [27] and in KO brains [45] further suggests a derangement of normal fatty acid targeting to phospholipid pools by Acsl 6 and increased targeting to neutral lipid pools via increased Acsl 1 or 3 activities (Fig. 1). Thus, Acsl have a functional role in the selective uptake and targeting of fatty acids to specific cellular lipid pools in an Acsl isoform-dependent manner. Herein, we propose that Snca interacts with specific Acsl thereby modulating 20:4n-6 metabolism and downstream events such as neuroinflammatory responses.

Snca and brain cholesterol metabolism

While the previous section is focused on brain phospholipid fatty acid metabolism, we have also demonstrated profound and convincing evidence that Snca deficiency impacts brain and astrocyte cholesterol metabolism [27, 45]. Because astrocytes are the major site for brain cholesterol biosynthesis [91, 92], our initial experiments were focused on examining astrocyte cholesterol metabolism. In Snca-deficient astrocytes, cholesteryl ester and cholesterol levels are significantly elevated [27], suggesting a derangement in astrocyte cholesterol metabolism. Fatty acid-targeting experiments further support this point as fatty acid targeting to cholesteryl esters is elevated between 4.3- and 8.0-fold, in a fatty acid-dependent manner [27]. This is consistent with our observed increases in cholesteryl ester levels found in astrocytes, suggesting increased cholesteryl ester synthesis. One mechanism that might account for this increase in cholesteryl esters is that excess cholesterol is deposited in lipid droplets after its esterification by acyl-coenzyme A:cholesterol acyltransferase (ACAT) to form cholesteryl esters [93]. This is important because astrocytes from Snca-deficient mice have an elevated level of cholesterol, but it is unknown whether or not this elevation results in increased cholesteryl ester formation. However, the increased targeting of fatty acids to cholesteryl esters is highly suggestive that this is the case and will be tested in future experiments.

Extending our observations in astrocytes to the whole brain, we have found changes similar to those observed in astrocytes for elevated levels of cholesteryl ester and cholesterol [45]. However, these increased levels are not the result of alterations in the expression of key enzymes associated with cholesterol synthesis and export, as the mRNA levels of these proteins are unchanged. Thus, we have found a new link between Snca and brain cholesterol metabolism; however, the mechanisms underlying this link are poorly defined.

Brain cholesterol and neurodegeneration

This novel link between Snca and brain cholesterol metabolism is important because cholesterol is an essential component of all mammalian cell membranes, and it is critical for a variety vital CNS processes such as synaptogenesis [94] and myelin formation [95, 96]. Disturbances in brain cholesterol homeostasis are associated with a variety of neurodegenerative diseases including Alzheimer's disease [97], Niemann-Pick C1 disease [98], and Huntington disease [99]. However, the role of cholesterol in the progression of Parkinson's disease (PD) is underappreciated. High dietary levels of cholesterol intake are suggested as being associated with an increased risk of PD [100], although it is important to reinforce that dietary cholesterol does not enter the brain. Rather, astrocytes are the primary sources for endogenous brain cholesterol [91, 92]. In cell culture studies, incubating cells with methyl-β-cyclodextrin to sequester membrane cholesterol results in the aggregation of Snca [101]. These findings suggest a role for cholesterol in facilitating Lewy body and inclusion body formation, both of which are hallmarks of PD. Interestingly, Snca accumulates in the brains of patients with Niemann-Pick C1 disease and in Niemann-Pick C1 mutant mice [102, 103], and in the brains of patients with Alzheimer's disease as the non-amyloid component of Alzheimer's disease plaques [104]. The established association of neurodegenerative diseases and the altered brain cholesterol homeostasis provides additional importance to our novel link between Snca and brain cholesterol metabolism.

Cholesterol metabolism in astrocytes

The brain is one of the most cholesterol-rich organs in the body and almost all of the cholesterol used in the brain is synthesized within the CNS [92, 97, 105, 106]. Although neurons in the immature brain can synthesize cholesterol, mature neurons are dependent on cholesterol synthesized in and exported from astrocytes [91, 92]. In the absence of the astrocyte exported cholesterol, neurons fail to form synapses in their culture [107] and have a significantly reduced efficacy of presynaptic transmitter release [108]. Cholesterol is exported from astrocytes via ABCA1 and ABCG1 transporters [109–112] after it is bound to ApoE and ApoJ

[113–115]. Although ApoJ is present in the CNS, ApoE is the major apolipoprotein in the CNS and it is highly expressed in astrocytes [116–118]. Astrocytes secrete ApoE in a complex of cholesterol and phospholipids in the form of small, high-density lipoprotein like particles [114]. Interestingly, Snca has a structural similarity to apolipoproteins [14, 33], suggesting it may have a role in cholesterol efflux.

Cholesterol efflux is regulated in part by liver-X receptor (LXR) activation [112, 119]. In addition, 24(*S*)-hydroxycholesterol, which is the major form of cholesterol that is exported from the brain [120, 121], also controls cholesterol efflux from astrocytes via its ability to interact with LXR [119]. It is important to note that we did not observe any increase in LXR expression in the absence of Snca nor any change in mRNA levels encoding other enzymes associated with cholesterol synthesis and export [45]. Thus, astrocytes are absolutely critical participants in the maintenance of brain cholesterol homeostasis, demonstrating the importance of our novel link between Snca and astrocyte cholesterol metabolism.

Snca and brain inflammatory response

PD, microglia, astrocytes, and neuroinflammation

PD brains demonstrate another striking histological change associated with the degenerating brain. This change is the maintained presence of reactive microglia [122–124]. A postmortem examination of idiopathic PD brains demonstrates increased CD68 microglial immunoreactivity with increased disease duration [125]. A similar study found a widespread increase in microglial reactivity in the PD brain compared to controls [126]. Positron emission tomography identified that the early stage PD brains have increased microglial reactivity, and it was inversely correlated with decreased dopamine transporter marker while being positively correlated with disease motor severity [127]. In MPTP-induced Parkinsonism, nigral microglial immunoreactivity is substantially increased and is maintained for 1–3 years [128, 129]. These data demonstrate that microglia are activated early during the disease process and that this activation is maintained at later stages of PD, suggesting that microglial activation is an important contributor to disease pathophysiology.

Fibrillar Snca deposition activates microglia in vivo and is capable of activating microglia in vitro, yet it remains unclear what roles microglia have in the neuronal loss found in PD [125, 130]. Microglial activation leads to NMDA-induced neuronal mitochondrial dysfunction leading to focal neuronal cytoskeleton collapse characterized by neuritic beading [131]. This is important because in the absence of Snca the mass and turnover of the mitochondrial-specific phospholipid, cardiolipin is reduced as in complex I and III coupling [64]. This suggests that the effect of Snca in microglial activation and the reduction in mitochondrial function could exert a combined effect on neuronal survival. Herein, we propose that Snca is a key regulator of neuroinflammatory responses because of its ability to modulate microglial activation [29].

Astrocytes are also an important component of neuroinflammatory responses [124, 132, 133]. However, in PD the number of reactive astrocytes in the substantia nigra is highly variable, unlike the robust microglial response in this region [134]. On the other hand, the number of reactive astrocytes is inversely correlated with dopaminergic damage [135]. In MPTP models of PD, astrocyte activation parallels with cell death and astrocytes remain activated even after neuronal death [136–138]. Similarly, injection of 6-OHDA into the nigrastriatal region results in a rapid, long-lasting increase in astrocyte activation [139]. Intranigral infusion of IL-1β leads to increased astrocyte activation, which appears to be neuroprotective against 6-OHDA induced neuronal death [140]. In addition, IL-1β transiently increases expression of the $P2X_7$ purinergic receptor in astrocytes [141], increases the levels of the endocannabinoid 2-arachidonyl glycerol formation [142], and cysteinyl leukotriene release [143]. Thus, astrocyte activation also has a role in neuroinflammatory responses. Combined with our observations of altered astrocyte lipid metabolism in the absence of Snca [27] and the effect of Snca on microglia activation [29], this role of astrocyte activation adds additional strength to our proposed importance of Snca to brain inflammatory responses.

Snca and microglial activation

Although extracellular Snca aggregates may serve as an activating stimuli for microglia, we hypothesize that expression of the protein itself within microglia also regulates microglia reactivity (Fig. 2) [29]. We have demonstrated that in the absence of Snca, microglia are basally activated, expressing more proinflammatory cytokines and membrane markers consistent with activated cells [29]. In addition, upon stimulation the Snca-deficient cells secrete seven- to eightfold more IL-6 and TNFα, two potent proinflammatory molecules [29]. Although these microglia are more proinflammatory, their phagocytic ability is reduced nearly 50%, indicating that, despite the profound proinflammatory state, these Snca-deficient microglia are dysfunctional. Collectively, our results using microglia isolated from gene-ablated mice suggest that alterations in lipid-mediated signaling events, particularly through the PLD- and PLA_2-dependent pathways, are altered both in vitro and in vivo [32, 43].

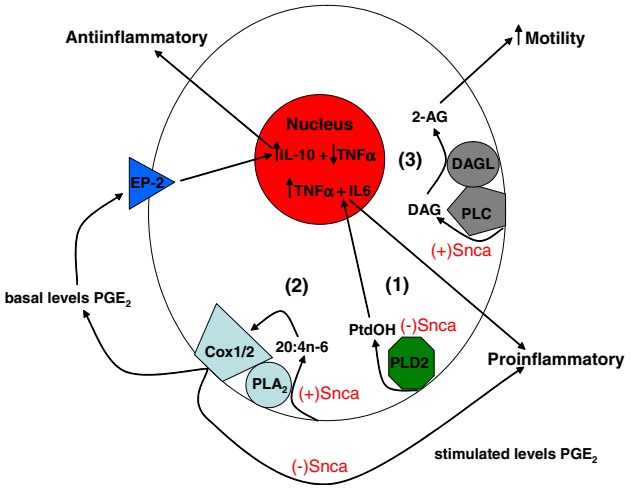

Fig. 2 α-Synuclein (Snca) is a key regulator of microglial function via its impact on lipid-mediated signal transduction. The presence of Snca can stimulate (+) or inhibit (−) key steps in microglial lipid signaling mechanisms. (1) Snca tonically inhibits PLD2 [43, 47, 48, 66], resulting in a reduction in phosphatidic acid (PtdOH) formation, which is a positive regulator of microglial activation via increasing proinflammatory cytokine production. In the absence of Snca or in the presence its mutant forms, including A53T, there will be increased PtdOH formation and increased simulation of proinflammatory cytokine production. (2) Snca impacts brain 20:4n-6 metabolism via its stimulation of microsomal Acsl activity, thereby increasing 20:4n-6 turnover [32]. Hence, Snca regulates brain 20:4n-6 metabolism and in its absence or in the presence of its mutant forms, including A53T, there will be increased substrate available for eicosanoid biosynthesis altering the microglial response. (3) Snca stimulates PLCβ [49], which is essential for providing diacylglycerol (DAG) for conversion by DAG lipase (DAGL) to 2-arachidonyl glycerol, which is a potent stimulator of microglial motility and phagocytosis. Hence, in the absence of Snca, PtdOH would be elevated causing increased proinflammatory cytokine production and upon stimulation, the increased release of 20:4n-6 in the presence of reduced reacylation would provide more 20:4n-6 for PGE_2 biosynthesis in the proinflammatory phase. In addition, reduction in 2-AG formation resulting in decreased motility and phagocytic ability. This is consistent with our findings in Snca-deficient microglia presented in the preliminary findings. In the absence of Snca or in the presence of its mutant forms, there will be reduced 2-AG formation that reduced phagocytosis and mobility. We hypothesize that the mutant forms of Snca behaves similar to the null [32], leading to an increased neuroinflammatory response in brain expressing this protein

Activity of these enzymes is critically important in regulating the microglial activation state. For example, PLD activity is required for macrophage complement-dependent phagocytosis [144] as well as integrin-dependent adhesion and subsequent activation of macrophages [145, 146]. PLD activation is also a required component of the macrophage activation response to a variety of proinflammatory stimuli including TNFα, LPS, phorbol ester, and zymosan [147–149]. PLA_2 activity is needed to liberate 20:4n-6 for subsequent downstream generation of proinflammatory eicosanoids following stimulation of microglia. In addition, PLA_2 activity is required for basal expression of cyclooxygenase-2 (COX-2) and subsequent prostaglandin generation [150]. The basal generation of prostanoids, particularly PGE_2, is required for tonic inhibition of microglial activity that is mediated through the EP2 receptor resulting in a variety of anti-inflammatory effects including increased IL-10 expression, an anti-inflammatory cytokine, downregulation of inducible nitric oxide synthase expression, decreased levels of major histocompatibililty complex class II antigen, and downregulating TNFα secretion [151, 152]. It is important to note that in LPS-stimulated microglia, there is an increase in PGE_2 synthesis only after a sequential increase in COX-2 expression followed by increased prostaglandin E_2 synthase levels [153]. Hence, the reduction in recycling in the whole brain may also alter the amount of free 20:4n-6 found in microglia, thereby having an important role in microglia prostanoid biosynthesis.

Summary

We have demonstrated that Snca has an important role in modulating brain lipid metabolism. The overall premise is that Snca functions as an adapter protein that enables membrane-associated proteins to more firmly interact with the membrane in a manner that facilitates function. Undoubtedly, the phosphorylation of the disordered tail region of Snca may have a regulatory role in this process. While we have demonstrated an important modulatory role for Snca in 20:4n-6 metabolism through Acsl activity, it is only until recently that this role has taken on physiological significance as we see that Snca is critical in modulating brain prostanoid synthesis.

This, coupled with the observed modulation of the microglia reactivity, suggests a profound and significant role for Snca in brain inflammatory responses.

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