FEBS Letters 585 (2011) 1001–1006

journal homepage: www.FEBSLetters.org

Review

Synuclein modulation of monoamine transporters

Adam W. Oaks, Anita Sidhu *

Department of Biochemistry, Molecular, Cellular Biology and Lombardi Cancer Center, Georgetown University, Washington, DC 20007, United States

ARTICLE INFO

Article history:
Received 2 February 2011
Accepted 4 March 2011
Available online 12 March 2011

Edited by Jesus Avila

Keywords:
Parkinson's disease
Depression
Mood disorder
Anti-depressant

ABSTRACT

Although well-studied in the context of neurodegenerative disease, a clear biological function for the synuclein proteins remains elusive. Emerging data indicate a role for synucleins in monoamine neurotransmitter homeostasis. A key regulatory component of monoamine neurotransmission is re-uptake of neurotransmitter by the dopamine transporter, norepinephrine transporter, and serotonin transporter, which are common drug targets in the treatment of depression and other mood disorders. Through interactions with these transporters, the neuronal cytoskeleton, and pre-synaptic scaffolding proteins, α-synuclein, β-synuclein, and γ-synuclein modulate trafficking, expression and function of monoamine transporters at the cell surface, thus playing a central role in regulating monoamine re-uptake.

1. Introduction

The synuclein family of proteins, and especially α-synuclein (α-Syn), has been linked to Parkinson's disease (PD), Alzheimer's disease (AD), and other neurodegenerative conditions. Studied primarily in this context, the synucleins continue to elude functional classification [1]. Emerging among competing hypotheses regarding their function is evidence of a role for synucleins in regulating homeostasis of the monoamine neurotransmitters dopamine (DA), norepinephrine (NE), and serotonin (5-hydroxytryptamine, 5-HT) [2–4]. Monoamine neurotransmission modulates many physiological processes and is regulated, in part, by presynaptic plasma membrane monoamine transporters (MAT), the transmembrane proteins solely responsible for re-uptake of synaptic DA, NE, and 5-HT [5]. Due to their essential role within the brain of recovering monoamine neurotransmitters, the MAT are important pharmacological targets in the treatment of several neuropsychiatric conditions, including depression, other mood disorders, and addiction [5]. Physical interactions between synuclein proteins and MAT indicate an important role for the synucleins in regulating transporter function, trafficking and distribution at the synapse. Further elucidation of these mechanisms will bridge gaps in our knowledge of the function of synuclein proteins in both normal and disease states. Thus, this review is constructed with three principal goals: (1) to summarize the characterization of synuclein proteins within the process of monoamine synthesis and release; (2) to present data that points to an interactive involvement of synucleins and MAT in the regulation of monoamine reuptake; and (3) to outline future directions for research into this novel mechanism of synuclein-dependent monoamine homeostasis.

2. Synucleins in monoamine neurotransmitter release and synthesis

Orthologous genes cloned from multiple species demonstrate that synucleins, a group of prevalent pre-synaptic proteins, are highly conserved but unique to vertebrate organisms [1]. This family of genes has been expanded to include multiple paralogues identified as α-Syn, β-synuclein (β-Syn), and γ-synuclein (γ-Syn). Expression of these proteins varies throughout the central nervous system (CNS) and also developmentally [1]. α-Syn, β-Syn, and γ-Syn share significant sequence identity: an N-terminal series of 11-residue repeats (7–87 in α-Syn), a centrally located hydrophobic region (61–95 in α-Syn), and an acidic C-terminal domain (96–140 in α-Syn). The central region of α-Syn composes the non-Aβ component of AD amyloid (NAC), and as such is known as the NAC domain [1]. Synucleins participate in numerous interactions with other proteins, lipid membranes, and nucleic acids, suggesting a possible role in the chaperoning or trafficking of biomolecules [1]. Indeed,

Abbreviations: α-Syn, α-synuclein; β-Syn, β-synuclein; γ-Syn, γ-synuclein; AD, Alzheimer's disease; CNS, central nervous system; DA, dopamine; DAT, dopamine transporter; DMI, desipramine; MAT, presynaptic plasma membrane monoamine transporters; NAC, non-Aβ component of AD amyloid; NE, norepinephrine; NET, norepinephrine transporter; PD, Parkinson's disease; 5-HT, serotonin, 5-hydroxytryptamine; SERT, serotonin transporter; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; Synt-1, syntaxin 1A; VMAT2, vesicular monoamine transporter 2; WIS, Wistar rat; WKY, Wistar-Kyoto rat

* Corresponding author. Address: Laboratory of Molecular Neurochemistry, The Research Building, Room W222, 3970 Reservoir Road, NW, Washington, DC 20007, United States. Fax: +1 202 687 0279.

E-mail address: sidhua@georgetown.edu (A. Sidhu).

0014-5793/$36.00
doi:10.1016/j.febslet.2011.03.009

the N-terminal portion of α-Syn shares 40% identity with the 14-3-3 proteins [6], and is the most conserved region among α-Syn, β-Syn, and γ-Syn, all of which possess chaperone-like activity [7]. Although studies from single, double, and triple synuclein knockout (KO) mice indicate that synucleins are not essential for viable development [8–10], data from these same mice have nonetheless shown repeatedly that the synucleins are required for normal presynaptic function [8–15].

Among possible presynaptic functions that synucleins may perform is regulation of the synthesis, release, and reuptake of monoamine neurotransmitters (Fig. 1). The involvement of synucleins in monoamine homeostasis has been explored in part to determine the connection between α-Syn and the profound loss of dopaminergic neurons that occurs in PD. α-Syn accumulates in Lewy bodies, a hallmark of PD, and is linked with both familial and idiopathic forms of the disease. Availability of DA depends in part on the activity of tyrosine hydroxylase (TH), an enzyme in the biosynthetic pathways of both DA and NE (Fig. 1(1)). It was shown that TH enzymatic activity can be regulated through direct interactions with α-Syn [16], and that expression of TH was increased in the retina of α-Syn/γ-Syn double knockout (KO) mice compared to wild type and single KO mice [14]. While no evidence exists for an interaction between β-Syn and TH, it has been suggested that β-Syn overlaps functionally with α-Syn [1]. No reports have emerged of a similar interaction between synucleins and tryptophan hydroxylase (TrH), the rate-limiting enzyme in the production of 5-HT. Loss of TrH neurons in serotonergic nuclei, however, has also been associated with neurodegenerative synucleinopathies [17].

In addition to regulation of neurotransmitter synthesis, synucleins are involved in the storage of neurotransmitters (Fig. 1(2)). Release of synthesized neurotransmitter by monoaminergic neurons in the brain requires packaging of DA, NE, or 5-HT into vesicles by the vesicular monoamine transporter 2 (VMAT2). VMAT2 co-localizes with α-Syn in the Lewy bodies of PD [18], and overexpression of α-Syn can disrupt VMAT2 function in various contexts [1]. The influence of β-Syn and γ-Syn upon VMAT2 expression and activity are not known.

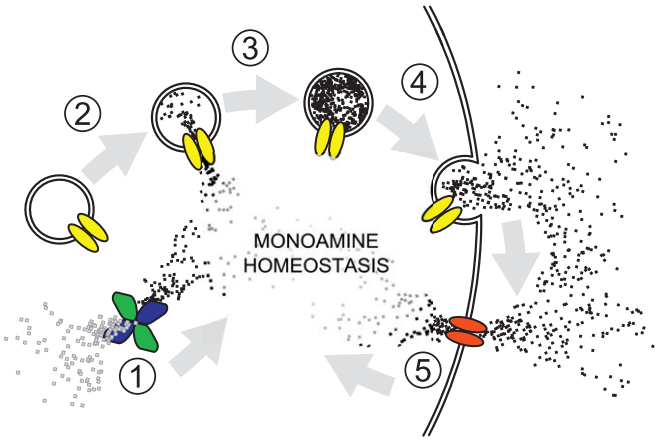

Fig. 1. Synucleins modulate monoamine homeostasis. Monoamine neurotransmitter signaling in the brain is regulated at several levels, including biosynthesis (1), vesicular refilling and release (2–4), and reuptake (5). All of these processes are impacted by at least one member of the synuclein family of proteins. The activity of tyrosine hydroxylase, which is on the biosynthetic pathway (1) of dopamine and norepinephrine, is modulated by α-Syn [16]. Synthesized monoamine neurotransmitters must be loaded into vesicles through the vesicular monoamine transporter (VMAT2). Expression and activity of VMAT2 can be modulated by α-Syn [1,18], thus regulating the rate of vesicular refilling (2). Synuclein levels alter the number and distribution of neurotransmitter vesicles [8,9,11,13,15], thus directly impacting the process of vesicle translocation (3). SNARE-mediated fusion of vesicles with presynaptic membrane releases monoamine neurotransmitters into the synapse (4). Formation of functional SNARE complexes is dependent on normal synuclein levels [10]. Finally, released neurotransmitter is cleared from the synapse by the monoamine transporters (MAT). MAT function is dependent on regulated trafficking to the cell surface (5), which is modulated by all three synucleins [3,4,23,24,27–39,46].

Synaptic vesicles filled by VMAT2 must be translocated prior to neurotransmitter release, and are subject to regulated trafficking to the cell surface (Fig. 1(3)). Maintenance of normal levels of the monoamine neurotransmitters is in part dependent on the number, size, and location of loaded vesicles. α-Syn KO mice have reduced DA and NE storage capacity, and ultrastructural studies show a depleted reserve pool of monoamine storage vesicles [11,13,15]. Dopaminergic and noradrenergic activity in these animals may be preserved through an increased rate of vesicular refilling, suggesting a compensatory mechanism that produces an outwardly normal phenotype, even in the absence of α-Syn expression [13,15]. Double α-Syn/β-Syn KO mice have synaptic ultrastructure similar to single KO mice, and striatal DA content in these animals is significantly reduced compared to single KO mice [9]. Studies on double α-Syn/γ-Syn KO mice did not reveal altered DA content [12], though a hyperdopaminergic phenotype supports the conclusion that the absence of synucleins alters the management of monoamines in presynaptic vesicle pools [8].

Release of neurotransmitters, including DA, NE, and 5-HT, requires fusion of loaded vesicles with the presynaptic membrane through a soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) dependent process [19]. SNARE-dependent vesicle activity occurs on a millisecond time scale, and is tightly regulated by other pre-synaptic proteins, including the co-chaperone cysteine-string protein α (CSPα) [19]. Deletion of CSPα produces a lethal neurodegenerative phenotype that is reversed by over-expression of α-Syn, restoring normal levels of SNARE complex assembly [19]. α-Syn interacts with the SNARE protein SNAP-25, and promotes SNARE assembly in vitro [10], suggesting that synucleins participate in the process of neurotransmitter release through an interaction with the vesicle exocytosis machinery (Fig. 1(4)). Indeed, triple α-Syn/β-Syn/γ-Syn KO mice, though viable, develop deficits in motor function and show impaired SNARE complex assembly in the brain [10]. This result indicates a direct and functionally consequential involvement of synucleins in SNARE-dependent presynaptic activity. While a key function of SNARE proteins is regulation of neurotransmitter release, the SNARE complex is also activated in other processes that require trafficking of vesicles or other membrane-bound structures to the presynaptic membrane, including the process of neurotransmitter reuptake that is performed by the dopamine transporter (DAT), norepinephrine transporter (NET), and serotonin transporter (SERT).

3. Synuclein modulation of MAT

DAT, NET, and SERT have highly similar primary sequences of 620, 617, and 630 amino acids, respectively [5]. They share 12 predicted α-helical transmembrane domains, and each transporter has extended intracellular tails as well as large extracellular loops that are subject to numerous post-translation modifications and may be involved in MAT regulation [5]. Localized to the plasma membrane of pre-synaptic neurons, MAT have a central role in monoamine homeostasis, providing the primary means by which their respective neurotransmitter substrates can be removed from the synapse (Fig. 1(5)) [5]. This reuptake action regulates neurotransmission by terminating signaling as DA, NE, or 5-HT are drawn back into the presynaptic neuron. Reuptake also serves as a first step in neurotransmitter recycling, placing recovered monoamines in position for reloading into synaptic vesicles [5]. MAT trafficking and activity at the cell surface is regulated by the SNARE

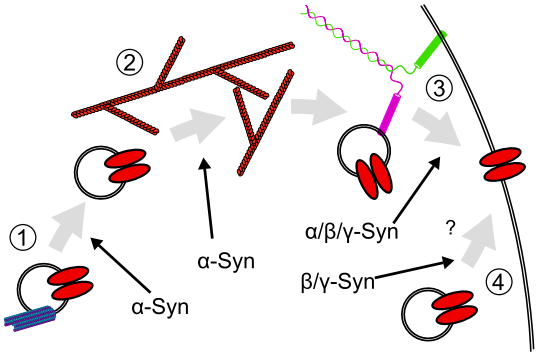

Fig. 2. Synuclein modulation of monoamine transporter trafficking. Monoamine transporters (MAT) are synthesized and transported to the pre-synaptic membrane on membrane-bound structures. Before arriving at the cell surface, MAT must travel along the axon via a microtubule network (1), transit the actin microfilament matrix (2), and fuse with the cell surface in a SNARE-dependent manner (3). These processes are each impacted by some or all of the synuclein proteins. α-Syn is involved in mediating microtubule-based MAT transport (1), as modulation of both DAT and NET by α-Syn is dependent upon an intact microtubule cytoskeleton [24,27–29]. α-Syn also mediates actin-based MAT transport (2), as modulation of NET by α-Syn is dependent on an intact actin cytoskeleton [27]. Synucleins enable normal SNARE complex formation [10], suggesting that SNARE-dependent insertion of MAT into the plasma membrane is also modulated by synucleins (3). Modulation of MAT by β-Syn and γ-Syn is not sensitive to agents that target the cytoskeleton [24], and may occur through a distinct, as yet un-identified mechanism (4).

protein syntaxin 1A (Synt-1) [20–22]. As SNARE complex assembly is in part dependent on the synucleins [10], it is possible that synucleins also play a role in mediating the effects on MAT of Synt-1 (Fig. 2(3)), although this has not been directly demonstrated.

In addition to putative modulation of MAT function through effects on SNARE proteins, synucleins appear to directly regulate MAT by another, possibly independent, mechanism (Fig. 2). Synucleins were identified as potential participants in MAT regulatory processes due to their overlap in localization and mutual involvement in diseases of the monoaminergic systems [23]. Through protein:protein interactions with DAT, NET, and SERT, it has been shown that α-Syn, β-Syn, and γ-Syn can each modulate trafficking of some or all of these transporters to the cell surface, thus influencing reuptake of neurotransmitters [2,4,24]. Trafficking of MAT is dependent upon interactions with the cytoskeleton and other pre-synaptic scaffolding proteins [25,26], and these interactions between MAT and the cytoskeleton can be modulated by the synucleins (Fig. 2(1) and (2)) [24,27–29]. Regulation of MAT function by different mechanisms can be variously positive (increases uptake) or negative (decreases uptake), and there is evidence that synucleins can influence MAT trafficking both positively and negatively [23,27]. Data in support of an important role for α-Syn, β-Syn, and γ-Syn in modulation of DAT, NET, and SERT continues to accumulate (Table 1), and will be described in detail below.

4. Synuclein modulation of DAT

The most thoroughly investigated synuclein-MAT interaction is that between α-Syn and DAT. Initial yeast two-hybrid and immunoprecipitation experiments identified a physical interaction between α-Syn and the carboxy terminal of DAT [23]. Affinity purification experiments showed that the NAC domain of α-Syn mediates the interaction with DAT [30]. Though little doubt remains as to the existence of a physical interaction between α-Syn and DAT, results characterizing the functional significance of this interaction are conflicting, showing that overexpression of α-Syn in DAT expressing cells can variously increase or decrease DAT trafficking to the cell surface under different conditions [23,31]. It was proposed that α-Syn overexpression enhanced neurodegenerative processes by promoting the influx of DA, a compound which in excess becomes cytotoxic through the induction of oxidative damage [23]. These results prompted further study, which uncovered inconsistencies in the observed phenomena.

Table 1
Characterization of synuclein–MAT interactions. Physical interactions determined by yeast two-hybrid screens or co-immunoprecipitation. Modulation of surface expression determined by neurotransmitter uptake assays, behavioral response to cocaine, cell fractionation, and biotinylation experiments. Microtubule and actin dependence of modulation determined in the presence of cytoskeleton disrupting agents. +, occurs; –, no effect/does not occur; ↑, increase; ↓, decrease; ↑/↓, concentration dependent; ND, not determined; N/A, not applicable. References are as noted.

	DAT	NET	SERT	References
Physical interaction with				
α-Synuclein	+	+	+	DAT: [23,26,29–33] NET: [3,24,27,28] SERT: [4,46]
β-Synuclein	ND	ND	–	SERT: [46]
γ-Synuclein	ND	+	+	NET: [24] SERT: [46]
Modulation of surface expression				
α-Synuclein	↑/↓	↑/↓	↓	DAT: [23,29–38] NET: [3,24,27,28] SERT: [29,46]
β-Synuclein	ND	↓	–	NET: [24] SERT: [46]
γ-Synuclein	↓	↓	↓	DAT: [39] NET: [24] SERT: [46]
Modulation is microtubule dependent				
α-Synuclein	+	+	ND	DAT: [29] NET: [24,27,28]
β-Synuclein	ND	–	N/A	NET: [24]
γ-Synuclein	ND	–	ND	NET: [24]
Modulation is actin dependent				
α-Synuclein	ND	+	ND	NET: [27]
β-Synuclein	ND	ND	N/A	N/A
γ-Synuclein	ND	ND	ND	N/A

Our work has established a large body of evidence demonstrating negative modulation of DAT by α-Syn. While a physical interaction between α-Syn and DAT was confirmed by co-immunoprecipitation, the positive influence of α-Syn on DAT trafficking has not been reconfirmed. Experiments in several cell lines as well as cultured rat neurons showed dose-dependent *reduction* of cell surface DAT expression by α-Syn [29–33]. Together, the data indicates that when co-expressed with DAT, the role of α-Syn is to reduce DAT function by limiting cell surface expression through increased trafficking away from the cell surface. This interaction does not appear to alter the function of the transporter, as affinity for substrate was unaffected. Rather, DAT availability is reduced, as indicated by the reduction of maximum uptake capacity observed in co-transfected cells [30].

Studies using lentiviral vectors to modify endogenous levels of α-Syn in the rat brain show that the locomotor response to cocaine is also α-Syn dependent [34]. Hyperlocomotion following administration of cocaine is thought to result from blockade of DAT, the effect of which is to prevent clearance of DA from the synapse. Extended DA signaling then causes abnormally high measures on a standard behavioral measure of locomotor activity, such as total distance traveled. Increases in α-Syn levels are associated with increases in the locomotor response, suggesting that α-Syn retards cell-surface localization of DAT. That is, with high levels of α-Syn, less DAT is available at the cell surface, and the cocaine blockade of DA transport is longer and more complete than it would be under normal conditions, producing the observed increase in the locomotor response [34]. These results are consistent with a model wherein DAT is negatively modulated by increases in endogenous

levels of α-Syn. Nonetheless, it is clear that α-Syn modulation of DAT is a homeostatic process that is extremely context dependent. The effects of α-Syn on DAT can be modulated by the adherent properties of co-expressing cells, the route of administration of α-Syn, or the means of regulation of α-Syn expression [32,35–37]. Data have shown that knockdown or overexpression of α-Syn can have unexpected effects on DAT, and may impact other aspects of DAT function, including synthesis and export from the Golgi apparatus [38]. Further assessment of the interaction between the endogenous regulatory processes controlling α-Syn expression and DAT activity is required to resolve the complex functional relationship between these proteins.

Given the overall sequence similarity of α-Syn and γ-Syn, and greater than 50% identity in the NAC domain, it was a logical progression to look for a similar interaction between γ-Syn and DAT. Indeed, knock-down of endogenous γ-Syn expression with lentiviral siRNA also significantly reduces the locomotor response to cocaine, while overexpression of γ-Syn and DAT resulted in increased locomotor activity [39]. Further investigation into this phenomenon is required, as the mechanism by which γ-Syn modulates DAT has not been addressed. Nonetheless, the finding that γ-Syn and α-Syn modulate DAT in a similar manner provides confirmation of the importance of the NAC domain in mediating MAT modulation synucleins. As predicted by this line of reasoning, no interaction has thus far been identified between β-Syn and DAT.

Another feature of synuclein–DAT interactions is the dependence of α-Syn modulation of DAT on an intact microtubule cytoskeleton. Nocodazole, a microtuble destabilizing agent, reverses the α-Syn dependent inhibition of DAT [29], indicating that the observed modulation of DAT by α-Syn requires a cytoskeletal substrate on which to anchor the transporter. The dependence of synuclein modulation of MAT on the cytoskeleton is discussed in greater detail below.

5. Synuclein modulation of NET

Noradrenergic projections are present throughout the CNS and the regulation of their NE release is tied to NET expression and cell surface localization. The nature of the interactions between synuclein proteins and NET is established by a small number of studies. Given the similarities in structure and function between the MAT, however, it is not surprising that a similar set of results emerged from cellular models of NET trafficking. An increasing level of α-Syn expression negatively modulates NET uptake activity by decreasing NET expression at the cell surface [3,28]. Physical interaction between α-Syn and NET was confirmed by co-immunoprecipitation from both cultured cells and rat brain tissue, and it was shown that the observed physical and functional interaction was dependent on the NAC domain [3].

Modulation of NET by α-Syn is not strictly monotonic, as different ratios of co-transfection are capable of producing either positive or negative effects on NET trafficking [28]. Low levels of α-Syn (0.5:1 co-transfection ratio) induce an increase in NET uptake capacity compared to α-Syn negative cells, while overexpression (3:1 co-transfection ratio) brings about a decrease in the V_{max} of NE uptake [28], without altering affinity for the NE substrate. Cell surface expression and physical interaction (co-immunoprecipitation of NET and α-Syn) follows a similar pattern. This finding supports a homeostatic role for synucleins, indicating that normal NET expression may depend upon a certain baseline level of synuclein–NET interaction, and that extremes of expression both above and below this level can result in negative modulation of NET activity. While this pattern of regulation has not been demonstrated within any single study on DAT (see above), what appear to be conflicting observations from separate studies may in fact be demonstrating the functional effects of expression either above or below the level for "optimal" synuclein–DAT interaction.

NET trafficking is also modulated by both β-Syn and γ-Syn, as increased expression of either synuclein reduces cell-surface NET in vitro [24]. Modulation of NET by β-Syn is surprising in that it must be NAC-domain independent, as this domain is almost entirely absent in the β-Syn protein. Though the effects of β-Syn and γ-Syn in co-transfected cells are monotonic, experiments on Wistar (WIS) and Wistar-Kyoto (WKY) rats have revealed a complex three-way interaction between α-Syn, γ-Syn, and NET expression [24], suggesting that multiple synuclein binding partners of NET may cooperate or compete presynaptically to accomplish transporter modulation.

WKY rats are an outbred strain of WIS rats, and have a depressive-like phenotype that is well characterized on traditional behavioral measures such as the forced swim test [40]. These animals have reduced expression of NET, which also occurs in patients with major depression [24,41]. Reduced expression of NET may be part of a homeostatic mechanism that attempts to compensate for reduced NE release in major depression [42]. NET is the target of many antidepressant drugs, the acute effect of which is to further limit re-uptake of synaptic NE. Chronic exposure to antidepressants also alters cell-surface levels of NET, reducing availability of the transporter in the brain [43]. Given that NET trafficking is modulated by α-Syn, β-Syn, and γ-Syn, it was hypothesized that synuclein expression and NET trafficking in the WKY strain would differ from the WIS control strain [24]. Furthermore, it was proposed on the basis of the available information on antidepressant effects on NET function and expression, that chronic treatment with the tri-cyclic antidepressant desipramine (DMI) would improve depressive-like behavior, possibly by a mechanism involving alterations in the expression, distribution, and interactions of synuclein proteins [24].

The findings were consistent with previous observations on the mechanisms underlying depression and antidepressant therapy in the human population. WKY rats displayed a more depressive-like behavioral phenotype and had lower overall NET expression, both of which were altered by chronic administration of DMI [24]. Differences in the baseline expression levels of α-Syn to γ-Syn were also detected between these animals. More importantly, chronic DMI treatment induced a shift in both the balance and the distribution of α-Syn and γ-Syn expression throughout the brain [24,41]. These changes in synuclein expression are associated with altered interactions with NET and the microtubule cytoskeleton [24]. It was not determined whether these shifts in expression were associated with alterations in synuclein transcription, and future work should address this question directly. Transcriptional regulation of synuclein mRNA expression is poorly understood, and the identification of a mode of regulation that responds to drug treatment could be an important means for addressing the broad range of mood and neurodegenerative conditions potentially impacted by synuclein imbalances.

The α-Syn-dependent modulation of NET cell-surface expression was reversed by treatment with the microtubule destabilizing agents nocodazole, colchicine, and vinblastine [27,28]. An intact, dynamic actin cytoskeleton was also required for the α-Syn modulation of NET to occur [27]. Modulation of NET by β-Syn and γ-Syn, however, was not affected by treatment of co-transfected cells with nocodazole [24]. In the rat brain, the effect of nocodazole on NE uptake was dependent on the relative expression of α-Syn and γ-Syn. In synaptosomes isolated from tissues where α-Syn is the predominant synuclein, NET activity is nocodazole sensitive [24]. Increased expression of γ-Syn, however, disconnects NET modulation from the integrity of the microtubule cytoskeleton. Distribution of γ-Syn in cultured cells was not altered by microtubule destabilizers [44], and that the efficacy of microtubule-directed chemotherapeu-

tic agents is limited in γ-Syn overexpressing cells [45]. In WKY rats with a depressive-like phenotype, chronic administration of the NET-selective antidepressant (DMI) decreased γ-Syn expression and increased the sensitivity of NET distribution to nocodazole [24]. Taken together, these findings suggest that γ-Syn is responsible for cytoskeleton-independent regulation of diverse cellular processes, including noradrenergic neurotransmission. β-Syn modulation of NET in co-transfected cells is also resistant to nocodazole [24], indicating that β-Syn may also act on NET in a microtubule independent manner, though more studies will be required to adequately describe this process.

6. Synuclein modulation of SERT

Physical and functional interactions between synuclein proteins and SERT have also been assessed, showing that the impact of α-Syn overexpression on SERT trafficking follows the general pattern observed with DAT and NET. Cell-surface expression and uptake activity of SERT in co-transfected cells are negatively modulated by α-Syn in an NAC-domain dependent manner [4]. A physical interaction between α-Syn and SERT was confirmed by immunoprecipitation both in cultured cells and in rat brain tissue [4], and interactions between SERT and γ-Syn have been demonstrated in co-transfected cells [46]. An assessment of γ-Syn and β-Syn interactions shows that while β-Syn does not modulate SERT activity, overexpression of γ-Syn can induce partial negative modulation of SERT distribution to the cell surface [46]. It is not yet clear whether modulation of SERT trafficking by the synucleins is dependent on the microtubule or actin cytoskeletons. Preliminary findings indicted that microtubule destabilizing agents disrupt α-Syn modulation of SERT, reversing the inhibition of uptake in co-transfected cells [A. Sidhu, unpublished data]. Also, rat brain synaptosomes have a microtubule-dependent component of SERT uptake capacity that can be accessed by treatment with nocodazole, which may therefore be modulated by the relative levels of α-Syn and γ-Syn expression [A. Sidhu, unpublished data].

7. Synuclein and MAT at the synapse

A key feature of all α-Syn–MAT interactions has been their requirement of the NAC domain, which is well-conserved between α-Syn and γ-Syn. Though this dependence has not been demonstrated clearly for γ-Syn modulation of MAT, both synucleins that contain the NAC domain are found in complex with MAT. While β-Syn, which is missing most of this sequence, can modulate NET when overexpressed [24], it does not modulate SERT, nor has a physical interaction with any MAT yet been demonstrated. One possible conclusion that emerges is that the NAC domain is not only *necessary* for interaction with MAT, it is also *sufficient* for modulation of transporter activity. Thus, synucleins, through interactions of this domain with MAT, may be displacing or cooperatively enhancing interactions with another MAT binding partner in a manner that either blocks insertion and or promotes endocytosis of the transporters. The trafficking of MAT, and ultimately the level of cell surface expression, appears to also depend on the cytoskeletal elements of the cell. A model emerges wherein synucleins tether MAT to the cytoskeleton (Fig. 2(1) and (2)), yet are differentially sensitive to targeted disruption of cytoskeletal components, enabling fine-tuning of MAT trafficking through diverse pre-synaptic protein–protein interactions (Fig. 2(3) and (4)).

8. Future studies

Investigation into the involvement of synuclein proteins in SNARE-mediated regulation of MAT trafficking and activity is warranted given the important contributions of this process to monoamine homeostasis [20–22]. Furthermore, it was recently demonstrated that SNARE complex formation is partially dependent on expression of the synuclein proteins [10], which strongly suggests that modulation of MAT by synucleins involves interactions with SNARE proteins, likely including Synt-1. A unified description of presynaptic MAT trafficking that involves both SNARE proteins and the synucleins is an attractive theory that would synthesize mechanisms that to this point have been described in isolation. Progress in this area will likely require continued utilization of α-Syn/β-Syn/γ-Syn triple KO animals, which are the only synuclein KO mice to display a prominent behavioral and neurochemical phenotype [8,10–13,15]. Indeed, studies of single α-Syn KO mice, along with other models of synuclein function, have produced some negative results that highlight the need for a more nuanced approach in this area of investigation [47,48]. Nonetheless, the body of work described here defines synucleins among the crowded presynaptic milieu as a means by which monoamine neurotransmission can be regulated. In particular, modulation of DAT, NET, and SERT by the synucleins stands out as especially important because of the central role of these transporters not only in normal neuronal signaling, but in a range of disease states including mood disorders and neurodegenerative conditions.

Future work should also attempt to address the mechanisms by which antidepressants and other MAT-directed drugs alter synuclein expression. This stems from the finding that chronic treatment with DMI induces changes in α-Syn and γ-Syn expression [24,41]. Additional studies have now linked antidepressant drugs with synuclein expression [49,50], and though it is not known how this effect is exerted, further investigation could help elucidate the mechanisms responsible for transcriptional and translational control of synucleins. Furthermore, the mechanism by which synuclein expression alters MAT distribution should be investigated to determine the influences of synuclein expression on Synt-1-dependent trafficking of MAT. Studies have indicated a clear role for α-Syn, β-Syn, and γ-Syn in the trafficking of MAT, and suggest that synucleins are likely to affect many other presynaptic proteins that take part in this complex, multi-faceted process.

Acknowledgment

The preparation of this manuscript was supported in part by grants from NIH: R01MH075020, R01AG28108, and R01NS060041 [A.S.].

References

[1] Surguchov, A. (2008) Molecular and cellular biology of synucleins. Int. Rev. Cell Mol. Biol. 270, 225–317.
[2] Sidhu, A., Wersinger, C. and Vernier, P. (2004) alpha-Synuclein regulation of the dopaminergic transporter: a possible role in the pathogenesis of Parkinson's disease. FEBS Lett. 565 (1–3), 1–5.
[3] Wersinger, C., Jeannotte, A. and Sidhu, A. (2006) Attenuation of the norepinephrine transporter activity and trafficking via interactions with alpha-synuclein. Eur. J. Neurosci. 24 (11), 3141–3152.
[4] Wersinger, C., Rusnak, M. and Sidhu, A. (2006) Modulation of the trafficking of the human serotonin transporter by human alpha-synuclein. Eur. J. Neurosci. 24 (1), 55–64.
[5] Torres, G.E., Gainetdinov, R.R. and Caron, M.G. (2003) Plasma membrane monoamine transporters: structure, regulation and function. Nat. Rev. Neurosci. 4 (1), 13–25.
[6] Ostrerova, N., Petrucelli, L., Farrer, M., Mehta, N., Choi, P., Hardy, J. and Wolozin, B. (1999) alpha-Synuclein shares physical and functional homology with 14-3-3 proteins. J. Neurosci. 19 (14), 5782–5791.
[7] Souza, J.M., Giasson, B.I., Lee, V.M. and Ischiropoulos, H. (2000) Chaperone-like activity of synucleins. FEBS Lett. 474 (1), 116–119.
[8] Senior, S.L., Ninkina, N., Deacon, R., Bannerman, D., Buchman, V.L., Cragg, S.J. and Wade-Martins, R. (2008) Increased striatal dopamine release and hyperdopaminergic-like behaviour in mice lacking both alpha-synuclein and gamma-synuclein. Eur. J. Neurosci. 27 (4), 947–957.

[9] Chandra, S., Fornai, F., Kwon, H.B., Yazdani, U., Atasoy, D., Liu, X., Hammer, R.E., Battaglia, G., German, D.C., Castillo, P.E. and Sudhof, T.C. (2004) Double-knockout mice for alpha- and beta-synucleins: effect on synaptic functions. Proc. Natl. Acad. Sci. USA 101 (41), 14966–14971.
[10] Burre, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M. and Sudhof, T.C. (2010) {alpha}-Synuclein promotes SNARE-complex assembly in vivo and in vitro. Science.
[11] Cabin, D.E., Shimazu, K., Murphy, D., Cole, N.B., Gottschalk, W., McIlwain, K.L., Orrison, B., Chen, A., Ellis, C.E., Paylor, R., Lu, B. and Nussbaum, R.L. (2002) Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged repetitive stimulation in mice lacking alpha-synuclein. J. Neurosci. 22 (20), 8797–8807.
[12] Robertson, D.C., Schmidt, O., Ninkina, N., Jones, P.A., Sharkey, J. and Buchman, V.L. (2004) Developmental loss and resistance to MPTP toxicity of dopaminergic neurones in substantia nigra pars compacta of gamma-synuclein, alpha-synuclein and double alpha/gamma-synuclein null mutant mice. J. Neurochem. 89 (5), 1126–1136.
[13] Yavich, L., Tanila, H., Vepsalainen, S. and Jakala, P. (2004) Role of alpha-synuclein in presynaptic dopamine recruitment. J. Neurosci. 24 (49), 11165–11170.
[14] Surgucheva, I., Ninkina, N., Buchman, V.L., Grasing, K. and Surguchov, A. (2005) Protein aggregation in retinal cells and approaches to cell protection. Cell. Mol. Neurobiol. 25 (6), 1051–1066.
[15] Yavich, L., Jakala, P. and Tanila, H. (2006) Abnormal compartmentalization of norepinephrine in mouse dentate gyrus in alpha-synuclein knockout and A30P transgenic mice. J. Neurochem. 99 (3), 724–732.
[16] Perez, R.G., Waymire, J.C., Lin, E., Liu, J.J., Guo, F. and Zigmond, M.J. (2002) A role for alpha-synuclein in the regulation of dopamine biosynthesis. J. Neurosci. 22 (8), 3090–3099.
[17] Benarroch, E.E., Schmeichel, A.M., Sandroni, P., Parisi, J.E. and Low, P.A. (2007) Rostral raphe involvement in Lewy body dementia and multiple system atrophy. Acta Neuropathol. 114 (3), 213–220.
[18] Yamamoto, S., Fukae, J., Mori, H., Mizuno, Y. and Hattori, N. (2006) Positive immunoreactivity for vesicular monoamine transporter 2 in Lewy bodies and Lewy neurites in substantia nigra. Neurosci. Lett. 396 (3), 187–191.
[19] Chandra, S., Gallardo, G., Fernández-Chacón, R., Schlüter, O.M. and Südhof, T.C. (2005) [alpha]-Synuclein cooperates with CSP[alpha] in preventing neurodegeneration. Cell 123 (3), 383–396.
[20] Sung, U., Apparsundaram, S., Galli, A., Kahlig, K.M., Savchenko, V., Schroeter, S., Quick, M.W. and Blakely, R.D. (2003) A regulated interaction of syntaxin 1A with the antidepressant-sensitive norepinephrine transporter establishes catecholamine clearance capacity. J. Neurosci. 23 (5), 1697–1709.
[21] Samuvel, D.J., Jayanthi, L.D., Bhat, N.R. and Ramamoorthy, S. (2005) A role for p38 mitogen-activated protein kinase in the regulation of the serotonin transporter: evidence for distinct cellular mechanisms involved in transporter surface expression. J. Neurosci. 25 (1), 29–41.
[22] Binda, F., Dipace, C., Bowton, E., Robertson, S.D., Lute, B.J., Fog, J.U., Zhang, M., Sen, N., Colbran, R.J., Gnegy, M.E., Gether, U., Javitch, J.A., Erreger, K. and Galli, A. (2008) Syntaxin 1A interaction with the dopamine transporter promotes amphetamine-induced dopamine efflux. Mol. Pharmacol. 74 (4), 1101–1108.
[23] Lee, F.J., Liu, F., Pristupa, Z.B. and Niznik, H.B. (2001) Direct binding and functional coupling of alpha-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis. FASEB J. 15 (6), 916–926.
[24] Jeannotte, A.M., McCarthy, J.G., Redei, E.E. and Sidhu, A. (2009) Desipramine modulation of alpha-, gamma-synuclein, and the norepinephrine transporter in an animal model of depression. Neuropsychopharmacology 34 (4), 987–998.
[25] González, M.I. and Robinson, M.B. (2004) Neurotransmitter transporters: why dance with so many partners? Curr. Opin. Pharmacol. 4 (1), 30–35.
[26] Maiya, R., Mayfield, R.D. and Lisa, A.N. (2004) Dopamine transporter network and pathways. International Review of Neurobiology, Academic Press. p. 79–96.
[27] Jeannotte, A.M. and Sidhu, A. (2008) Regulated interactions of the norepineprhine transporter by the actin and microtubule cytoskeletons. J. Neurochem. 105 (5), 1668–1682.
[28] Jeannotte, A.M. and Sidhu, A. (2007) Regulation of the norepinephrine transporter by alpha-synuclein-mediated interactions with microtubules. Eur. J. Neurosci. 26 (6), 1509–1520.
[29] Wersinger, C. and Sidhu, A. (2005) Disruption of the interaction of alpha-synuclein with microtubules enhances cell surface recruitment of the dopamine transporter. Biochemistry 44 (41), 13612–13624.
[30] Wersinger, C., Prou, D., Vernier, P. and Sidhu, A. (2003) Modulation of dopamine transporter function by alpha-synuclein is altered by impairment of cell adhesion and by induction of oxidative stress. FASEB J. 17 (14), 2151–2153.
[31] Wersinger, C. and Sidhu, A. (2003) Attenuation of dopamine transporter activity by alpha-synuclein. Neurosci. Lett. 340 (3), 189–192.
[32] Wersinger, C., Vernier, P. and Sidhu, A. (2004) Trypsin disrupts the trafficking of the human dopamine transporter by alpha-synuclein and its A30P mutant. Biochemistry 43 (5), 1242–1253.
[33] Wersinger, C., Prou, D., Vernier, P., Niznik, H.B. and Sidhu, A. (2003) Mutations in the lipid-binding domain of alpha-synuclein confer overlapping, yet distinct, functional properties in the regulation of dopamine transporter activity. Mol. Cell. Neurosci. 24 (1), 91–105.
[34] Boyer, F. and Dreyer, J.L. (2007) alpha-Synuclein in the nucleus accumbens induces changes in cocaine behaviour in rats. Eur. J. Neurosci. 26 (10), 2764–2776.
[35] Adamczyk, A., Kazmierczak, A. and Strosznajder, J.B. (2006) [alpha]-Synuclein and its neurotoxic fragment inhibit dopamine uptake into rat striatal synaptosomes: relationship to nitric oxide. Neurochem. Int. 49 (4), 407–412.
[36] Fountaine, T.M. and Wade-Martins, R. (2007) RNA interference-mediated knockdown of alpha-synuclein protects human dopaminergic neuroblastoma cells from MPP(+) toxicity and reduces dopamine transport. J. Neurosci. Res. 85 (2), 351–363.
[37] Fountaine, T.M., Venda, L.L., Warrick, N., Christian, H.C., Brundin, P., Channon, K.M. and Wade-Martins, R. (2008) The effect of alpha-synuclein knockdown on MPP+ toxicity in models of human neurons. Eur. J. Neurosci. 28 (12), 2459–2473.
[38] Gosavi, N., Lee, H.-J., Lee, J.S., Patel, S. and Lee, S.-J. (2002) Golgi fragmentation occurs in the cells with prefibrillar alpha-synuclein aggregates and precedes the formation of fibrillar inclusion. J. Biol. Chem. 277 (50), 48984–48992.
[39] Boyer, F. and Dreyer, J.L. (2008) The role of gamma-synuclein in cocaine-induced behaviour in rats. Eur. J. Neurosci. 27 (11), 2938–2951.
[40] Lahmame, A., del Arco, C., Pazos, A., Yritia, M. and Armario, A. (1997) Are Wistar-Kyoto rats a genetic animal model of depression resistant to antidepressants? Eur. J. Pharmacol. 337 (2–3), 115–123.
[41] Jeannotte, A.M., McCarthy, J.G. and Sidhu, A. (2009) Desipramine induced changes in the norepinephrine transporter, alpha- and gamma-synuclein in the hippocampus, amygdala and striatum. Neurosci. Lett.
[42] Klimek, V., Stockmeier, C., Overholser, J., Meltzer, H.Y., Kalka, S., Dilley, G. and Ordway, G.A. (1997) Reduced levels of norepinephrine transporters in the locus coeruleus in major depression. J. Neurosci. 17 (21), 8451–8458.
[43] Hebert, C., Habimana, A., Elie, R. and Reader, T.A. (2001) Effects of chronic antidepressant treatments on 5-HT and NA transporters in rat brain: an autoradiographic study. Neurochem. Int. 38 (1), 63–74.
[44] Surguchov, A., Surgucheva, I., Solessio, E. and Baehr, W. (1999) Synoretin – a new protein belonging to the synuclein family. Mol. Cell. Neurosci. 13 (2), 95–103.
[45] Zhou, Y., Inaba, S. and Liu, J. (2006) Inhibition of synuclein-gamma expression increases the sensitivity of breast cancer cells to paclitaxel treatment. Int. J. Oncol. 29 (1), 289–295.
[46] Wersinger, C. and Sidhu, A. (2009) Partial regulation of serotonin transporter function by gamma-synuclein. Neurosci. Lett. 453 (3), 157–161.
[47] Schlüter, O.M., Fornai, F., Alessandrí, M.G., Takamori, S., Geppert, M., Jahn, R. and Südhof, T.C. (2003) Role of [alpha]-synuclein in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in mice. Neuroscience 118 (4), 985–1002.
[48] Pena-Oliver, Y., Buchman, V.L. and Stephens, D.N. (2010) Lack of involvement of alpha-synuclein on unconditioned anxiety in mice. Behav. Brain Res.
[49] Lee, J.H., Ko, E., Kim, Y.E., Min, J.Y., Liu, J., Kim, Y., Shin, M., Hong, M. and Bae, H. (2010) Gene expression profile analysis of genes in hippocampuses from antidepressant treated rats using DNA microarray. BMC Neurosci. 11 (1), 152.
[50] McHugh, P., Rogers, G., Glubb, D., Joyce, P. and Kennedy, M. (2009) Proteomic analysis of rat hippocampus exposed to the antidepressant paroxetine. J. Psychopharmacol.