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.

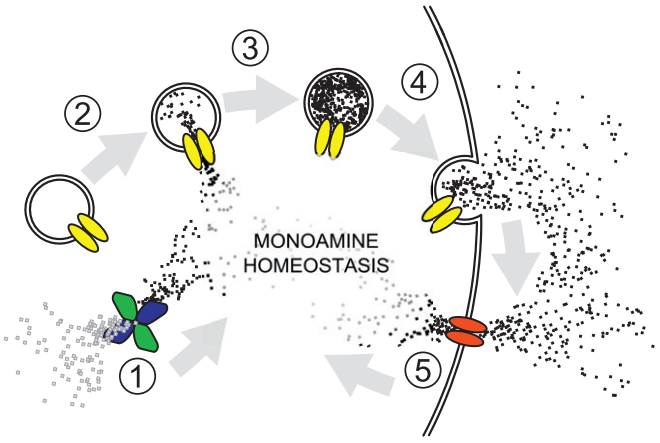

Fig. 1. Synucleins modulate monoamine homeostasis. Monoamine neurotransmitter signaling in the brain is regulated at several levels, including biosynthesis (1), vesicular refilling and release (2–4), and reuptake (5). All of these processes are impacted by at least one member of the synuclein family 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