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Review

α-synuclein: between synaptic function and dysfunction

G. Di Rosa, D. Puzzo, A. Sant'Angelo, F. Trinchese and O. Arancio
Department of Psychiatry, Physiology and Neuroscience, New York University School of Medicine, New York, NY and Nathan Kline Institute for Psychiatric Research, Dementia Research Center, Orangeburg, NY, USA

Summary. Alpha-synuclein belongs to a family of vertebrate proteins, encoded by three different genes: α, ß, and γ. The protein has become of interest to the neuroscience community in the last few years after the discovery that a mutation in the α-synuclein gene is associated with familial autosomal-dominant early-onset forms of Parkinson Disease. However, it is not yet clear how the protein is involved in the disease. Several studies have suggested that α-synuclein plays a role in neurotransmitter release and synaptic plasticity. This hypothesis might help elucidate how α-synuclein malfunctioning contributes to the development of a series of disorders known as synucleinopathies.

Key words: Synuclein, Neurotransmission, Parkinson disease, Plasticity

Introduction

Synucleins (SYNs) are small (113-143 amino acids) proteins, highly conserved among vertebrates (Clayton and George, 1998), with up to 87% homology between the human and the zebra finch sequence (Maroteaux et al., 1988; Ueda et al., 1993; George et al., 1995; Goedert, 1997). Three different genes, α, ß and γ, encode αSYN, ßSYN, and γSYN, respectively.

SYNs were first described in 1988 when a novel protein was isolated in the electrical organ of *Torpedo Californica* and in rat brain (Maroteaux et al., 1988). The protein was named SYN because of its synaptic and nuclear localization. A second intrinsic peptide component of human Alzheimer's Disease (AD) amyloid, named 'non-amyloid-beta-component precursor protein' (NACP), belonging to the SYN family, was reported in 1993 (Ueda et al., 1993). Later, in 1995, data were presented documenting the presence of αSYN in the telencephalic song control circuit of the zebra finch (George et al., 1995). αSYN was subsequently found in Parkinson's Disease (PD) neuropathologic hallmarks, named Lewy bodies (LBs) and Lewy neurites, and in cytoplasmic inclusions of patients affected by dementia with LBs and multiple system atrophy (Spillantini et al., 1997; Arima et al., 1998; Baba et al., 1998; Spillantini et al., 1998; Takeda et al., 1998a; Tu et al., 1998; Wakabayashi et al., 1998; Culvenor et al., 1999). αSYN-positive-LBs have also been found in LB variant of AD, familial AD and Down's syndrome (Lippa et al., 1998, 1999; Takeda et al., 1998b), as well as in neurodegenerative disorders with iron accumulation type 1: Hallervorden-Spaz disease (Arawaka et al., 1998; Wakabayashi et al., 1999). Because of the presence of SYN deposits, all these neurodegenerative disorders are now grouped under the name of synucleinopathies (Galvin et al., 2001).

Among the different types of SYNs, αSYN has received much attention because of the discovery that a mutation of the protein is involved in familial PD (Polymeropoulos et al., 1997). The gene for α-SYN has been mapped to chromosome 4 in humans. αSYN is expressed throughout the brain: the molecular layer of cerebellum, hippocampus, amygdala, retina, neocortex, nucleus accumbens, striatum and olfactory mucosa. The primary sequence of αSYN consists of 140 residues. The amino-terminal sequence (residues 7-87) contains a series of amphipathic α-helical domains that could be responsible for the multiple interactions of αSYN with membranes or other proteins (Goedert, 1997). This region is characterized by a strictly conserved periodicity of 11 amino acids that resembles the amphipathic helices of apolipoproteins and contains two lysine residues on each side of the helix, highly conserved in all SYNs and, at the same time, hallmark of class A2 helices. The central region of αSYN (residues 61-95) is very hydrophobic (Ueda et al., 1993). The carboxyl-terminal region is enriched of glutamate residues and quite acidic (Nakajo et al., 1993). The secondary structure of αSYN changes with the environment. The protein has an unfolded random coil structure in aqueous solution (Weinreb et al., 1996), an α-helical structure when it binds acidic phospholipid

Offprint requests to: Dr. Ottavio Arancio, NYIU School of Medicine/Dementia Research Center, Nathan Kline Institute for Psychiatric Research, 140 Old Orangeburg Road, Orangeburg, NY 10962., USA. e-mail: arancio@nki.rfmh.org

vesicles (Davidson et al., 1998), and it can be organized in insoluble fibrils with a ß-sheet structure, mimicking the insoluble filaments found in LB (Giasson et al., 1999).

The association of αSYN deposits in LBs with selective loss of dopaminergic (DA) neurons from the substantia nigra pars compacta (SNpc) and with extracellular melanin released from degenerating cells characterizes the PD brain (Giasson et al., 2000; Clayton and George, 1999; Goedert, 1999; Hashimoto and Masliah, 1999; Dickson, 2000; Dunnet and Bjorklund, 1999). In association to DA neurons, other populations of neuromelanin-containing and non-neuromelanin-containing neurons degenerate and accumulate LBs in brainstem and basal forebrain of patients affected by PD (Giasson et al., 2000; Galvin et al., 2001). Interestingly, the neuropathologic features in the DA neurons, the massive degeneration of DA nigral neurons and the concurrent modifications of the other neuronal populations correlate all with either the primary clinical features of PD: bradykinesia, cogwheel rigidity, resting tremor, postural instability, and the secondary clinical features of the disease including autonomic instability, seborrhea, sleep disturbances and possibly dementia.

Role of αSYN in neurotransmission

The mechanism(s) by which αSYN plays a role in the pathogenesis of synucleinopathies is (are) not yet clear. This is due, at least in part, to the fact that the physiologic function of αSYN still remains unknown. Numerous studies strongly support the idea that αSYN plays a role in neurotransmission. Indeed, the protein is located in proximity to synaptic vesicles in presynaptic terminals (Iway et al., 1995) where it colocalizes with ß-SYN (Kahle et al., 2000). Interestingly, a small fraction of αSYN is associated with vesicular membranes (Irizarry et al., 1996). Moreover, recent studies, examining structural synaptic changes after treatment of cultured hippocampal neurons with an antisense (AS) oligonucleotide, a manipulation that reduces αSYN expression, have demonstrated a marked selective reduction of the presynaptic vesicle distal pool size in treated neurons (Cabin et al., 2002; Murphy et al., 2000). These findings are interpreted as support for a role of αSYN in interaction and regulation of vesicles storage and turnover that might be compromised in synucleinopathies (Murphy et al., 2000; Cabin et al., 2002).

Developmental studies have also stressed the importance of αSYN for synaptic function. Differently from synapsin I, another presynaptic protein that appears in cell cultures as early as 24 hours after plating, αSYN does not show until day 5 in cultured hippocampal neurons (Withers et al., 1997). Consistent with this observation, immunostaining with policlonal antibodies against the NAC domain of αSYN in normal fetal cortex neuroectodermal precursor cells is higher in the soma, to become more elevated in synaptic terminals of adult cortex (Bayer et al., 1999). These findings suggest a potential role for αSYN in maturation and modulation of previously existing synapses rather than in development of new ones (Hsu et al., 1998). Furthermore, it is possible that αSYN is up-regulated during cell death. In vivo infusion of quinolinic acid into the striatum of rodents causes apoptotic death also in approximately 10% neurons of controlateral SNpc, accompanied by 35% αSYN-positive neurons (Kholodilov et al., 1999; Vila et al., 2000). This observation is consistent with the finding that αSYN is involved in the pathogenesis of familial forms of PD (Polymeropoulos et al., 1997; Kruger et al., 1998), a condition in which DA neurons of SNpc degenerate because of programmed cell death (Mochizuki et al., 1996; Tompkins et al., 1997). In summary, evidence has been provided suggesting that αSYN might also be expressed during neuronal injury as a result of plastic and regenerative phenomena. New synapse formation and sprouting would ensure protection for the survivor cells (Coyle, 1997; Kholodilov et al., 1999).

αSYN and synaptic plasticity

Considering dementia is one of the clinical features of PD and AD (Baba et al., 1998; Dunnet and Bjorklund, 1999; Galvin et al., 1999; Dickon, 2000; Giasson et al., 2000; Hurtig et al., 2000) and observing the prominent expression of αSYN in the hippocampus (Kahle et al., 2000; Murphy et al., 2000; Masliah et al., 2000; Abeliovich et al., 2000), it has also been speculated that αSYN might play a role in synaptic plasticity. This hypothesis is supported by increasing evidence suggesting that SYNs are developmentally regulated gene products in the major telencephalic nucleus of a song-control circuit in the zebra finch (Clayton and George, 1998). αSYN gene in the song control nucleus of the bird is mainly expressed within the first month of life and remains elevated for another 1-2 weeks, when the protein levels are higher and a lot of neuronal and synaptic rearrangements take place (Jin and Clayton, 1997). Interestingly, this period corresponds to the interval of time when song plasticity is greatest. Conversely, αSYN is absent in the song control nucleus of adult birds. It is possible that higher expression of αSYN is merely a consequence of synaptic changes or metabolic stress of the early stages of development (Stamoudis et al., 1996). However, it is likely that the regulation of the protein expression is intrinsic and exists regardless of environmental influence (Jin and Clayton 1997). This has been confirmed in studies on birds raised in isolation with no song stimuli, which show a normal developmental pattern of αSYN.

More evidence supporting an involvement of αSYN in synaptic plasticity has been offered by studies on the olfactory epithelium where all SYNs are expressed. Since olfactory cells are capable of regenerating continuously throughout life, it seems likely that SYNs may play a role in regeneration and plasticity of adult

human olfactory epithelium (Duda et al., 1999). However, the conclusion that αSYN is part of plastic phenomena contrasts with observations on wild-type and αSYN knock-out mice demonstrating that CA1 and mossy fiber long term potentiation (LTP), a well-known type of synaptic plasticity that might underlie learning and memory, is normal in these animals (Abeliovich et al., 2000; Castillo et al., 1997; Schluter et al., 1999). Nonetheless, it is possible that the phenotypic changes in the genetically modified mice are relatively mild because of functional redundancy of ßSYN, a protein with very similar pattern of expression in mammal brains as αSYN (Jakes et al., 1994). In addition, long-term compensatory changes may develop following chronic depletion of αSYN, as demonstrated for other presynaptic proteins (Augustine et al., 1996).

αSYN and dopaminergic nigrostriatal neurotransmission

Because of the involvement of αSYN in PD, many studies have evaluated the role of αSYN in regulation of DA release and reuptake at dopaminergic terminals. Studies on human wild-type αSYN transgenic mice have shown a massive degeneration of DA nigral neurons and loss of DA terminals in the basal ganglia together with motor impairment (Masliah et al., 2000). The protein might work as an activity-dependent negative regulator of DA neurotrasmission (Abeliovich et al., 2000). Furthermore, a reduction of DA content in αSYN knock-out mice brain has been described, whereas the levels of the major DA metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) were comparable both in mutant and wild-type transgenic mice. The observation that the DOPAC/DA ratio, an index of DA degradation rate (Horger et al., 1998), is mildly higher in αSYN knock-out mice, suggests that the deficit might affect DA synthesis, and/or storage or dynamic release (Abeliovich et al., 2000). However, the application of a single electrical stimulus or a train of stimuli, resembling the burst firing of nigral DA neurons in vivo (Grace and Bunney., 1984), generates a peak of extracellular DA, via a Ca^{2+}-dependent mechanism (Wightman and Zimmermann, 1990), that is similar between wild type and mutant mice. Thus, αSYN can not be involved in DA release, reuptake and diffusion regulation evoked after a single stimulus or a series of stimuli (Abeliovich et al., 2000).

Recent studies have linked αSYN with a form of synaptic depression known as Paired Stimulus Depression (PSD), occurring at nigrostriatal synaptic terminals. PSD is normally evoked by pairs of electrical stimuli applied on mouse brain slice preparations. αSYN knock-out mice show a faster recovery of extracellular DA peak when slices are exposed to paired electrical stimuli. This would indicate that αSYN might play an inhibitory role in PSD (Abeliovich et al., 2000). However, it is still not clear how αSYN participates to this activity-dependent modulation of DA neurotrasmission. The autoreceptor dependent inhibitory system (Kennedy et al., 1992) is a candidate mechanism. The autoreceptor dependent pathway is basically mediated by DA D2 presynaptic receptors. D2 receptor activation leads to a decrease in DA release. Conversely, the presence of a DA D2 antagonist seems to accelerate the time course of synaptic recovery (Kennedy et al., 1992), as it happens in αSYN knock-out mice (Abeliovich et al., 2000). Therefore, it is plausible that the differences between mutant and wild-type mice depend on autoreceptor activity. However, these data are not supported by results obtained by using sulpiride, a D2 receptors antagonist. Synaptic recovery from train stimulation is significantly accelerated by sulpiride regardless of the genotype, following train stimulation but not after a single stimulus. This suggests that αSYN influences PSD via a D2-receptor-independent mechanism (Abeliovich et al., 2000).

Another candidate mechanism for the involvement of αSYN in PSD is the replenishment of a readily releasable pool (RRP) of synaptic vesicles (Dittman and Regher, 1998; Stevens and Wesseling, 1998; Wang and Kaczmarek, 1998; Gomis et al., 1999). It has been previously described that αSYN plays a regulatory function in synaptic vesicle storage and turnover (Clayton and George, 1998; Murphy et al., 2000). This observation together with the evidence that αSYN specifically binds acidic phospholipid vesicles (Davidson et al., 1998) and highly inhibits phospholipase D2 (PLD2), the enzyme that produces phosphatidic acid (PA) and diacylglycerol (DAG) from phosphatidylcholine (Jenco et al., 1998), strongly supports the idea that αSYN might participate to PSD via a regulatory activity on a RRP of vesicles. This pool is thought to be localized at synaptic level. Its depletion is specifically followed by depressed synaptic responses (Goda and Sudhorf, 1997). PSD might thus represent the time necessary for RRP to refill after stimulus and αSYN would act modulating the rate of these processes and, consequently, the length of PSD (Abeliovich et al., 2000). Furthermore, the refilling rate of RRP appears to be accelerated by high extracellular levels of Ca^{2+} suggesting that αSYN may play a similar role on the same target of extracellular Ca^{2+} with the consequence of depletion of RRP (Abeliovich et al., 2000). It is also possible that DA D2 receptor inhibition and the RRP refilling rate cooperate when modulating PSD. In addition, based on the observation that αSYN highly inhibits PLD2 (Jenco et al., 1998), it is plausible that DAG increases the size and recovery rate of RRP directly acting on protein kinase C (Osterova et al., 1999), and PA stimulates synaptic vesicle formation (Frohman and Morris, 1996). Thus, as αSYN is mutated, there would be an alteration of the regulation of synaptic vesicle release.

Finally, αSYN has been linked to amphetamine-regulated DA release. It is known that amphetamines increase DA nigral neurotransmission and locomotion by increasing DA release and reducing DA reuptake (Seiden et al., 1993; Wise, 1996). Interestingly, a dramatic

reduction in amphetamine stimulated locomotion has been described in αSYN knock-out mice after intraperitoneal injection of D-amphetamine, but no differences were reported between mutant mice and wild-type mice in locomotor activity, when animals were placed in a novel environment (Rebec, 1997; Garris et al., 1999). Taken together, these data provide support for the idea that αSYN is involved in modulation of DA neurotransmission and that neuropathological, biochemical and neurophysiological impairment of DA structures and activity in PD are due to either malfunction or absence or mutation of the wild-type protein (Polymeropoulos et al., 1997; Kruger et al., 1998).

Mutations in αSYN determine common features of neurodegenerative diseases

Interest in αSYN has risen after the discovery of a mutation in the αSYN gene accounting for a familial form of PD. An alanine to threonine mutation at codon 53 (A53T) is linked to an autosomal dominant early-onset familial form of PD in Italian-American and Greek families (Polymeropoulos et al., 1997). An additional missense mutation (A30P) in a family of German origin is also pathologically relevant (Kruger et al., 1998). From these studies αSYN appears to be involved in neurodegenerative diseases by interacting with membrane lipids and axonal transport.

Mutation of αSYN, fatty acid binding and membrane interaction

The role of αSYN in lipid transport has been hypothesized after noticing the similarity between the amphipathic α-helical domain (11-residue repeats) of the protein and that of A2 apolipoproteins, specialized in carrying lipid molecules (Epand, 1993). A structural transition of αSYN from the unfolded random coil to the α-helical structure has been seen in presence of acidic phospholipids (Davidson et al., 1998). αSYN has also been involved in membrane biogenesis and cleavage of membrane lipids. This is supported by the evidence that αSYN is a highly specific inhibitor of PLD2, which produces PA and DAG from phosphatidylcholine. αSYN recognizes local domains enriched of PA, binds to them and inhibits PLD2-dependent production of PA. Conversely when PA is depleted or there is no interaction between αSYN and PLD2, the enzyme is disinhibited and causes an increase of membrane lipid cleavage. This appears to act as a regulatory system that controls membranes turnover and plasticity in which αSYN might be centrally involved. Recent studies performed by thin layer chromatography overlay on human brain extracts have demonstrated that αSYN strongly binds phosphatidylethanolamine (PE) and phosphatidylinositol (PI), whereas it weakly binds phosphatidylserine (PS), and does not have any interaction with phosphatidylcholine, phosphatidic acid, sphingomyelin or cholesterol (Jo et al., 2000). Additionally, planar lipid bilayers are disrupted by the association of αSYN and a close inspection reveals aggregates and fibrils on the bilayer surface.

Can mutated αSYN modify lipids and membrane interactions? One of the two point mutations, A30P, would abolish the ability of αSYN to bind lipid vesicles (Jensen et al., 1998). This could affect synaptic function by modifying a regulatory role of the presynaptic protein αSYN. The other point mutation, A53T, disrupts lipid bilayers at a slower rate than wild-type αSYN, rendering the protein less effective in membrane turnover regulation. The reduction in membrane binding would lead to a higher cytosolic concentration of αSYN, fibrils formation and accumulation and finally to the cellular degeneration processes of early-onset familial PD. Neuronal toxicity of the beta-pleated sheet fibrillary structure (El Agnaf et al., 1998; Kakizuka, 1998) is due to the accumulation of these insoluble filaments within axons and cell bodies, which interferes with the physiological transport of vital proteins from soma to axon terminals. Lack of essential proteins in distal regions of axons and dendrites would impair structural and functional integrity of neurons and synapses and alter the connections between SNpc and basal ganglia in the case of PD (Galvin et al., 1999; Trojanowski et al., 1999; Giasson et al., 2000). All these observations strongly suggest that lipidic vesicle binding is a specific property of αSYN and that significative changes both in structure and function of neurons occur when this property is altered.

Mutation of αSYN and axonal transport

Although the secretory process is basically similar in neurons and in other secretory cells, the neuronal site of secretion, the axon terminal, is considerably distant from cell body and dendrites, sites of synthesis, rendering absolutely necessary a specific transport mechanism to guide the secretory products and the newly-formed membranous organelles to the end of the axon. Neurons essentially possess two different forms of axonal transportation: the fast axonal transport and the slow axonal transport. Membrane organelles (synaptic vesicle precursor membranes, large dense-core vesicles, mithocondria, and smooth endoplasmic reticulum elements) move toward the nerve terminal (anterograde transport) and back toward the cell body (retrograde transport) by fast axonal transport, whereas cytosolic proteins and cytoskeletal elements move only in the anterograde direction, by slow axonal transport (Brady, 1985; Hirokawa, 1998). Proteins with multiple associations, such as αSYN, move at multiple rates (Brady., 1985; Jensen et al., 1998). Based on studies on rat visual system models it has been proposed that the A30P point mutation of αSYN impairs the vesicle-binding activity of the protein and its fast axonal transportation. This promotes the redistribution of the protein that can move only by slow axonal transport,

facilitating the accumulation of αSYN in cell bodies and leading to formation of LBs and Lewy neurites (Jensen et al., 1998, 1999).

Despite these observations, the relationship between αSYN and axonal transport is far more complex. Kahle et al., (2000) have described a normal synaptosomal localization and presynaptic distribution of wild-type and [A30P] αSYN in transgenic mouse brain, indicating that the anterograde transportation of αSYN *in vivo* is not abolished by the mutation. Mutated αSYN apparently would accumulate in pathological aggregates inside neurons because of its overexpression rather than failure of its axonal transportation (Kahle et al., 2000). In addition, the absence of any gross morphological modifications in striatal matrix architecture and in protein composition of presynaptic vesicles in mice lacking αSYN suggests that the protein is not essential for neuronal development or for the formation of presynaptic terminal (Abeliovich et al., 2000). Interestingly, the A53T point mutation in transgenic animals affects neither the vesicle binding of αSYN nor the axonal transportation of the protein. This might be explained by the fact that the human [A53T] αSYN resembles the wild-type αSYN in rats and that both proteins bear a threonine at position 53 (Maroteaux et al., 1988; Maroteaux and Sheller, 1991). That Thr 53 is wild-type in other species but yet linked to PD in humans could have many possible explanations and the normal phenotype in other species might be the result of a compensation by a second difference in the SYN sequence.

Influence of the environment on αSYN and pathogenesis of PD

If it is plausible that a mutated protein can be more vulnerable and functionally defective, it is not clear why a normal protein starts to accumulate and precipitate into insoluble filaments causing cellular degeneration and death. It is still not even known if αSYN is the *actual primum* movens of the neurodegenerative process or is just an epi-phenomenon, a consequence of neuronal death. In addition to the two point mutations, there could be some risk factors that facilitate and increase the formation and accumulation of the pathological fibrils and that become responsible of the outcome of the disease, even without any genetic involvement. Recent studies have strongly implicated oxidative stress in the pathogenesis of neurodegenerative diseases. Normally, cells possess antioxidant protective systems to eliminate the reactive oxygen and nitrogen species. Unfortunately, in case of excessive production, these protective mechanisms are saturated and fail to carry out their physiological functions. The result of this impairment is a serious damage to tissues, cells and molecules (proteins, lipids, nucleic acids) (O'Donnell et al., 1999; White et al., 1999; Giasson et al., 2002). Tyrosine residues of proteins can be nitrated in presence of CO or other reactive oxygen species, into 3-nitrotyrosine (3-NT). Immunoreactive 3-NT-αSYN has been found in LBs of PD brain (Good et al., 1998; Duda et al., 2000). In addition, specific monoclonal antibodies against nitrated αSYN stain pathological inclusions such as LBs and label the insoluble, filamentous αSYN that forms these lesions (Giasson et al., 2000). These findings are confirmed by the appearance of perinuclear aggregates in conditions under which peroxynitrite is generated (Paxinou et al., 2001). Similar αSYN aggregates are observed in 20-30% of cells overexpressing wild-type or A53T αSYN and in 5% of cells overexpressing A30P αSYN (Paxinou et al., 2001). Taken together, these results suggest an important link between oxidation and nitration processes and neurodegenerative disorders. It might be hypothesized that environmental and genetic factors cooperate in the pathogenesis of PD and that there might be a sort of "synergic" relationship between the two components. Mutations could increase the susceptibility to environmental toxins (Junn and Mouradian, 2002). However, both transgenic mice expressing A30P mutation and wild-type control mice after exposure to methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) show no difference in either number of DA neurons or catecholamine concentration in SN and striatum (Rathke-Hartlieb et al., 2001).

Failure of ubiquitination of αSYN by mutant parkin in autosomal recessive PD

Recent reports have raised the possibility that the inherited and the sporadic, idiopathic, form of PD can be related to a similar metabolic pathway. Mutations of parkin, an E3 ubiquitin ligase, cause an autosomal recessive juvenile-onset form of PD (Kitada et al., 1998, 2000; Lucking et al., 2000; Hattori et al., 2000; Mizuno et al., 2001; Shimura et al., 2000; Schlossmacher et al., 2002; Wang et al., 2001). The relationship between parkin and αSYN in the pathogenesis of PD is supported by the evidence that: a) both proteins co-localize in LBs of brainstem, b) αSYN is a substrate of parkin in the ubiquitination pathway and, finally, c) mutant parkin fails to polyubiquitinate αSYN (Shimura et al., 2001). Ubiquitination is a process during which proteins are conjugated with multimers of ubiquitin by a cascade of enzymatic activations. After this process they become more susceptible of degradation by proteosomes (Tanaka et al., 2001). Wild-type parkin participates to the process as a ubiquitin ligase, whereas the mutant one does not, which causes nonubiquitinated substrates to accumulate and generate cellular dysfunction (Shimura et al., 2000). This is probably what happens with αSYN, which is specifically recognized by parkin; in fact, αSYN glycosilated isoform, αSYNp22, has been found to be part of a stable protein complex including parkin and an enzyme of ubiquitination cascade, UbcH7 (Shimura et al., 2001). Furthermore, it is likely that parkin is responsible for the production of LBs being that: a) LBs are absent in autosomal recessive PD where parkin is lacking (Takahashi et al., 1994; van de Warrenburg et al.,

2001), b) parkin and polyubiquitinated αSYN are present in LBs (Trojanowski et al., 1999), and c) parkin ubiquitin ligase activity shows specificity for αSYNp22 and its product, the ubiquitinated αSYN (Shimura et al., 2001). In summary, it is possible that when parkin is mutated, as in autosomal recessive PD with absence of LBs, αSYN fails to be ubiquitinated and, being less degradable by proteosomes, accumulates. Conversely, when there is no genetic involvement, such as in sporadic PD with presence of LBs, parkin is working correctly but, although αSYN is properly ubiquitinated, other factors impair its complete degradation by proteosomes, resulting in protein accumulation and cellular dysfunction (Shimura et al., 2001). These hypotheses link two etiologically and pathogenetically different forms of PD, justifying the onset of PD without αSYN mutations.

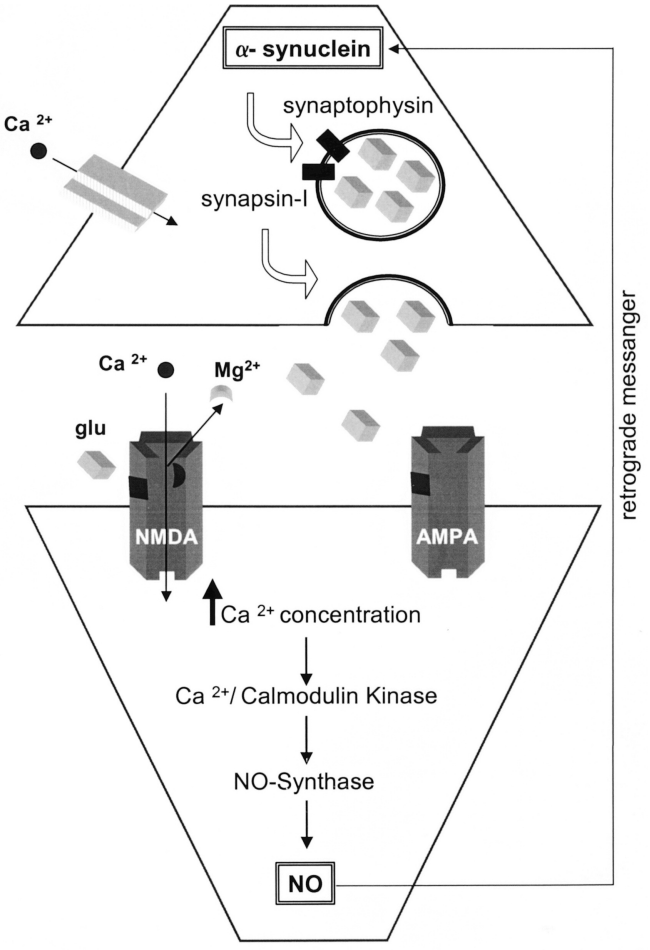

Fig. 1. Schematic depiction of the possible role of αSYN in neurotransmitter release during synaptic plasticity. Glutamate (glu) release during high frequency stimulation of the presynaptic neuron leads to Ca^{2+} entrance through postsynaptic NMDA receptors that activates a series of second messengers including the nitric oxide synthesizing enzyme (NO-synthase). NO travels retrogradely to the presynaptic neuron where it binds to α-synuclein and leads to long-lasting increase in neurotransmitter release.

Conclusions

It is amazing how this small protein alone could be responsible of all these functions and dysfunctions! Many hypotheses have been formulated but none indeed satisfies neuroscientists completely: how can a single protein produce such a great variety of pathological conditions? What is the physiological function of αSYN? It has been speculated that αSYN plays a role in normal neurotransmission as a modulator in interaction and regulation of vesicle storage and turnover. Recent studies have shown that αSYN inhibits the activity-dependent modulation of DA neurotransmission. A role in lipid transport has also been investigated because of the resemblance of αSYN molecular structure with the structure of A2 apolipoproteins, suggesting that the protein might act in lipid vesicle binding and transportation. Interestingly, αSYN is up-regulated in neurodegenerative processes either as a compensatory response or a direct contribution to neuronal death. Finally, several lines of evidence have converged to demonstrate αSYN expression in vivo and in vitro in developmental models of synaptic plasticity. Figure 1 illustrates an interesting working hypothesis that our group is currently investigating. Induction of synaptic plasticity by glutamate release from the presynaptic terminal would activate the NMDA subtype of the glutamate receptors allowing the entrance of Ca^{2+} into the presynaptic terminal. The elevation of Ca^{2+} levels would trigger an enzyme known as NO-synthase, leading to production of the retrograde messenger, NO. The gas would interact with αSYN causing a long-lasting increase in neurotransmitter release. Intriguingly, as we have pointed out above, oxidation and nitration processes of αSYN have been linked to neuro-degenerative diseases (Paxinou et al., 2001).

So many hypotheses have led only to a few conclusions! It would certainly help understand more the whole picture to clearly elucidate how αSYN acts in healthy neurons and what indeed the peculiar molecular mechanism that leads to a real dysfunction is. Maybe this could be the first step towards a new pathogenetic and eventually therapeutic approach to neurodegenerative diseases. Furthermore, another member of the SYN family, ßSYN, might play similar functions as αSYN. Considering that both α- and ß-SYN share similar molecular structure and immunochemical properties (Jakes et al., 1994) together with similar presynaptic localization respectively in hippocampus, temporal and cerebellar cortex of mouse brain (Kahle et al., 2000; Murphy et al., 2000), it would be interesting to further investigate about the possibility of a synergic action of the two proteins, in either the genetic or the sporadic fashion, in the pathogenesis of PD.

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