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Alpha-synuclein inhibits poly (ADP-ribose) polymerase-1 (PARP-1) activity via NO-dependent pathway

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Abstract

α-Synuclein (ASN) is a brain-enriched protein that functions as a molecular chaperone and regulator of the synaptic vesicle cycle. However, if ASN is overexpressed and in prefibrillar oligomeric forms it activates free radical formation and has been implicated in neurodegeneration. The nuclear target for the free radical cascade is poly (ADP-ribose) polymerase-1 (PARP-1), a DNA-binding enzyme and transcriptional regulator that decides on cell survival or death. Our previous data indicated that soluble oligomeric form of ASN significantly stimulated nitric oxide synthase (NOS) activity and by oxidative stress leads to mitochondria failure and cell death. The aim of this study was to investigate the effect of ASN on PARP-1 protein level and on its activity in the rat brain using radiochemical and immunochemical methods. It was found that ASN (10 μM) had no effect on PARP-1 protein level. However, ASN inhibited this enzyme activity by 35% in rat brain cortex and hippocampus investigated together and in striatum by 18%. An inhibitor of constitutive NOS isoform, N^G-nitro-L-arginine (NNLA, 100 μM), partially prevented ASN-evoked PARP-1 inhibition. The NO pool liberated by ASN could be involved in the decrease of PARP-1 activity. The direct interaction between ASN and PARP-1 protein should be taken into consideration.

Key words: *brain, alpha-synuclein, poly (ADP-ribose) polymerase-1, nitric oxide.*

Introduction

α-Synuclein (ASN) is a small soluble acidic protein enriched in the brain that plays an important role in the regulation of synaptic nerve terminal function. However, it has also been shown that it is implicated in the pathogenesis of several neurodegenerative diseases, which are collectively known as synucleinopathies [6]. The function of ASN under normal physiological conditions as well as its role in neurodegenerative diseases depends on the concentration and the assembly state of this protein [20]. At nanomolar concentration, ASN plays an important role in synaptic plasticity and regulation of vesicle transport, acts as a chaperone protein, protects neurons against oxidative stress and inhibits apoptosis [24]. However, if it is overexpressed at high concentration and in oligomeric forms ASN is cytotoxic [9]. This protein is localized within nerve terminals in the central nervous system [7,8,14]. It has also been found in the nucleus [19,23,31-33]. Recent data have emphasized

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extracellular localization of this protein [2,15,18]. ASN could be secreted from cells via exocytosis and could be involved in alteration of the signalling pathway from the extracellular compartment of the cells [16]. Our previous data indicated that it is liberated from synaptoneurosomes into the extracellular space during oxidative and nitrosative stress [2]. Extracellular ASN was shown to potentiate Ca^{2+} influx into rat synaptoneurosomes [5] and inhibit dopamine uptake into rat striatal synaptosomes by NO-mediated dopamine transporter alteration [3]. The nuclear localization of ASN suggests its role in the regulation of gene transcription and cell division [33]. ASN was shown to form a complex with histones and affect histone acetylation and in consequence its function [11,13]. The nuclear accumulation of full-length ASN [12] or its C-terminal fragment [31] is stimulated by oxidative stress.

The free radical damage of DNA single and double strand breaks activates the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1), which plays an important role in the cellular processes including gene transcription, signal transduction and defence against oxidative stress [1,27,30]. Activated PARP-1 rapidly cleaves substrate NAD^+ molecules to form ADP-ribose polymers on histones and other cellular proteins as part of the cellular defence and DNA repair programme [21]. Over-activated PARP-1, on the other hand, may be a cause of cell death due to its rapid depletion of cellular energy sources such as NAD+ and ATP [29]. The role of ASN in the modulation of PARP-1 protein is as yet unknown. Therefore, the aim of the present study was to examine the effect of ASN on PARP-1 protein level and activity and the role of NO in ASN-evoked PARP-1 alteration.

Material and Methods

All experiments on animals were accepted by the Polish National Ethics Committee, and were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

ASN was obtained from rPeptide, USA. Protease inhibitor cocktail was from Roche, Mannheim, Germany. Adenine [^{14}C]NAD (252mCi/mmol) was obtained from Amersham, Buckinghamshire and all other chemicals were purchased from Sigma (St. Louis, MO, USA).

Determination of PARP-1 protein level

The rat cerebral cortex including the hippocampus or striatum were homogenized in ice cold lysis buffer pH 7.4 (in mM: 10 Tris-HCl, 250 sucrose, 50 NaCl, 1 EDTA, 1 DTT and protease inhibitor cocktail) and centrifuged for 5 min at 500 × g at 4°C. Pelleted nuclei were suspended in lysis buffer, and incubated in the presence of ASN (10 µM) for 30 min at 37°C. Aliquots of crude nuclear fraction were diluted 1 : 1 with 2 × electrophoresis sample buffer and heated at 95°C for 5 min. Equal amounts of protein (40 µg) were separated on 7.5% SDS/PAGE gels and transferred to PVDF membranes. Then membranes were incubated in 1% bovine serum albumin in PBS with Tween 20 for 1 h and exposed overnight to mouse anti-PARP (1 : 500) antibody (Sigma Aldrich). After treatment for 1 h with the corresponding horseradish peroxidase-coupled secondary antibodies (anti-mouse from Amersham Biosciences), the protein bands were detected by ECL reagent (Amersham Biosciences).

Determination of PARP-1 activity

A 10% homogenate (w/v) from the cerebral cortex, including the hippocampus and the striatum, was prepared in a Dounce-type glass homogenizer in ice-cold 0.25 M sucrose with 50 mM NaCl, 1 mM EDTA and 1 mM dithiothreitol (DTT) in 10 mM Tris/HCl, pH 7.4, with "Complete™" protease inhibitors. The homogenate was pre-incubated for 30 min at 37°C in the absence (control) and presence of ASN (10 µM) or ASN + NNLA (100 µM). The PARP-1 activity was assayed as described previously [26]. The total reaction mixture in a final volume of 100 µl contained 400 µM [adenine-^{14}C]NAD as a substrate (2×10^5 d.p.m., sp. activity: 252 mCi/mmol), 100 mM Tris-HCl buffer pH 8.0, 10 mM $MgCl_2$, 1 mM DTT and 200 µg of protein. The reaction was carried out for 1 min at 37°C and stopped with 800 µl of ice-cold 25% trichloroacetic acid (TCA). The precipitate was collected on Whatman GF/B filters, washed five times with ice-cold 5% TCA and processed for determination of radioactivity in Bray's scintillation fluid using a scintillator counter (Wallac 1409 LKB).

Results

ASN (10 µM) significantly inhibited PARP-1 activity in rat brain cortex including hippocampus by 35%

and in striatum by 18% (Table I). Our previous results indicated that this protein significantly stimulated nitric oxide synthase (NOS) activity in rat brain. The results of the present study demonstrated that during the 30-min period of pre-incubation an inhibitor of constitutive NOS isoforms, N^G-nitro-L-arginine (NNLA, 100 µM), partially prevented ASN-evoked PARP-1 inhibition (Fig. 1). During this short period of ASN action it had no effect on PARP-1 protein level, indicating that inhibition of this enzyme activity is not connected with alteration of protein content (data not shown).

Table I. ASN decreased PARP-1 activity in rat brain cortex including hippocampus and in striatum

	PARP-1 activity [pmol × mg of protein^{-1} × min^{-1}]	
brain structure	control	ASN
cortex + hippocampus	16.93 ± 1.52	11.03 ± 1.07 (*)
striatum	51.19 ± 3.27	42.11 ± 1.65 (**)

*PARP-1 activity was measured in rat brain cortex including hippocampus and in striatum after 30-min incubation in the absence (control) and presence of ASN at 10 µM concentration, as described in experimental procedure. Data represent the mean value ± S.E.M. for 3 separate experiments, each carried out in triplicate. Significance of data was determined with one-way ANOVA followed by Bonferoni post-hoc test, *, **p < 0.05, 0.01 versus appropriate control.*

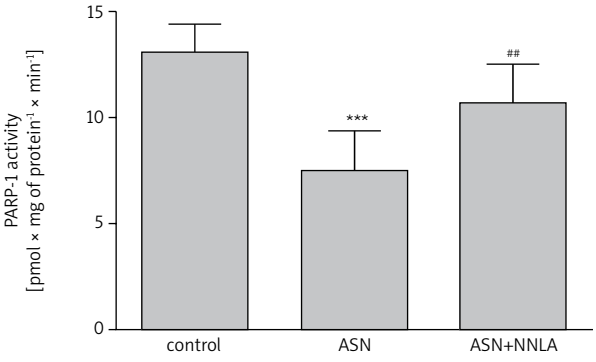

Fig. 1. NNLA prevented ASN-evoked PARP-1 inhibition in rat brain. PARP-1 activity was measured in rat brain cortex including hippocampus after 30-min pre-incubation in the absence (control) and presence of ASN (10 µM) or ASN + NNLA (100 µM). Data represent the mean value ± S.E.M. for 4 separate experiments, each carried out in triplicate. Significance of data was determined with one-way ANOVA followed by Bonferoni test, *** $p < 0.001$ versus control, ## $p < 0.01$ versus ASN.

Discussion

ASN is a brain-enriched protein unevenly distributed in axons, presynaptic terminals, cytoplasm, mitochondria and nucleus in the neuron [17,18,33]. It is also released by neurons into the extracellular space either as part of its normal cellular processing or under oxidative/nitrosative stress and neurodegenerative processes [2,10,28]. In this study, we have shown for the first time that ASN significantly decreased activity of PARP-1, the DNA-bound enzyme that participates in the maintenance of genome integrity. The inhibition of NOS partially prevented the effect of ASN on PARP-1 activity in rat brain. Moreover, we observed a lack of ASN influence on PARP-1 protein level. Although it is well documented that PARP-1 is involved in cell death machinery and plays an important role in neurodegenerative processes, so far there are no data concerning the role of ASN in PARP-1 function in the brain. This subject is important because both these proteins are present in the nucleus and their cross-talk may influence the function of several genes. The present results indicate that a short period (30 min) of ASN action is sufficient to decrease PARP-1 activity. During this period of incubation ASN had no effect on PARP-1 protein level. In addition, our study indicated that caspase-3 activity was not altered by ASN during 30 min of its action (data not shown). These data show that ASN during its early stage of action is involved in inhibition of PARP-1 activity independent of protein lowering. However, our preliminary data demonstrated that prolonged time of incubation (24 h) in the presence of ASN caused caspase-3 activation and in consequence PARP-1 cleavage [4].

Our previous results [3] showed that ASN stimulated NOS activity and NO release in the rat brain. According to the classical hypothesis, the excessive liberation of NO could initiate the free radical cascade, causing DNA damage that subsequently could lead to PARP-1 activation [34]. In contrast, Sidorkina et al., 2003 reported an inhibitory effect of NO on PARP-1 activity through degradation of the zinc finger motif in this protein [25]. In addition, results of Pytlowany et al., 2008 indicated that the NO donor sodium nitroprusside strongly decreased PARP-1 activity in PC12 cells [22].

The detailed mechanism of ASN action and the role of NO in the regulation of PARP-1 activity remain to be clarified. However, on the basis of the pre-

sent data we suggest that in the case of ASN action, excessively liberated NO probably interacts directly with the PARP-1 molecule, leading to its inhibition. However, since the inhibitor of NOS (NNLA) only partially prevents the decrease of PARP-1 activity evoked by ASN, we suggest that the direct interaction between ASN and PARP-1 protein could also play an important role.

In conclusion, our findings indicate that ASN through inhibition of PARP-1 activity may affect the efficiency of this enzyme in DNA repair and in regulation of transcription.

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