Original article

Folia Neuropathologica

Sphingosine kinases modulate the secretion of amyloid β precursor protein from SH-SY5Y neuroblastoma cells: the role of α-synuclein

Henryk Jesko[1], Taro Okada[2], Robert P. Strosznajder[3], Shun-ichi Nakamura[2]

[1]Department of Cellular Signalling, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland,
[2]Division of Biochemistry, Department of Molecular and Cellular Biology, Kobe University Graduate School of Medicine, Kobe, Japan,
[3]Laboratory of Preclinical Research and Environmental Agents, Department of Neurosurgery, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Folia Neuropathol 2014; 52 (1): 70-78 DOI: 10.5114/fn.2014.41745

Abstract

Sphingosine kinases (SphK 1&2) are involved in the regulation of cell survival, differentiation and neurotransmitter secretion. Current data suggest potential links between sphingolipid signalling, α-synuclein (ASN) and Alzheimer's disease (AD). Our aim was to investigate the possible role of SphKs and ASN in the regulation of the production and secretion of the amyloid β precursor protein (APP). We have previously shown that ASN intensified the secretion and toxicity of amyloid β (Aβ) to the point where it caused cell death. Our current results show that APP, the precursor protein for Aβ, is also influenced by ASN. The stable overexpression of wtASN in SH-SY5Y cells caused a three-fold, significant increase of the cellular APP level. This suggests that the influence of ASN on Aβ metabolism may actually occur at the level of APP protein rather than only through the changes of its cleavage into Aβ. To elucidate the mechanisms of APP modulation the cells were exposed to S1P and an SphK inhibitor (SKI). 72 h S1P treatment at 5 μM caused a nearly 50% reduction of the cellular APP signal. S1P also caused a tendency towards higher APP secretion, though the results were insignificant. The inhibition of SphKs decreased medium APP levels in a dose-dependent manner, reaching significance at 5 μM SKI with a correspondingly elevated intracellular level. Thus, it is reasonable to expect that in fact the influence of SphK activity on APP might be pro-secretory. This would also be in agreement with numerous articles on SphK-dependent secretion in the literature. The chronic nature of AD further suggests that subtle alterations in APP metabolism could have the potential to drive important changes in brain condition.

Key words: *sphingosine kinase, S1P, SH-SY5Y, Aβ, α-synuclein, neurodegeneration, AD.*

Introduction

Sphingosine kinases (SphK1&2) and their signalling pathways are key regulators of cell survival, proliferation and differentiation. Moreover, SphKs influence crucial functions of neurons such as the secretion of neurotransmitters or neurite shape. The SphK product sphingosine-1-phosphate (S1P) may play the role

Communicating author:
Robert P. Strosznajder, Laboratory of Preclinical Research and Environmental Agents, Department of Neurosurgery, Mossakowski Medical Research Centre, Polish Academy of Sciences, 5 Pawińskiego Str., 02-106 Warsaw, Poland, phone: +48 22 608 64 11, e-mail: rstrosznajder@yahoo.com

of an intracellular second messenger or act through cell surface receptors. The extracellular presence of S1P is dependent on the translocation of SphK1 to the plasma membrane; SphK2 can also undergo intracellular translocation, but the compartment and targets of S1P produced in such cases are different.

Accumulating evidence suggests the involvement of sphingosine kinases in a number of pathological conditions. Ageing is the crucial risk factor for Alzheimer's and Parkinson's diseases (AD and PD, respectively). The enhanced neuronal vulnerability to oxidative stress in ageing may be a result of the dysregulation of membrane lipids including sphingolipids [15]. Disturbed S1P levels could also potentially facilitate neurodegeneration-linked events such as ischaemic brain damage. S1P and especially its precursor, sphingosine, have already been found to possess some protective effects in the ischaemic heart model; it is tempting to speculate that it could be of interest in the brain ischaemia as well [36]. However, the results are highly unclear as S1P can be both cytoprotective [36] and sensitise the cells to oxidative stress-induced calcium dysregulation [5]; its changes in various organs seem to take different courses. The role of S1P in glutamatergic neurotransmission constitutes another important aspect of its potential influence of neuronal survival. The ischaemic damage is largely linked to a wave of uncontrolled neurotransmitter secretion. S1P takes part in the regulation of neuronal excitability and learning and memory phenomena [19]. It has been shown to trigger glutamate release and, importantly, to enhance depolarisation-induced secretion of the neurotransmitter [16].

It remains to be explained if conditions such as AD or PD can be affected by the age-related disturbances of sphingosine signalling and if these changes could create vulnerable background for the development of such diseases. Some results indeed point to the possible role of SphKs in AD. Sphingolipid signalling influences amyloid beta (Aβ)-induced inflammation [18]. Moreover, Aβ inhibits SphK1 activity in SH-SY5Y neuroblastoma [10] and selectively modulates the expression of some S1P receptor subtypes in monocytes [18]. Disturbances in sphingolipid metabolism are noted in AD starting from the earliest stages [20] with the levels of pro-apoptotic ceramide increased and activated catabolism of the usually pro-survival compound S1P. Importantly, changes observed correlate with cognitive decline. This phenomenon may potentially constitute a crucial factor of neuronal death and the resulting dementia. The levels of ceramides (Cer16, Cer18, Cer20, and Cer24) are changed in AD brains [8] and serum d18:1-C16:0 and d18:1-C24:0 ceramides are associated with an increased AD risk [25]. SphK overexpression reduces the toxic effect of Aβ [10]. Inhibitors of selected enzymes involved in sphingolipid metabolism may be extremely promising tools in AD research. Moreover, ceramide stabilises beta-secretase and boosts Aβ levels while glycosphingolipid depletion inhibits APP maturation and transport [29,34]. Thus, the ceramide-S1P 'biostat' shows vast potential as a possible important point of AD-related pathology.

Amyloid β and its precursor protein (APP) have long been viewed among the central elements of neuronal survival and death. However, more recent research has suggested that α-synuclein (ASN) may also be a factor of AD pathology. ASN is a small peptide of up to 140 amino acids; β- and γ-synucleins have also been identified. All synucleins are enriched in the central nervous system, particularly in the structures affected by AD: the neocortex, hippocampus and substantia nigra.

Despite classically being viewed as an intracellular protein, ASN has recently been found to undergo secretion [11] and uptake to the surrounding cells [22]. ASN is found in intracellular Lewy bodies present in Parkinson's disease and in ca. 60% of Alzheimer's disease (AD) cases. Moreover, a fragment of α-synuclein dubbed non-Aβ component of AD amyloid (NAC) is found in Aβ plaques in Alzheimer's and other diseases [35]. The immunoreactivity of ASN it self was also found in mature core amyloid plaques [3]. α-synuclein enhances amyloid β secretion from cells in culture twofold [21]. ASN also potentiates Aβ toxicity; this leads to mitochondrial damage and programmed cell death in a mode probably dependent on the Aβ load [21]. ASN could facilitate Aβ aggregation [24], though ASN-deficient models can also show accelerated Aβ aggregation [17]. The ASN level is increased in AD [3]. Finally, membrane-bound ASN physically interacts with amyloid β [24], though its mechanism and exact significance are highly unclear.

The currently available data suggest potential links between sphingolipid signalling and α-synuclein. Moreover, ASN may be engaged in Alzheimer's disease and modify the metabolism and fate of amyloid β precursor protein. The aim of our work was

to investigate the possible link between sphingosine kinase signalling, ASN and the production and secretion of APP.

Material and methods

Dulbecco's modified Eagle's medium (DMEM)/F12 and other cell media as well as inorganic reagents were from Wako. All-*trans* retinoic acid was from Sigma-Aldrich. Anti-APP (clone# 22C11) antibody was purchased from Millipore. SphK inhibitors were from Calbiochem; S1P from Biomol.

The human SH-SY5Y neuroblastoma cell line is known to be able to both proliferate and differentiate in culture and is able to express a number of neuronal features including beta dopamine hydroxylase activity and cholinergic and glutamatergic phenotypes. Low-passage (< 16), stably transfected wtASN-expressing SH-SY5Y cells were used for quantitative studies of S1P and SKI influence. As α-synuclein undergoes proteolytic degradation in certain cell states and all-*trans* retinoic acid (ATRA)-induced cell differentiation inhibits this phenomenon [30], we treated the cultures for five days with 10 μM ATRA. ATRA induced morphological changes towards a more arborised phenotype as shown in Fig. 1A. The survival of the differentiated cell line was found to be unaffected by the concentrations of S1P or SKI used, which differs somewhat from the situation in non-differentiated cells.

As the endogenous ASN levels were still very low (data not shown), a stably transfected cell line was prepared for use in the ASN quantification experiments. Standard molecular biology methods were employed. A full-length human ASN sequence was cloned from total RNA using proofreading RT-PCR

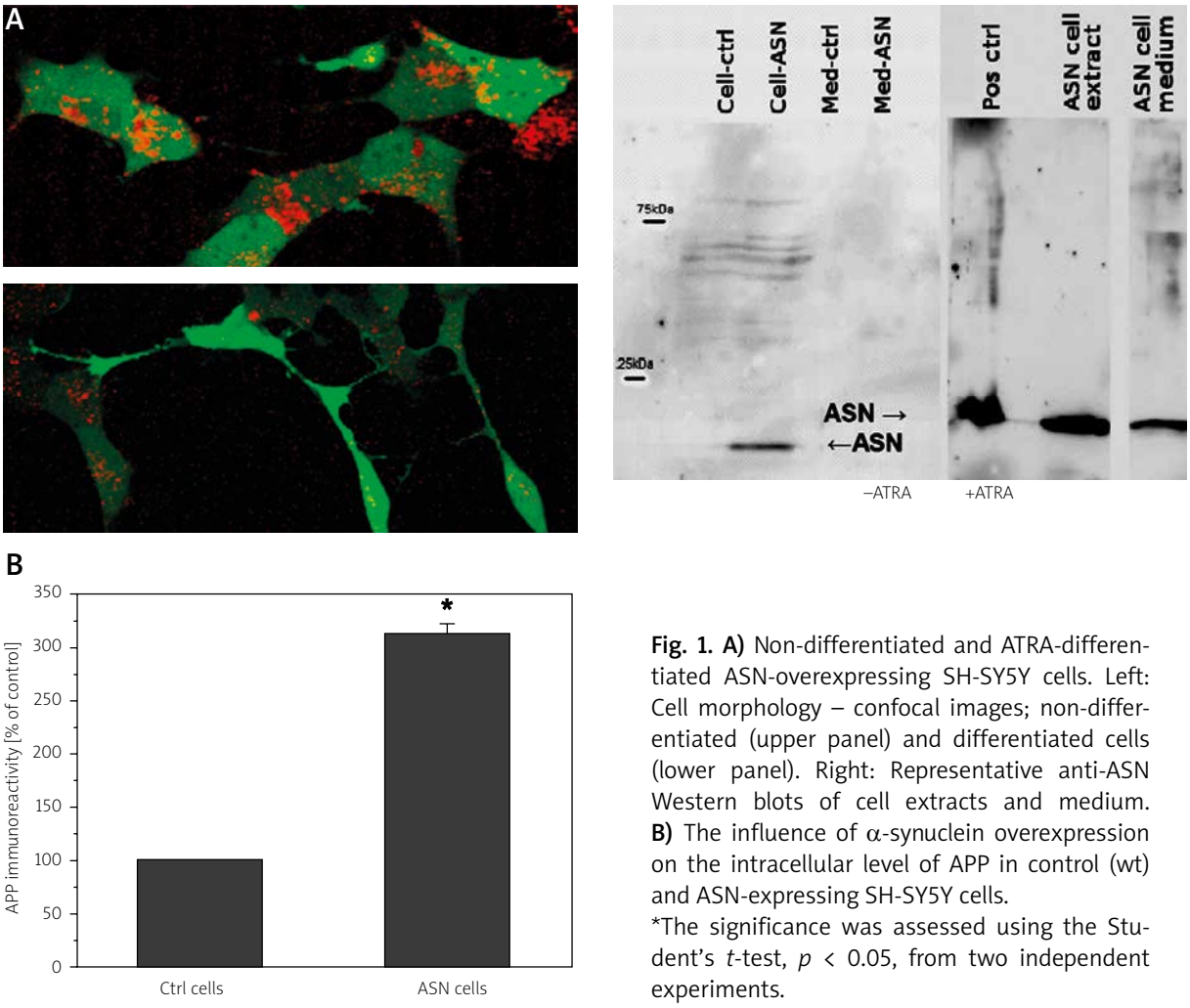

Fig. 1. A) Non-differentiated and ATRA-differentiated ASN-overexpressing SH-SY5Y cells. Left: Cell morphology – confocal images; non-differentiated (upper panel) and differentiated cells (lower panel). Right: Representative anti-ASN Western blots of cell extracts and medium. **B)** The influence of α-synuclein overexpression on the intracellular level of APP in control (wt) and ASN-expressing SH-SY5Y cells.
*The significance was assessed using the Student's *t*-test, $p < 0.05$, from two independent experiments.

(Roche Transcriptor Hi-Fi cDNA Synthesis & KODplus Neo kits) with primers:

5'-cgaggtaccatggatgtattcatgaaagg-3' (sense);

5'-cagggtaccttaggcttcaggttcgtagtc-3' (antisense),

purified using Promega Wizard SV Gel and PCR Clean-Up System according to the manufacturer's protocol, then inserted into a pCMV5 vector. The vector was ligated and amplified in commercially obtained competent DH5α bacteria from TaKaRa, PCR-checked using EmeraldAmp MasterMix (TaKaRa) and isolated with the Promega Wizard Plus miniprep kit. The insert was verified through sequencing using the BigDye® Terminator v3.1 kit from Applied Biosystems. The vector was co-transfected into SH-SY5Y cells with a pSV2 vector using the FuGene 6 reagent (Promega). Stably transfected SH-SY5Y clones were selected with 5 µg/ml blasticidin and used for the experiments.

The SphK product, sphingosine-1-phosphate (S1P), and the potent, selective sphingosine kinase inhibitor (SKI) were both used at concentrations of 0.1, 1.0 and 5.0 µM, respectively.

For the measurements of intracellular and secreted APP low-passage, wtASN-expressing cells were seeded at 0.8 × 10^5/well onto six-well dishes and cultured overnight in DMEM/F12 with 10% foetal calf serum, penicillin + streptomycin and 5 µg/ml blasticidin to achieve ~10% confluence. 10 µM ATRA was then added fresh every day for 120 h. After differentiation the cells were switched to serum-free DMEM/F12 medium and APP was collected for three days. The medium was centrifuged and denatured in a Laemmli sample buffer. The cells were washed twice with phosphate-buffered saline (PBS), scraped in 0.9 ml of PBS, centrifuged and lysed for 30 minutes at 4°C in 0.25 ml of 50 mM Tris-HCl pH 7.5 containing 150 mM NaCl, 0.5% Nonidet P-40. 5 mM EDTA and Complete™ protease inhibitors. The lysates were cleared by centrifugation and denatured. After measurements of protein contents the denatured medium and cell extracts were resolved using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) together with Bio-Rad Precision Plus Protein WesternC standards. After transfer to nitrocellulose membranes, the non-specific binding was blocked with 5% skim milk for two hours at room temperature and western-blots were performed using 1 : 100 "22C11" anti-APP antibody in Tween-20-containing Tris-buffered saline (TTBS) and after washes with 1 : 10 000 anti-mouse horseradish peroxidase (HRP)-linked secondary IgG together with 1 : 10 000 Streptactin-HRP conjugate in 0.5% milk/TTBS for 1 h. The chemiluminescent (ECL) signal was measured with a LAS-1000 apparatus (Fuji) and quantitated using ImageJ software. The statistical significance of the obtained results was assessed using Student's *t*-test.

Results

The endogenous level of APP was detectable in the SH-SY5Y cell line; it was possible to quantitate the secretion of APP protein after three days of incubation in control conditions. The expression of wtASN (Fig. 1A) caused an increase of the cell extract APP signal in non-differentiated SH-SY5Y cells to 313% of the values observed in non-transfected cells (Fig. 1B). Moreover, preliminary results suggest that a similar change could occur also in secreted APP (data not shown).

To characterise the molecular mechanisms of APP modulation we used the sphingosine kinase product S1P and an inhibitor of both SphK isoforms termed SKI. The 72 h treatment with 5 µM S1P caused a reduction of APP signal in the extracts of differentiated SH-SY5Y cells to 51% of the control ($p < 0.05$; Fig. 2A) with an insignificant increase in secretion (Fig. 2B). The effect of S1P on the APP level in the extract was nearly identical (46% of the appropriate control, not shown in the figures) when non-ASN transfected cells were used.

5 µM SKI administered to the differentiated ASN-transfected cells in the same conditions caused a significant increase of the cellular APP level (5 µM: 121%; $p < 0.01$; Fig. 3A). Correspondingly, the APP signal in the medium was reduced (54% of control; $p < 0.02$; Fig. 3B) while lower concentrations did not induce significant changes (APP secretion at 98% of the control level for 0.1 µM SKI and 80% for 1 µM SKI).

Discussion

The dysregulation of membrane lipids takes place in ageing, which is the most important risk factor for neurodegenerative disorders [15]. Disturbed sphingolipid metabolism could also directly affect age-related neurodegenerative diseases such as AD, PD or cerebral ischaemia. This could take place e.g. through the numerous functions of S1P in neuron generation, survival, neurite retraction/extension, neurotransmitter release [1,28], and its ambiguous role in oxidative

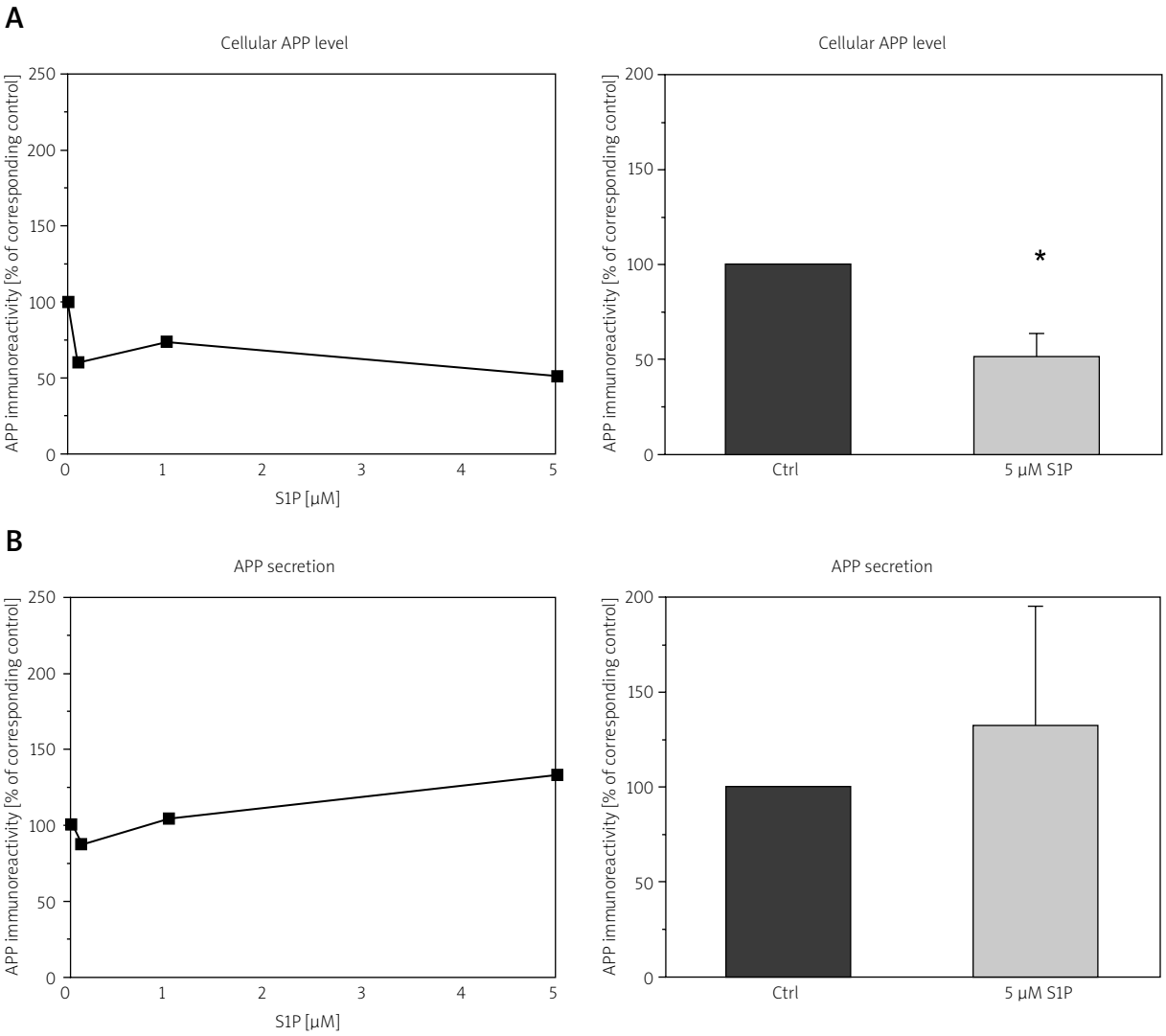

Fig. 2. The intracellular level (cell extract, **A**) and secretion (medium, **B**) of amyloid precursor protein in retinoic acid (ATRA)-differentiated, ASN-transfected SH-SY5Y cells treated with various concentrations of SKI vs. untreated cells.
*$p < 0.05$, Student's *t*-test, 3-4 independent experiments.

stress-linked events (glutathione depletion leading to H_2O_2 sensitivity in PC12 cells vs. heart protection against ischaemia/reperfusion injury) [5,36]. In the current work, we assessed the role of S1P in the ASN-dependent expression and secretion of APP in SH-SY5Y neuroblastoma cells.

ASN is enriched in the central nervous system (CNS) structures particularly affected by AD. Our previous work suggested a link between ASN and Aβ. ASN was able to increase the Aβ secretion and toxicity to a point where it caused cell death [21]. Other results suggest that ASN could influence Aβ aggregation, though the results are far from clear [17,24]. The two proteins can also physically bind each other [24]. Our current work shows that the precursor protein for Aβ is also influenced by ASN. The expression of wt α-synuclein has led to a significant increase of endogenous APP in SH-SY5Y cells. This suggests that APP level and/or secretion might be positively dependent on the intracellular level of ASN. In the study by Kazmierczak *et al*. [21], ASN was added externally and it was possible that its action could have been mediated by cell surface structures or even occurred outside the cell [21]. Our current results suggest that

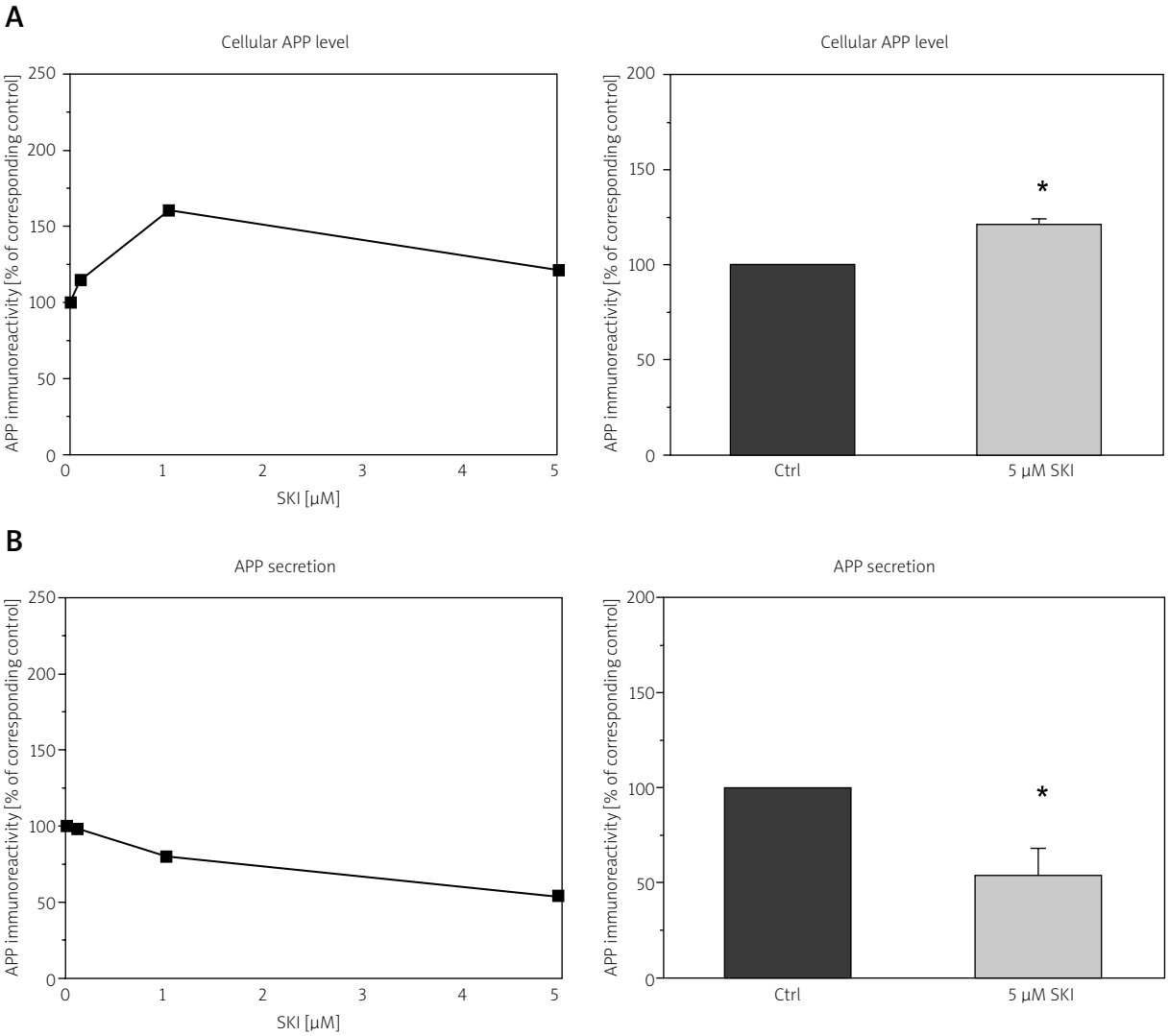

Fig. 3. The intracellular level (cell extract, **A**) and secretion (medium, **B**) of amyloid precursor protein in retinoic acid (ATRA)-differentiated, ASN-transfected SH-SY5Y cells treated with various concentrations of S1P vs. untreated cells.
*$p < 0.05$, Student's *t*-test, three independent experiments.

the phenomenon should be linked to the metabolism of Aβ precursor protein rather than occurring (only) at the level of APP cleavage/Aβ.

Sphingosine kinases undergo precise regulation through post-translational modifications [6], differential subcellular translocation [6,14], binding partners [9,12], and have partially different targets [14,27,28]. Each may be an important factor of AD albeit in different ways. Sphingolipid signalling may influence Aβ production [29,32,34] and siRNA/inhibition of SphK1/SphK2 reduces β-cleavage of APP leading to lower the secretion of Aβ and sAPPβ [33]. SphKs and sphingolipids may also modulate Aβ toxicity [7,10] and the inflammation it causes [18] as well as the resulting brain tissue damage [2]. In turn, Aβ inhibits S1P production [10] and modulates S1P receptors [18]. A correlation of sphingolipid disturbances with cognitive decline was observed in AD [20] and the protective influence of SphKs in Aβ toxicity has been reported [10].

We found that the SphK product S1P lowered the cellular level of APP. The influence of S1P on the secretion of APP showed a tendency towards activation, though the results were insignificant. SKI caused a dose-dependent decrease of medium APP levels, reaching a statistical significance at 5 μM.

The cellular level of APP was significantly elevated. Based on these results it is reasonable to suggest that in fact the influence of S1P on APP might be pro-secretory. Such a conclusion would be consistent with the ubiquitous [4,16,26] and physiologically relevant [19] pro-secretory influence of S1P [28]. Results suggesting the influence of the ceramide/sphingolipid signalling on the processing of APP have been published [23]. It is thus reasonable to expect that its actual influence on APP secretion will follow the general trend despite the fact that the various examples seem to employ an array of somewhat different mechanisms. S1P has been found to directly bind (probably the transmembrane and intracellular domains) and modulate the activity of BACE1, the rate-limiting enzyme for Aβ production [33]. The tendency towards higher APP levels in the medium and the reduction of its cellular content in the SH-SY5Y line strongly suggest that the secretion of whole APP probably masks the reduction that would stem from the mentioned increased cleavage. This is analogous to some degree to the situation in hamster ovary cells where the secretion of $A\beta_{1-42}$ follows the profile of APP secretion [31]. In these cells the influence of sphingolipids seemed to be different from its role in neuroblastoma (and probably the brain). Sphingolipid deficiency enhanced the secretion of the products of non-amyloidogenic α-cleavage of overexpressed human APP75 (with amyloidogenic cleavage products unchanged or reduced), an effect mediated by the MAPK/ERK pathway and rescued by sphingosine treatment [31]. This suggests that the various steps of the sphingolipid pathway may influence different aspects of APP/Aβ metabolism in opposite ways; moreover, sphingolipid action may differ significantly between the CNS and the periphery. The chronic nature of AD and the extremely elongated period of changes preceding its clinical manifestation further suggest that even very subtle changes in APP metabolism could have tremendous effects on the state of CNS either taken alone or in combination with simultaneously occurring pathological changes.

Although both ASN and APP undergo regulation by the SphK pathway, APP does not appear to follow the S1P metabolism-induced changes of ASN. We observed rather unexpected results regarding the influence of the S1P pathway on ASN secretion. Both S1P and SKI elevated the ASN signal in the medium without affecting the cellular level significantly (unpublished own results). Somewhat analogous results were obtained by Takasugi *et al*. [33] in a study on APP cleavage suggesting differential roles of cellular vs. exogenous S1P in these processes. It is possible that the influence of sphingolipid metabolism on ASN (which is limited to its secretion) does not modify the production of APP, which could be dependent mainly on the intracellular ASN level. Another explanation is that APP might undergo an additional level of SphK-related regulation working between ASN and APP production, as suggested by the different types of effects of SKI and S1P on APP vs. ASN.

Our results suggest a potentially important link between ASN and APP/Aβ. The interactions between these proteins may open new views on the devastating pathologies and possibly lead to a more comprehensive understanding of these processes.

Acknowledgements

This work was supported in part by the Statutory Budget of the M. Mossakowski Medical Research Centre, Polish Academy of Sciences (theme no. 1 and 17), NCN grant no. 5870/B/P01/2011/40 and research fellowships from the Japan Society for the Promotion of Science (H.J., R.P.S.).

References

1. Agudo-López A, Miguel BG, Fernández I, Martínez AM. Involvement of mitochondria on neuroprotective effect of sphingosine-1-phosphate in cell death in an in vitro model of brain ischemia. Neurosci Lett 2010; 470: 130-133.
2. Asle-Rousta M, Kolahdooz Z, Oryan S, Ahmadiani A, Dargahi L. FTY720 (fingolimod) attenuates beta-amyloid peptide (Aβ42)-induced impairment of spatial learning and memory in rats. J Mol Neurosci 2013; 50: 524-532.
3. Broe M, Shepherd CE, Mann DMA, Milward EA, Gai W-P, Thiel E, Halliday GM. Insoluble alpha-synuclein in Alzheimer's disease without Lewy body formation. Neurotox Res 2005; 7: 69-76.
4. Cantrell Stanford J, Morris AJ, Sunkara M, Popa GJ, Larson KL, Özcan S. Sphingosine 1-phosphate (s1p) regulates glucose-stimulated insulin secretion in pancreatic beta cells. J Biol Chem 2012; 287: 13457-13464.
5. Denisova NA, Fisher D, Provost M, Joseph JA. The role of glutathione, membrane sphingomyelin, and its metabolites in oxidative stress-induced calcium "dysregulation" in PC12 cells. Free Radic Biol Med 1999; 27: 1292-1301.
6. Ding G, Sonoda H, Yu H, Kajimoto T, Goparaju SK, Jahangeer S, Okada T, Nakamura S. Protein kinase D-mediated phosphorylation and nuclear export of sphingosine kinase 2. J Biol Chem 2007; 282: 27493-27502.
7. Doi Y, Takeuchi H, Horiuchi H, Hanyu T, Kawanokuchi J, Jin S, Parajuli B, Sonobe Y, Mizuno T, Suzumura A. Fingolimod phosphate attenuates oligomeric amyloid β-induced neurotoxicity

via increased brain-derived neurotrophic factor expression in neurons. PLoS One 2013; 8: e61988.
8. Filippov V, Song MA, Zhang K, Vinters HV, Tung S, Kirsch WM, Yang J, Duerksen-Hughes PJ. Increased ceramide in brains with Alzheimer's and other neurodegenerative diseases. J Alzheimers Dis 2012; 29: 537-547.
9. Fujita T, Okada T, Hayashi S, Jahangeer S, Miwa N, Nakamura S. Delta-catenin/NPRAP (neural plakophilin-related armadillo repeat protein) interacts with and activates sphingosine kinase 1. Biochem J 2004; 382: 717-723.
10. Gomez-Brouchet A, Pchejetski D, Brizuela L, Garcia V, Altié M-F, Maddelein M-L, Delisle M-B, Cuvillier O. Critical role for sphingosine kinase-1 in regulating survival of neuroblastoma cells exposed to amyloid-beta peptide. Mol Pharmacol 2007; 72: 341-349.
11. Hasegawa T, Konno M, Baba T, Sugeno N, Kikuchi A, Kobayashi M, Miura E, Tanaka N, Tamai K, Furukawa K, Arai H, Mori F, Wakabayashi K, Aoki M, Itoyama Y, Takeda A. The AAA-ATPase VPS4 regulates extracellular secretion and lysosomal targeting of α-synuclein. PLoS One 2011; 6: e29460.
12. Hayashi S, Okada T, Igarashi N, Fujita T, Jahangeer S, Nakamura S-I. Identification and characterization of RPK118, a novel sphingosine kinase-1-binding protein. J Biol Chem 2002; 277: 33319-33324.
13. He X, Huang Y, Li B, Gong C-X, Schuchman EH. Deregulation of sphingolipid metabolism in Alzheimer's disease. Neurobiol Aging 2010; 31: 398-408.
14. Igarashi N, Okada T, Hayashi S, Fujita T, Jahangeer S, Nakamura S. Sphingosine kinase 2 is a nuclear protein and inhibits DNA synthesis. J Biol Chem 2003; 278: 46832-46839.
15. Joseph JA, Denisova NA, Bielinski D, Fisher DR, Shukitt-Hale B. Oxidative stress protection and vulnerability in aging: putative nutritional implications for intervention. Mech Ageing Dev 2000; 116: 141-153.
16. Kajimoto T, Okada T, Yu H, Goparaju SK, Jahangeer S, Nakamura S. Involvement of sphingosine-1-phosphate in glutamate secretion in hippocampal neurons. Mol Cell Biol 2007; 27: 3429-3440.
17. Kallhoff V, Peethumnongsin E, Zheng H. Lack of alpha-synuclein increases amyloid plaque accumulation in a transgenic mouse model of Alzheimer's disease. Mol Neurodegener 2007; 2: 6.
18. Kaneider NC, Lindner J, Feistritzer C, Sturn DH, Mosheimer BA, Djanani AM, Wiedermann CJ. The immune modulator FTY720 targets sphingosine-kinase-dependent migration of human monocytes in response to amyloid beta-protein and its precursor. FASEB J 2004; 18: 1309-1311.
19. Kanno T, Nishizaki T, Proia RL, Kajimoto T, Jahangeer S, Okada T, Nakamura S. Regulation of synaptic strength by sphingosine 1-phosphate in the hippocampus. Neuroscience 2010; 171: 973-980.
20. Katsel P, Li C, Haroutunian V. Gene expression alterations in the sphingolipid metabolism pathways during progression of dementia and Alzheimer's disease: a shift toward ceramide accumulation at the earliest recognizable stages of Alzheimer's disease? Neurochem Res 2007; 32: 845-856.
21. Kazmierczak A, Strosznajder JB, Adamczyk A. alpha-Synuclein enhances secretion and toxicity of amyloid beta peptides in PC12 cells. Neurochem Int 2008; 53: 263-269.
22. Lee H-J, Suk J-E, Bae E-J, Lee S-J. Clearance and deposition of extracellular alpha-synuclein aggregates in microglia. Biochem Biophys Res Commun 2008; 372: 423-428.
23. Li H, Kim WS, Guillemin GJ, Hill AF, Evin G, Garner B. Modulation of amyloid precursor protein processing by synthetic ceramide analogues. Biochim Biophys Acta 2010; 1801: 887-895.
24. Mandal PK, Pettegrew JW, Masliah E, Hamilton RL, Mandal R. Interaction between Abeta peptide and alpha synuclein: molecular mechanisms in overlapping pathology of Alzheimer's and Parkinson's in dementia with Lewy body disease. Neurochem Res 2006; 31: 1153-1162.
25. Mielke MM, Bandaru VVR, Haughey NJ, Xia J, Fried LP, Yasar S, Albert M, Varma V, Harris G, Schneider EB, Rabins PV, Bandeen-Roche K, Lyketsos CG, Carlson MC. Serum ceramides increase the risk of Alzheimer disease: the Women's Health and Aging Study II. Neurology 2012; 79: 633-641.
26. Milara J, Mata M, Mauricio MD, Donet E, Morcillo EJ, Cortijo J. Sphingosine-1-phosphate increases human alveolar epithelial IL-8 secretion, proliferation and neutrophil chemotaxis. Eur J Pharmacol 2009; 609: 132-139.
27. Okada T, Ding G, Sonoda H, Kajimoto T, Haga Y, Khosrowbeygi A, Gao S, Miwa N, Jahangeer S, Nakamura S-I. Involvement of N-terminal-extended form of sphingosine kinase 2 in serum-dependent regulation of cell proliferation and apoptosis. J Biol Chem 2005; 280: 36318-36325.
28. Okada T, Kajimoto T, Jahangeer S, Nakamura S. Sphingosine kinase/sphingosine 1-phosphate signalling in central nervous system. Cell Signal 2009; 21: 7-13.
29. Puglielli L, Ellis BC, Saunders AJ, Kovacs DM. Ceramide stabilizes beta-site amyloid precursor protein-cleaving enzyme 1 and promotes amyloid beta-peptide biogenesis. J Biol Chem 2003; 278: 19777-19783.
30. Satoh JI, Kuroda Y. Ubiquitin C-terminal hydrolase-L1 (PGP9.5) expression in human neural cell lines following induction of neuronal differentiation and exposure to cytokines, neurotrophic factors or heat stress. Neuropathol Appl Neurobiol 2001; 27: 95-104.
31. Sawamura N, Ko M, Yu W, Zou K, Hanada K, Suzuki T, Gong J-S, Yanagisawa K, Michikawa M. Modulation of amyloid precursor protein cleavage by cellular sphingolipids. J Biol Chem 2004; 279: 11984-11991.
32. Takasugi N, Sasaki T, Ebinuma I, Osawa S, Isshiki H, Takeo K, Tomita T, Iwatsubo T. FTY720/fingolimod, a sphingosine analogue, reduces amyloid-β production in neurons. PLoS One 2013; 8: e64050.
33. Takasugi N, Sasaki T, Suzuki K, Osawa S, Isshiki H, Hori Y, Shimada N, Higo T, Yokoshima S, Fukuyama T, Lee VM-Y, Trojanowski JQ, Tomita T, Iwatsubo T. BACE1 activity is modulated by cell-associated sphingosine-1-phosphate. J Neurosci 2011; 31: 6850-6857.
34. Tamboli IY, Prager K, Barth E, Heneka M, Sandhoff K, Walter J. Inhibition of glycosphingolipid biosynthesis reduces secretion of the beta-amyloid precursor protein and amyloid beta-peptide. J Biol Chem 2005; 280: 28110-28117.

35. Uéda K, Fukushima H, Masliah E, Xia Y, Iwai A, Yoshimoto M, Otero DA, Kondo J, Ihara Y, Saitoh T. Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. Proc Natl Acad Sci U S A 1993; 90: 11282-11286.
36. Vessey DA, Kelley M, Li L, Huang Y. Sphingosine protects aging hearts from ischemia/reperfusion injury: Superiority to sphingosine 1-phosphate and ischemic pre- and post-conditioning. Oxid Med Cell Longev 2009; 2: 146-151.