Phosphorylated α-synuclein in normal mouse brain

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Abstract α-Synuclein phosphorylated at Ser129 is the main component of Lewy bodies of Parkinson's and closely related diseases. We studied, by quantitative immunoblotting, changes in the phosphorylation level of α-synuclein in the mouse brains subjected to cold water stress. Relative basal level of α-synuclein phosphorylation at Ser129 was 40% higher in the striatum compared with the hippocampus. The phosphorylation level decreased to 57% in the striatum 20 min after 5 min of cold water stress, and also in the hippocampus and cortex to lesser degrees. Recovery to basal levels took place over several hours. The stress-induced temporary dephosphorylation was of smaller magnitude in the striatum of aged (18 months) mice. These results show that α-synuclein phosphorylation level at Ser129 in vivo responds to physiological stimuli. Relative prominence and age sensitivity of this phenomenon in the striatum may be relevant to the pathogenesis of Parkinson's disease.

Keywords: α-Synuclein; Phosphorylation; Parkinson's disease; Striatum; Stress; Mouse

1. Introdution

α-Synuclein is a major protein component of Lewy bodies (LB), the histopathological hallmark of Parkinson's disease (PD) and dementia with LBs (DLB) [1,2]. Two mutations in the α-synuclein gene have been found to cause familial forms of PD characterized by extensive loss of nigral dopaminergic neurons and loss of dopamine in the striatum, St [3,4]. A recent study linked triplication of the α-synuclein locus to a familial PD [5]. Studies of transgenic rodents and *Drosophila* models strongly implicate α-synuclein in the degeneration of dopaminergic neurons, but its molecular mechanisms remain to be clarified [6].

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Abbreviations: CK, casein kinase; CWS, cold water stress; DLB, dementia with LBs; Hc, hippocampus; HRP, horseradish peroxidase; LB, Lewy body; PD, Parkinson's disease; SDS, sodium dodecylsulfate; St, striatum; VP, ventroposterior quadrant of the cerebral hemisphere

α-Synuclein is a neuronal protein particularly abundant in forebrain structures in rodents and humans [7,8], localized mainly in pre-synaptic terminals and in the neighborhood of synaptic vesicles [9–11], and suggested to be involved in trafficking of synaptic vesicles [12,13]. A drastic increase in the transcription of synelphin, the zebra finch α-synuclein homologue, during the development of the song control system has been observed [14]. α-Synuclein knockout mice with normal brain architecture exhibit a defect in synaptic physiology [15,16]. These findings indicate that α-synuclein has a physiological role in synaptic function.

Recent analyses have shown that most of the α-synuclein deposited in brains with DLB as well as other related disorders is phosphorylated at Ser129 [17], and a fraction of the phosphorylated α-synuclein is further monoubiquitinated at two lysines [18]. Interestingly, tau protein in neurofibrillary tangles, a hallmark of Alzheimer's disease, is also hyperphosphorylated and ubiquitinated [19,20]. We previously reported reversible tau hyperphosphorylation in normal mice in response to physical stress such as starvation and cold water [21–23]. Here, we took advantage of a specific phosphorylation dependent α-synuclein antibody to determine the effect of a brief cold water stress (CWS) on the phosphorylation level of α-synuclein in the St and other regions of the normal mouse brain.

2. Materials and methods

2.1. Animals

Male C57BL/6Njcl mice (10–13 weeks or 18 months, Clea Japan, Tokyo) weighing 25.0 ± 3.0 g were allowed free access to a standard chow and water, maintained at 23 °C and under light period of 08:00–20:00, and used in compliance with the protocols approved by the Animal Care and Use Committee of Mitsubishi Kagaku Institute of Life Sciences.

2.2. Cold water stress

CWS was given between 13:00 and 18:00. Mice were put in ice-chilled water of 7 cm depth in a 16 cm diameter ceramic container for 5 min, after which they were gently wiped dry and returned to the cages [23]. Mice were killed by cervical dislocation at designated times after CWS.

2.3. Western blot analysis

The brains were removed immediately after sacrifice and placed in ice-chilled saline. Brain regions were quickly removed by manual dissection under a dissecting microscope: right St, both hippocampi, right ventroposterior quadrant of the cerebral hemisphere (VP) and either half of cerebellum. The tissues were immediately homogenized using a Physcotron microhomogenizer (NS-310E, Microtech Nichion)

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in 300 μl of O+ buffer (62.5 mM Tris–HCl, pH 6.8, 10% (w/v) glycerol, 5% (v/v) 2-mercaptoethanol, 2.3% (w/v) sodium dodecylsulfate (SDS), 5 mM NaF, 100 μM orthovanadate, 1 μM okadaic acid, 1 mM PMSF, 1 mM EDTA, and 1 mM EGTA) [22]. The tissues were handled quickly, so that homogenation was within 2.5–4 min of cervical dislocation, as the postmortem lability of phosphate bound to α-synuclein is known [17]. Protein concentration was determined after dilution with saline by Bio-Rad Protein Assay with BSA in O+ buffer as standard.

Samples (15 μg protein/lane) were run on 15% acrylamide SDS–PAGE followed by electrophoretic transfer to PVDF membrane (Immobilon-P, Millipore) for 45 min at 8 V in a Trans-Blot SD Cell (Bio-Rad). Membrane was reacted with anti-phospho-α-synuclein antibody (PSer129) [17,24] overnight and then with blotting grade affinity purified goat anti-mouse IgG peroxidase conjugated (170-6516, Bio-Rad). For anti-α-synuclein (S-3062, Sigma), anti-synaptophysin (mouse monoclonal, 171B5) [25], and anti-phosphorylated tau (mouse monoclonal, AT8), nitrocellulose membrane (Protran BA 85, Schleicher & Schuell) was used, and the secondary antibodies were goat anti-rabbit or mouse IgG-horseradish peroxidase (HRP) (sc-2030, sc-2031, Santa Cruz). Chemiluminescence signals from the blots were obtained and quantified using SuperSignal West Pico (Pierce) and LAS-1000plus image analyzer with Image Gauge 3.0 software (Fujifilm). Band signal intensity was approximately linear to the input protein under the conditions used. Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Tukey/Kramer test of pairwise multiple comparisons. In all figures, error bars represent standard deviation, and * and ** indicate significant difference with $P < 0.05$ and $P < 0.01$, respectively.

3. Results

3.1. Immunoblot identification of phosphorylated α-synuclein in the mouse brain

Anti-PSer129 is a monoclonal antibody specific to α-synuclein phosphorylated on Ser129 as previously shown with extracts of human brain [24]. C-terminal 18 amino acids encompassing Ser129 are totally conserved between human and mouse [7]. Previous analysis estimated that ~4% of α-synuclein is phosphorylated in the normal rat brain [17]. The antibody PSer129 was used to identify the band of phosphorylated α-synuclein in immunoblots of mouse brain whole extracts (Fig. 1). An immunoreactive band of a ~17 kDa was detected (lane4), which registered with the α-synuclein band as detected with the anti-α-synuclein antibody (lane 2) when the two halves of the split lane were juxtaposed (lane 3). Thus, phosphorylation of α-synuclein at Ser129 does not cause a detectable mobility shift of the α-synuclein band.

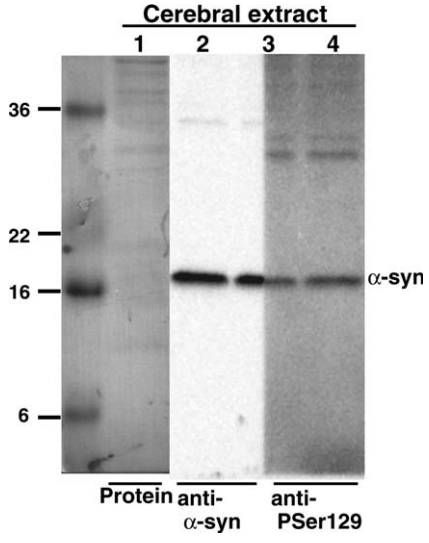

Fig. 1. Immunoblot identification of mouse α-synuclein band in a cerebral extract. Aliquots of mouse cerebral extract (10 μg protein) were immunoblotted. Blot carrying lane 1 was stained with Ponseau S for protein. The strip for lanes 2–4 was split in the middle and probed with anti-α-synuclein or anti-phospho-α-synuclein PSer129 antibodies as indicated. Molecular weights in kDa are given on the left.

3.2. α-Synuclein is more highly phosphorylated in the St than in the hippocampus or ventroposterior cerebral hemisphere

Relative content of α-synuclein was determined for St, hippocampus (Hc), VP, and cerebellum (Fig. 2A). VP comprises the amygdala, entorhinal cortex, and some neocortex, and has been shown to exhibit robust tau hyperphosphorylation after CWS [23]. The content of α-synuclein was highest in the Hc and VP followed by the St, while in the cerebellum the abundance was 11% of that of the Hc. Content of α-synuclein phosphorylated at Ser129 was highest in the St and VP (Fig. 2B). Synaptophysin, another presynaptic protein, was also more abundant in the St, Hc and VP, but the cerebellar synaptophysin content was about 52% of that of the Hc (Fig. 2C).

The ratio of anti-PSer129 to anti-α-synuclein signals for the four brain regions of each mouse was calculated as a measure of the fraction of α-synuclein phosphorylated at Ser129 (Fig. 2D). The ratio, averaged over six animals, was highest for the St (1.3, arbitrary unit) compared with the Hc (0.9) and VP (1.0). Meaningful values were not obtained for the cerebellum due to weak signals.

3.3. Cold water stress induces α-synuclein dephosphorylation most prominently in the St

To gain clues to the physiological significance of α-synuclein phosphorylation, we tested whether stressful stimuli to mice will cause changes in the phosphorylation level of this protein in the brain. Batches of six young adult mice were subjected to forced swimming in ice-chilled water for 5 min, and after various periods of recovery, three brain regions were quickly taken. The tissue extracts were individually analyzed by quantitative immunoblotting with anti-α-synuclein and phosphorylation-dependent α-synuclein antibody anti-PSer129.

No changes in the protein level of α-synuclein were observed in each of the three regions (Fig. 3A–D, white bars). By contrast, phosphorylation level at Ser129 decreased to approximately 57% of control in the St at 20 min after CWS (Fig. 3A and B, black bars). Thereafter, the phosphorylation level slowly returned toward the control level, but did not show full recovery at 24 h after CWS. In these striatal extracts, robust phosphorylation of tau could be demonstrated, as illustrated by a phosphorylation-dependent anti-tau antibody, AT8 (Fig. 3A, bottom).

In the Hc the decrease in the phosphorylation level of α-synuclein was significant at 20 min after CWS (Fig. 3C). A clear decrease to 60% at 20 min was observed in the VP and the gradual recovery was complete after 24 h (Fig. 3D). We previously reported on sharp and temporary hyperphosphorylation of tau in the Hc and VP after CWS [23]. Thus, brief CWS induced temporary decreases in the phosphorylation level of α-synuclein most prominently in the St. Similar changes were observed in the VP and less robustly in the Hc.

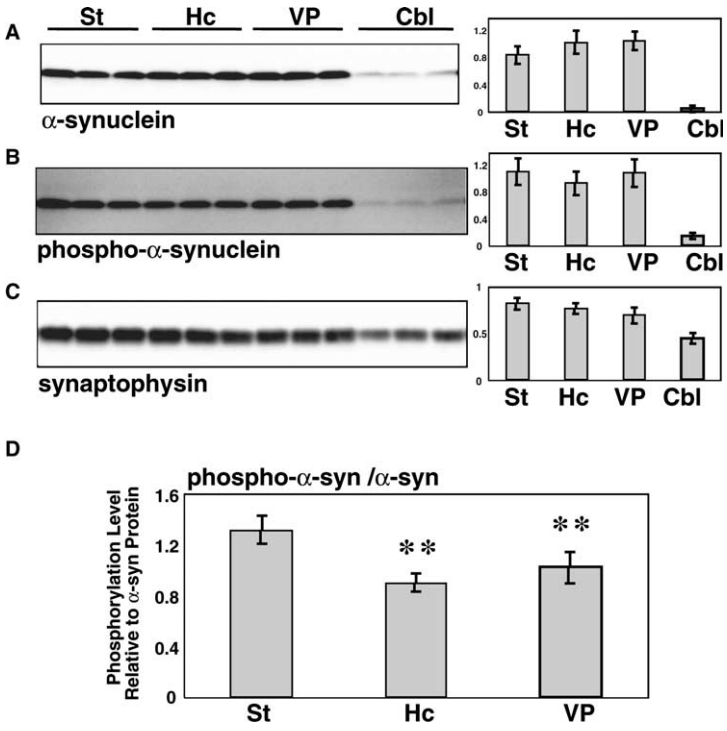

Fig. 2. Relative levels of α-synuclein, phospho-α-synuclein, and synaptophysin in selected regions of mouse brain. Extracts (15 μg protein) of St, Hc, ventroposterior quadrant of the cerebral hemisphere (VP) and cerebellum (Cbl) from six untreated mice were analyzed. The immunoblots in A–C illustrate the results of three mice with the three antibodies as indicated. Band intensities were quantified and normalized with respect to internal reference samples. Graphs summarize band signal quantification (arbitrary unit) based on analyses of six mice. Panel D shows relative phosphorylation levels of α-synuclein (phospho-α-synuclein signal divided by α-synuclein signal) in the three brain regions. Data are presented as means ± S.D.; ** indicates significant difference from St at $P < 0.01$.

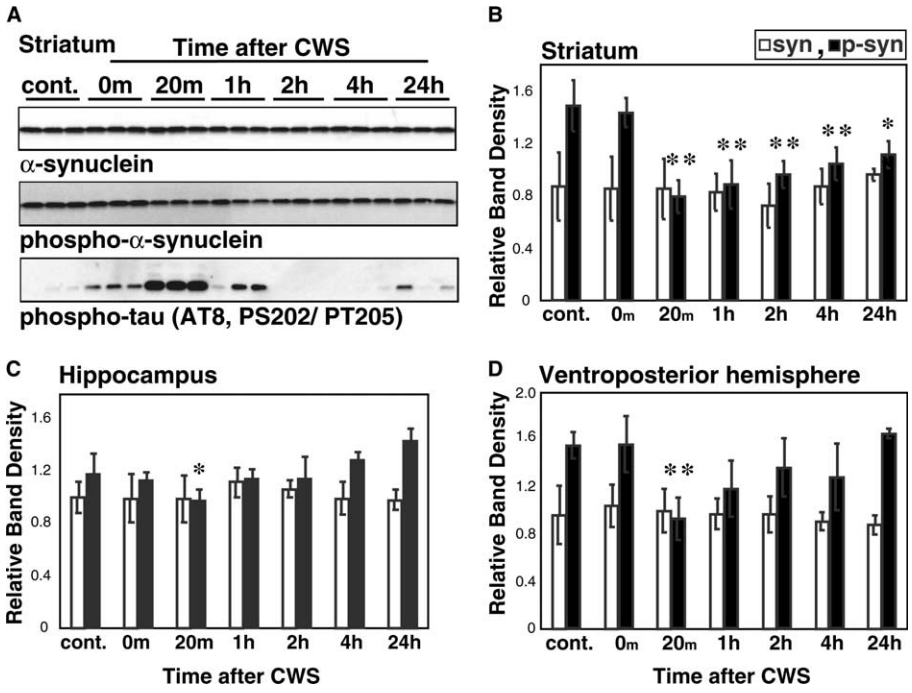

Fig. 3. Dephosphorylation of α-synuclein after CWS. Young adult (3 months) mice were subjected to 5 min of CWS and allowed to recover for various periods. Control mice received no CWS (cont.). Panel A shows representative immunoblots with anti-α-synuclein, anti-phospho-α-synuclein, and anti-phosphorylated tau antibodies for the St with three mice for each condition. Band intensities were quantified and normalized with respect to internal reference samples (Panels B–D) for α-synuclein protein (white bars) and the phosphorylated protein (p-syn, black bars). Graphs present quantitative results with six mice for each time point for the brain regions indicated. ** in Panels B and D indicates significant difference from respective control and 0 min values at $P < 0.01$, while * indicates significant difference from the control value in the hippocampus (C), or from control and 0 min values in the St (B) at $P < 0.05$.

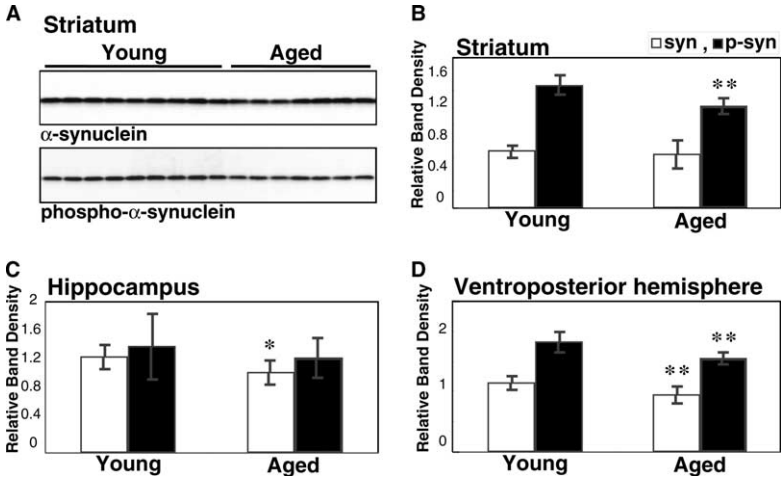

Fig. 4. Comparison of α-synuclein and phospho-α-synuclein levels in selected brain regions between young adult (3 months) and aged(18 months) mice. Panel A shows immunoblots of the striatal extracts from 9 young and 7 aged mice probed with anti-α-synuclein (upper blot) and anti-phospho-α-synuclein (PSer129) antibodies (lower blot). Panels B–D present quantitative summary of band intensities for the three brain regions. * and ** indicate significant difference from young adults at $P < 0.05$ and $P < 0.01$, respectively.

3.4. *Phosphorylation level of α-synuclein is decreased in the aged St*

Decrease in dopamine in the St is an early and the most conspicuous neurochemical change known in aging brains of mice as well as humans [26,27]. It was therefore of interest to study changes in α-synuclein and its phosphorylation level in the St and other selected regions in aging mice.

Quantitative immunoblots probed with anti-α-synuclein antibody showed no difference in the abundance of this protein in the St between young adult (3 months) and aged (18 months) mice (Fig. 4A, upper blot; 4B). Blotting with anti-phospho-α-synuclein antibody revealed a significant decrease in its phosphorylation level in the aged mice (Fig. 4A, lower; 4B). In the Hc, a tendency toward decreased levels of both α-synuclein protein and phosphorylation was observed (Fig. 4C), but statistical significance for the latter was not attained. In the VP, a significant decrease in α-synuclein was accompanied by a comparable decrease in the level of phosphorylated α-synuclein (Fig. 4D). Thus, the St characteristically showed a decrease in the phosphorylated fraction of α-synuclein with aging.

3.5. *α-Synuclein dephosphorylation response to CWS in aged mice*

Effect of aging on the stress response of α-synuclein phosphorylation level was studied using 26 mice of 18 months of age. No significant changes in the α-synuclein protein levels were observed in each of the three brain regions studied during up to 24 h after CWS (Fig. 5A–C, white bars). Significant decreases in the phosphorylation levels were observed in the St at 20 min and at 4 h after CWS (Fig. 5A, black bars). The decrease at 20 min was 21% from the control, a smaller decrease compared to the young mice (Fig. 3B). In the Hc and VP (Fig. 5B and C), decreases in the phosphorylation level were observed with significance at 20 min after CWS, and the return to the control levels was complete by 4 h. These results indicate that the stress response to CWS in terms of temporary decreases in the phosphorylation level of α-synuclein becomes attenuated in the aged St.

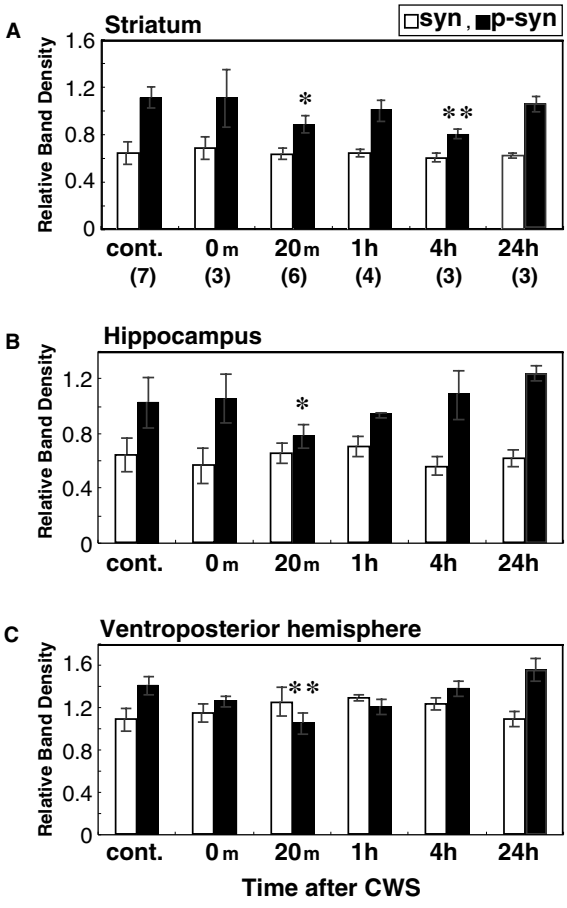

Fig. 5. α-Synuclein and phospho-α-synuclein levels in the three brain regions of aged (18 months) mice after 5 min of CWS and various periods of recovery. Control mice received no CWS (cont.). Numbers in parenthesis at the bottom of Panel A show the number of animals analyzed for each condition. * and ** indicate significant difference from controls at $P < 0.05$ and $P < 0.01$, respectively.

4. Discussion

We found that the phosphorylated fraction of α-synuclein in the mouse brain varies according to the regions: in the St it was 44% higher than in the Hc and 30% higher than in the cortex neighboring the Hc (VP). This phosphorylation level showed a 43% decrease 20 min after 5 min of forced swimming in cold water. Decreases were also observed in the Hc and VP. The phosphorylation level gradually returned to the basal levels over hours. This temporary dephosphorylation of α-synuclein was also observed in the three brain regions of aged mice (18 months), although the extent of change was less marked.

4.1. α-Synuclein phosphorylation

Studies with transfected cells showed initially that α-synuclein is phosphorylated constitutively [28] and experiments with a rat brain extract demonstrated in vitro incorporation of radioactive phosphate into α-synuclein [29]. Casein kinase 2 (CK2) and 1 (CK1) [28], and G protein-coupled receptor kinases [29] were implicated in α-synuclein phosphorylation in the cultured cells. Fujiwara et al. [17] showed pathological phosphorylation of α-synuclein at Ser129 in human brains and α-synuclein phosphorylation was demonstrated in *Drosophila* transgenically expressing human α-synuclein [30]. We here studied physiological levels of Ser129 phosphorylation in four regions of the normal mouse brain. Our finding that α-synuclein is relatively highly phosphorylated in the normal St suggests that certain neuronal subsystems in which Ser129 phosphorylation plays an important role may be more prevalent in the St than in other regions of the brain.

4.2. Physiological α-synuclein dephosphorylation

We report for the first time that a physiological stimulus induces changes in the phosphorylation level of α-synuclein in the brain. It has been shown that stress such as starvation and CWS causes reversible hyperphosphorylation of tau protein in the Hc and cortex of the mouse brain [20–23]. Since there seemed to be a certain parallel between tau and α-synuclein, two "natively unfolded" proteins that form intraneuronal deposits in Alzheimer's disease and PD, respectively, it was of interest to examine how phosphorylation of α-synuclein in vivo would respond to stressful stimuli. Tail shock stress has been shown to elicit dopamine release in the rat St [31], and stress in general has been discussed as a key factor in the loss of dopamine neurons [32].

Our discovery of clear temporary dephosphorylation of α-synuclein after CWS indicates that phosphorylation of α-synuclein is likely to have a physiological significance different from that of tau hyperphosphorylation. A difference is notable in the kinetics of tau hyperphosphorylation and α-synuclein dephosphorylation after CWS. Tau hyperphosphorylation was clearly observed immediately after 5 min of CWS, maximal at 20 min, and largely returned to the control level by 60–90 min (Fig. 3A) [23], whereas α-synuclein phosphoryration level was no different immediately after CWS, lowest at 20 min, and slowly recovered over several hours. Thus, changes in the phosphorylation level of α-synuclein are likely to be associated with certain longer-term neuronal processes.

A knockdown study of α-synuclein in cultured cells [33] and electrophysiological and ultrastructural studies of α-synuclein knockout mice [15,16] have shown an impairment in the regulatory aspect of synaptic transmission, and ultrastructurally, a reduction in the distal pool of synaptic vesicles, implicating α-synuclein in replenishment of synaptic vesicles at dopamine synapses after intense activities. Ser129 phosphorylation may be involved in the regulation of this distal pool. Unregulated hyperphosphorylation in a pathological context can possibly interfere with the mechanism sustaining the logistics of synaptic vesicles.

A low percentage (~4%) estimated for the phosphorylated fraction of α-synuclein in the rat brain [17] may invite skepticism as to its significance. Our demonstration of stress-responsive regulation of the phosphorylation level, however, supports a physiological role. It is tempting to speculate that this relatively abundant brain protein may occur in multiple functional compartments, and the phosphorylation is called for in a particular one.

The observed decrease in α-synuclein phosphorylation after CWS may reflect an aspect of neural responses to stress, as our preliminary observation indicated that this phenomenon occurs also after ether anesthesia, a classic experimental stress paradigm.

4.3. α-Synuclein phosphorylation and PD

α-Synuclein phosphorylated on Ser129 has been identified as the main component of LB characteristic of PD and closely related diseases [17]. Possible significance of α-synuclein phosphorylation to the etiology of α-synucleinopathy has been suggested by studies of effect of phosphorylation on the lipid binding property of α-synuclein [29] and increased tendency to fibril formation of phosphorylated α-synuclein [17]. A significant decrease of dopamine content in the St is the first neurochemically observable change in aging mouse brains [26]. Progressive decline of striatal dopamine content with age is also known in the humans [27]. It is of interest in this context that the temporary dephosphorylation of α-synuclein induced by CWS in the aged mouse brain was of lesser magnitude, particularly in the St compared with the young adults. Further understanding of the role of α-synuclein phosphorylation in the St should provide clues to the particular vulnerability of this brain region to PD.

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References

[1] Spillantini, M.G., Schmidt, M.L., Lee, V.M.-Y., Trojanowski, J.Q., Jakes, R. and Goedert, M. (1997) Nature 388, 839–840.
[2] Baba, M., Nakajo, S., Tu, P.-H., Tomita, T., Nakaya, K., Lee, V.M.-Y., Trojanowski, J.Q. and Iwatsubo, T. (1998) Am. J. Pathol. 152, 879–884.
[3] Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Srenroos, E.S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W.G., Lazzarini, A.M., Duvoisin, R.C., Iorio, G.D., Golbe, L.I. and Nussbaum, R.L. (1997) Science 276, 2045–2047.
[4] Kruger, R., Kuhn, W., Muller, T., Woitalla, D., Graeber, M., Kosel, S., Przuntek, H., Epplen, J.T., Schols, L. and Riess, O. (1998) Nat. Genet. 18, 106–108.

[5] Singleton, A.B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R., Lincoln, S., Crawley, A., Hanson, M., Maraganore, D., Adler, C., Cookson, M.R., Muenter, M., Baptista, M., Miller, D., Blancato, J., Hardy, J. and Gwinn-Hardy, K. (2003) Science 302, 841.
[6] Dauer, W. and Przedborski, S. (2003) Neuron 39, 889–909.
[7] Clayton, D.F. and George, J.M. (1998) Trends Neurosci. 21, 249–254.
[8] Kahle, P.J., Haass, C., Kretzschmar, H.A. and Neumann, M. (2002) J. Neurochem. 82, 449–457.
[9] Iwai, A., Masliah, E., Yoshimoto, M., Ge, N., Flanagan, L., Rohan de Silva, H.A., Kittel, A. and Saitoh, T. (1995) Neuron 14, 467–475.
[10] Jakes, R., Spillantini, M.G. and Gordert, M. (1994) FEBS Lett. 345, 27–32.
[11] Irizarry, M.C., Kim, T.W., McNamara, M., Tanzi, R.E., George, J.M., Clayton, D.F. and Hyman, B.T. (1996) J. Neuropathol. Exp. Neurol. 55, 889–895.
[12] Davidson, W.S., Jonas, A., Clayton, D.F. and George, J.M. (1998) J. Biol. Chem. 273, 9443–9449.
[13] Perrin, R.J., Woods, W.S., Clayton, D.F. and George, J.M. (2000) J. Biol. Chem. 275, 34393–34398.
[14] George, J.M., Jin, H., Woods, W.S. and Clayton, D.F. (1995) Neuron 15, 361–372.
[15] Abeliovich, A., Schmitz, Y., Farinas, I., Choi-Lundberg, D., Ho, W.-H., Castillo, P.E., Shinsky, N., Verdugo, J.M.G., Armanini, M., Ryan, A., Hynes, M., Phillips, H., Sulzer, D. and Rosenthal, A. (2000) Neuron 25, 239–252.
[16] 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) J. Neurosci. 22, 8797–8807.
[17] Fujiwara, H., Hasegawa, M., Dohmae, N., Kawashima, A., Masliah, E., Goldberg, M.S., Shen, J., Takio, K. and Iwatsubo, T. (2002) Nat. Cell Biol. 4, 160–164.
[18] Hasegawa, M., Fujiwara, H., Nonaka, T., Wakabayashi, K., Takahashi, H., Lee, V.M.-Y., Trojanowski, J.Q., Mann, D. and Iwatsubo, T. (2002) J. Biol. Chem. 277, 49071–49076.
[19] Hasegawa, M., Morishima-Kawashia, M., Takio, K., Suzuki, M., Titani, K. and Ihara, Y. (1992) J. Biol. Chem. 267, 17047–17054.
[20] Morishima-Kawashima, M., Hasegawa, M., Takio, K., Suzuki, M., Titani, K. and Ihara, Y. (1993) Neuron 10, 1151–1160.
[21] Yanagisawa, M., Planel, E., Ishiguro, K. and Fujita, S.C. (1999) FEBS Lett. 461, 329–333.
[22] Planel, E., Yasutake, K., Fujita, S.C. and Ishiguro, K. (2001) J. Biol. Chem. 276, 34298–34306.
[23] Okawa, Y., Ishiguro, K. and Fujita, S.C. (2003) FEBS Lett. 535, 183–189.
[24] Saito, Y., Kawashima, A., Ruberu, N.N., Fujiwara, H., Koyama, S., Sawabe, M., Arai, T., Nagura, H., Yamanouchi, H., Hasegawa, M., Iwatsubo, T. and Murayama, S. (2003) J. Neuropathol. Exp. Neurol. 62, 644–654.
[25] Obata, K., Nishiye, H., Fujita, S.C., Shirao, T., Inoue, H. and Uchizono, K. (1986) Brain Res. 375, 37–48.
[26] Finch, C.E. (1973) Brain Res. 52, 261–276.
[27] Carlsson, A. and Winblad, B. (1976) J. Neural Transm. 38, 271–276.
[28] Okochi, M., Walter, J., Koyama, A., Nakajo, S., Baba, M., Iwatsubo, T., Meijer, L., Kahle, P.J. and Haass, C. (2000) J. Biol. Chem. 275, 390–397.
[29] Pronin, A.N., Morris, A.J., Surguchov, A. and Benovic, J.L. (2000) J. Biol. Chem. 275, 26515–26522.
[30] Takahashi, M., Kanuka, H., Fujiwara, H., Koyama, A., Hasegawa, M., Miura, M. and Iwatsubo, T. (2003) Neurosci. Lett. 336, 155–158.
[31] Abercrombie, E.D., Keefe, K.A., DiFrischia, S.S. and Zigmond, M.J. (1989) J. Neurochem. 52, 1655–1658.
[32] Smith, A.D., Castro, S.L. and Zigmond, M.J. (2002) Physiol. Behav. 77, 527–531.
[33] Murphy, D.D., Rueter, S.M., Trojanowski, J.Q. and Lee, V.M.-Y. (2000) J. Neurosci. 20, 3214–3220.