Neuroscience Letters 472 (2010) 204–209

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Fasting induces a high level of 3-nitrotyrosine in the brain of rats

Yanying Liu[a,b], Rongqiao He[a,b,*]

[a] State Key Laboratory of Brain and Cognitive Sciences, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing, 100101, China
[b] Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing, 100101, China

ARTICLE INFO

Article history:
Received 7 November 2009
Received in revised form 1 February 2010
Accepted 2 February 2010

Keywords:
Nitration
3-Nitrotyrosine
α-Synuclein
Nitric oxide

ABSTRACT

Although the relationship between hyperglycemia (using diabetic animal model) and plasma nitrotyrosine level has been studied, the effect of hypoglycemia on nitrotyrosine level in the brain has not been addressed. Here, we evaluated nitration of protein, the colocalization of nitration with α-synuclein, activity of inducible nitric oxide synthase, and nitric oxide content using fasting and diabetic animal models. The results showed that signals of α-synuclein were widely distributed in most parts of the pallium, midbrain, hippocampus and cerebellum, as indicated by immunohistochemistry. Most signals of the 3-nitrotyrosine were colocalized with those of α-synuclein in the midbrain of fasting rats. The level of proteins containing 3-nitrotyrosine was significantly increased in the brain of fasting rats in Western blotting, especially in the midbrain, compared with control rats. In addition, the 3-nitrotyrosine signals increased in hippocampus of diabetic rats. Immunoprecipitation showed that α-synuclein was nitrated in the fasting rats. The iNOS activity and nitric oxide levels were significantly increased in both fasting and diabetic animals. The enhanced 3-nitrotyrosine level in the brain of fasting rats suggests that nitration of protein including α-synuclein in the midbrain is more affected by hypoglycemia in fasting than hyperglycemia in diabetic rats.

Nitrative injury has been implicated in the pathogenesis of some common diseases such as diabetes [9,29], neurodegenerative disorders [2,7] and vascular diseases [12,16,28]. Tyrosine nitration is a common protein modification that occurs in disease states associated with oxidative stress and increased nitric oxide synthesis [10,20]. The reaction of superoxide anion and nitric oxide (NO) *in vivo* produces peroxynitrite, which specifically modifies the tyrosine residues of proteins to generate 3-nitrotyrosine and other biomolecules such as thiol, aldehyde, and lipid [19,21,27]. The level of 3-nitrotyrosine and dityrosine, two biomarkers of nitration injury, are elevated in the brain and cerebrospinal fluid of Alzheimer's patients, and both the 3-nitrotyrosine concentration and the 3-nitrotyrosine/tyrosine ratio in cerebrospinal fluid increase significantly [25]. This elevation correlates positively with decreased cognitive functions.

α-Synuclein is a 140-amino acid protein (~17 kDa) that is abundant in presynaptic terminals and in Lewy bodies [24], which has been implicated in synaptic plasticity, neurotransmitter release, neuronal differentiation and regulation of neuronal viability [17]. This protein is regarded as a target for reactive nitrogen species in lesions caused by synucleinopathies, and this protein is specifically nitrated [7,8]. Although the precise function of α-synuclein in normal and diseased tissues is unknown, several lines of evidence suggest that hallmark lesions of neurodegenerative synucleinopathies contain α-synuclein that is modified by nitration of tyrosine residues and possibly by inter-protein cross-linking of tyrosine residues (to produce dityrosine adducts) that generate stable oligomers [11]. The presence of α-synuclein in the brains of Alzheimer's disease (AD) patients and animal models has been demonstrated by immunohistochemical and electron microscopic studies [15,24]. Giasson and colleagues reported that selective α-synuclein nitration has been found in oxidatively damaged tissues linked to neurodegeneration [7]. This is confirmed by another study showing that α-synuclein can be nitrated leading to changes of the structure and function of the protein [18].

As reported, a significant positive correlation between plasma nitrotyrosine levels and glucose tolerance is evident only in the presence of a reduced intake of some antioxidant vitamins in humans [3]. The fasting nitrotyrosine level is also significantly increased in diabetic patients [5]. However, Wang et al. showed that diabetes has no effect on plasma nitrotyrosine levels in Mexican Americans [26]. Recently, increased levels of inducible nitric oxide synthase (iNOS) mRNA and nitrotyrosine content have been observed in parallel with pathological alterations of the ultrastructure of liver mitochondria in diabetic rats [22]. The nitration of mitochondrial proteins induced by peroxynitrite may be responsible for structural damage to liver mitochondria.

* Corresponding author at: State Key Laboratory of Brain and Cognitive Sciences, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing, 100101, China. Tel.: +86 1064889876; fax: +86 1064853625.
E-mail addresses: herq@sun5.ibp.ac.cn, rongqiaohe@gmail.com, llldj@sina.com (R. He).

0304-3940/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2010.02.005

Although it has been established that plasma nitrotyrosine level correlates with severity of diabetes in rats, the relationship between hypoglycemia and nitrotyrosine levels in the brain—especially the nitration of α-synuclein—has not been reported. Thus, to elucidate the effect of hypoglycemia on the nitration of neural protein, we studied the nitration of protein, including α-synuclein, in the brain using fasted Sprague–Dawley (SD) rats.

Eight-week-old male Sprague–Dawley rats were used for the experiments. Diabetes (type-I) was induced by rapid intraperitoneal injection of streptozotocin (60 mg/kg). Blood glucose was determined using the Super Glucocard GT-1640 test meter (Arkray Inc., Japan). Animals were considered as diabetic when blood glucose level was higher than 16.7 mmol/l in streptozotocin-treated animals ($n = 30$) and was maintained at or above this level for at least 1 week. At 3- and 4-month intervals, animals were decapitated. The brain and blood were taken and frozen at −80 °C for later analysis. For the fasting animal model, 8-week-old male SD rats (bred in our animal facility) weighing 220–250 g were housed singly in cages. Rats were fasted for 48 h (but with water available), after which they were decapitated and the brains and blood collected. The different brain regions were immediately dissected and homogenized for Western blot and immunoprecipitation analysis. Brain homogenate and sera were obtained from the fasting group ($n = 12$), the diabetic group ($n = 30$), and the normal group ($n = 12$). The samples were immediately frozen and stored at −70 °C for use. Experimental protocols were performed in accordance with animal ethics statutes and were approved by the Animal Care and Use Committee in the Institute of Biophysics, Chinese Academy of Sciences.

Plasma glucose levels were determined by the glucose oxidase method [4]. Fifteen microliters of serum from SD rats were put into the working solution supplied with the glucose oxidase kit (Applygen Company, China) and analyzed according to the manufacturer's instruction. iNOS activity was determined using an iNOS assay kit (Nanjing Jiancheng Bioengineering Institute, China). Briefly, 10% brain homogenate (30 μl) or serum (15 μl) was mixed with the working solution supplied in the kit, and after incubation at 37 °C for 15 min the reaction was terminated by adding the terminating solution provided in the kit. The absorbance at 530 nm was recorded on a Hitachi U-2010 spectrophotometer (Japan), and iNOS activity was calculated in the reference of a standard curve. The amount of NO generated in samples was determined by measuring the absorbance at 550 nm.

Protein in samples was quantified with the BCA™ Protein Assay Kit (PIERCE, USA). Total soluble protein (10–30 μg) from tissues was resolved on 12% SDS-PAGE and transferred to a nitrocellulose membrane. The nitrocellulose membrane was blocked for 1 h in 5% (w/v) nonfat dry milk, and four duplicate membranes were probed overnight at 4 °C with polyclonal anti-α-synuclein (anti-Syn, Chemicon, USA) diluted 1:1000, rabbit anti-NOS-2 (iNOS, BIOS, China) diluted 1:300, monoclonal anti-3-nitrotyrosine (mouse IgG1 isotype) diluted 1:1000, or anti-actin (Santa Cruz Biotechnology Inc., USA) diluted 1:1000. Each membrane was washed three times with Tris-buffered saline (23 mM Tris, 150 mM NaCl) with 0.1% (v/v) Tween-20 (TBST, pH 7.4), then incubated with either horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG for 1 h at 37 °C. The membranes were again washed three times with TBST, and then immunoreactive bands were visualized using enhanced chemiluminescence. The protein bands were quantified by Quantity One 1D analysis software 4.5.2 (Bio-Rad, USA).

Rats were sacrificed 48 h after fasting, and the midbrain was quickly dissected out and homogenized in 9 volumes (m/v) of ice-cold immunoprecipitation buffer (Beyotime Inc., China). After spinning the homogenate at 14,000 × *g* for 15 min at 4 °C, supernatant (500 μl) was incubated with 2 μg (per 100–500 μg of total protein) of anti-α-synuclein antibody (Chemicon, USA) overnight at 4 °C with gentle shaking. The Ag/antibody immunocomplex was captured by further incubating with 100 μl of PBS-washed protein A/G agarose beads for 2 h and spun down (800 × *g*, 4 °C, 2 min). The beads were washed three times with PBS and resuspended in 10 μl 4× sample buffer. After boiling in water bath for 5 min, the supernatant was collected by centrifugation (14,000 × *g*, 25 °C, 1 min). Finally, the proteins were analyzed by Western blotting with monoclonal anti-3-nitrotyrosine (mouse IgG1 isotype) diluted 1:1000 to detect the nitrated α-synuclein.

SD rats were anesthetized with 0.3% sodium pentobarbital (1 ml/100 g body weight) and perfused through the heart with ice-cold sodium phosphate-buffered saline (0.01 M PBS), pH 7.4, followed by ice-cold 4% paraformaldehyde. For immunohistochemistry, we took brains from the SD rats and immersed them in 4% paraformaldehyde/0.5% glutaraldehyde in PBS overnight at 4 °C as described [13]. Brain samples were subsequently immersed in 20% sucrose in PBS for 24 h, followed by 30% sucrose in PBS for 48 h. Brain sections (20 μm thick) were cut on a freezing microtome and processed for immunohistochemical detection of α-synuclein and 3-nitrotyrosine. Sections were double-immunostained with rabbit polyclonal anti-α-synuclein (1:3000) and mouse monoclonal anti-3-nitrotyrosine primary antibody (1:500) overnight at 4 °C. After secondary incubation with a mixture of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse and tetramethyl rhodamine isothiocyanate (TRITC)-conjugated goat anti-rabbit (Sigma, USA) for 1 h, sections were stained with Hoechst 33342 for 5 min to identify nuclei. Sections were then washed three times again in PBS and then mounted and visualized by confocal microscopy. The 488-nm line of a krypton/argon laser was used for fluorescence excitation of 3-nitrotyrosine, the 568-nm line for the fluorescence excitation of α-synuclein and the 364-nm line was used for excitation of Hoechst 33342. Images were collected at the same time of exposure. TIFF filed were processed in Adobe Photoshop 8.0 for simultaneous 3-nitrotyrosine, α-synuclein and Hoechst 33342 visualization.

Data were analyzed using packages for the Social Sciences 11.5 statistical software (SPSS Inc., USA). One-way ANOVA followed by least significant differences post hoc tests were used to determine the statistical significance of differences of the means. Data are presented as the mean ± SD. p values less than 0.05 were considered significant.

Before measuring 3-nitrotyrosine levels in the rat brain, each animal's blood glucose was determined [4]. Fasting rats had a low level of blood glucose (6.08 ± 1.58 mmol/l, $n = 12$), and diabetic rats had a high level (41.23 ± 7.34 mmol/l, $n = 30$), compared with control rats (10.62 ± 2.14 mmol/l, $n = 12$). These results confirmed that the fasting and diabetic animal models were successfully generated.

To investigate whether α-synuclein was nitrated in fasting rats, monoclonal anti-3-nitrotyrosine and anti-α-synuclein were used to detect the location of nitrated α-synuclein in the rat brain (Fig. 1). The 3-nitrotyrosine signals were observed in the midbrain of 48-h fasted rats. α-Synuclein was widely distributed in most regions of the brain of the fasting, diabetic, and control rats. The majority of the 3-nitrotyrosine signals became yellow in the merged panel (Fig. 1), suggesting their colocalization with those of α-synuclein in the midbrain of the fasting rats. Under the experimental conditions, the 3-nitrotyrosine signals of diabetic rats partially colocalized with α-synuclein in the brain, but no substantial colocalization was observed in the control group. Hoechst 33342 staining exhibited the nuclei of cells in the brain of SD rats (Fig. 1). Relative quantification of the 3-nitrotyrosine fluorescent signals showed that significant difference was found between fasting and control group ($p < 0.05$, data not shown). Similar result was observed between the diabetic and control group rats. The relative fluorescence intensity of 3-NT between fasting

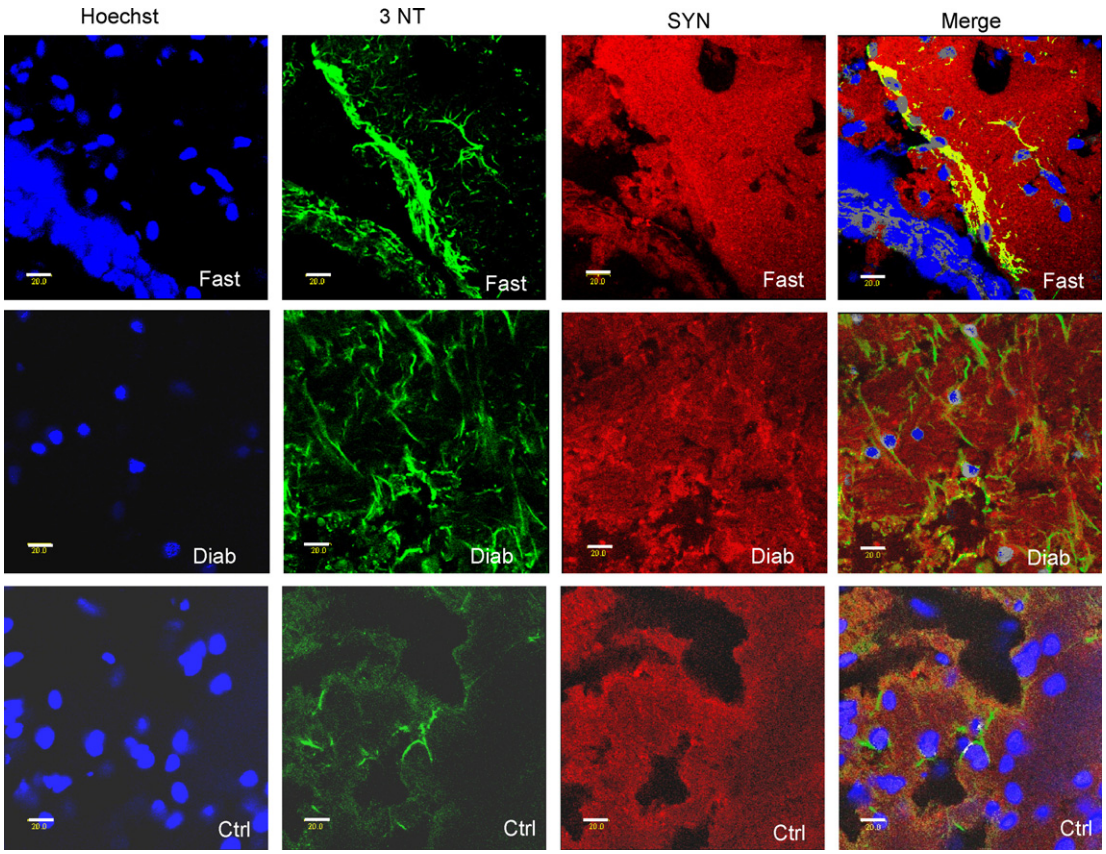

Fig. 1. Colocalization of α-synuclein and 3-nitrotyrosine in the midbrain of SD rats. Brain sections were double-immunostained with polyclonal anti-α-synuclein and mouse monoclonal anti-3-nitrotyrosine (3-NT-keyhole lympet hemocyanin, KLH) and observed by confocal microscopy. α-Synuclein (SYN) (TRITC, red), 3-nitrotyrosine (3-NT) (FITC, green), nuclei (Hoechst 33342, blue) and merged images are indicated. *Abbreviations*: Fast, fasting rats; Diab, diabetic rats; Ctrl, control. Scale bars: 20 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

and diabetic rats was not significantly different. This suggests that the α-synuclein nitration level may be elevated in the brain of the fasting SD rats.

To investigate the level of α-synuclein nitration in fasting rats, Western blotting was carried out using different brain sections. As shown in Fig. 2C, α-synuclein was widely distributed in different regions of the brain including the pallium, midbrain, hippocampus and cerebellum. There is a decreased level of α-synuclein expression at the hippocampus and cerebellum regions in the diabetic rats compared to that of control rats. In the fasting rats, however, an increased level of α-synuclein expression was found in the hippocampus sector and a decreased level of α-synuclein expression in the cerebellum parts of the rats. In average, there was no significant difference in α-synuclein expression in these regions among fasting, diabetic and control SD rats (Fig. 2D). Similar to the results presented in Fig. 1, the levels of 3-nitrotyrosine-containing proteins (55 kDa) in fasting rats were significantly increased in the midbrain and cerebellum, compared with the diabetic and control group (Fig. 2A). Furthermore, anti-3-nitrotyrosine could recognize a 17-kDa protein whose molecular mass was consistent with α-synuclein, which was increased in midbrain and cerebellum. For diabetic rats, however, the signals of 17-kDa were decreased in midbrain, hippocampus and cerebellum. It appears that the 3-NT signals of diabetic rats higher than those of control group (Fig. 1) are resulted from some other proteins. Quantitative analysis also showed that the 3-nitrotyrosine level increased slightly in the brain of diabetic rats (Fig. 2B). This suggests again that nitration of α-synuclein is increased in the brain of fasting rats.

To further confirm that this 17-kDa protein was α-synuclein and whether α-synuclein was nitrated in the brain of SD rats, we measured the nitration of α-synuclein by immunoprecipitation and detected a significantly increased level of nitrated α-synuclein in the midbrain of fasting rats ($p < 0.01$) (Fig. 2E and F). These results together suggest that the level of nitrated α-synuclein is increased in the brain of fasting rats.

To study why nitration of α-synuclein increased in the fasting rats, the expression and enzymatic activity of iNOS were also measured. The expression of iNOS was elevated in the brain of fasting and diabetic animals in comparison with the control group (Fig. 3A and B). However, there was no significant difference of the iNOS activity in the brains between fasting and diabetic rats (Fig. 3C). Similar result was observed in the blood of SD rats (Fig. 3E). To confirm iNOS was indeed active in these animals, we analyzed NO generation in the brain and serum. The results showed a higher level of NO in both the fasting and diabetic rats compared with control rats (Fig. 3D and F). However, there was no significant difference in NO production between the fasting and diabetic groups, suggesting that fasting is related to both the expression and activity of iNOS in these animals.

In the present study, we demonstrated that the level of 3-nitrotyrosine protein was increased in the brain of fasting rats, but not in diabetic rats. Furthermore, the iNOS activity in the brain of fasting rats increased significantly, and the levels of NO and iNOS activity in both brain and plasma also increased. According to Abdelmegeed et al., the oxidative stress induced by fasting may result in the generation of high levels of superoxide and NO [1], leading to the generation and abundance of peroxynitrite, which promotes tyrosine nitration *in vivo*. This suggests that fasting induces oxidative stress and leads to increased protein nitration, including nitration of α-synuclein.

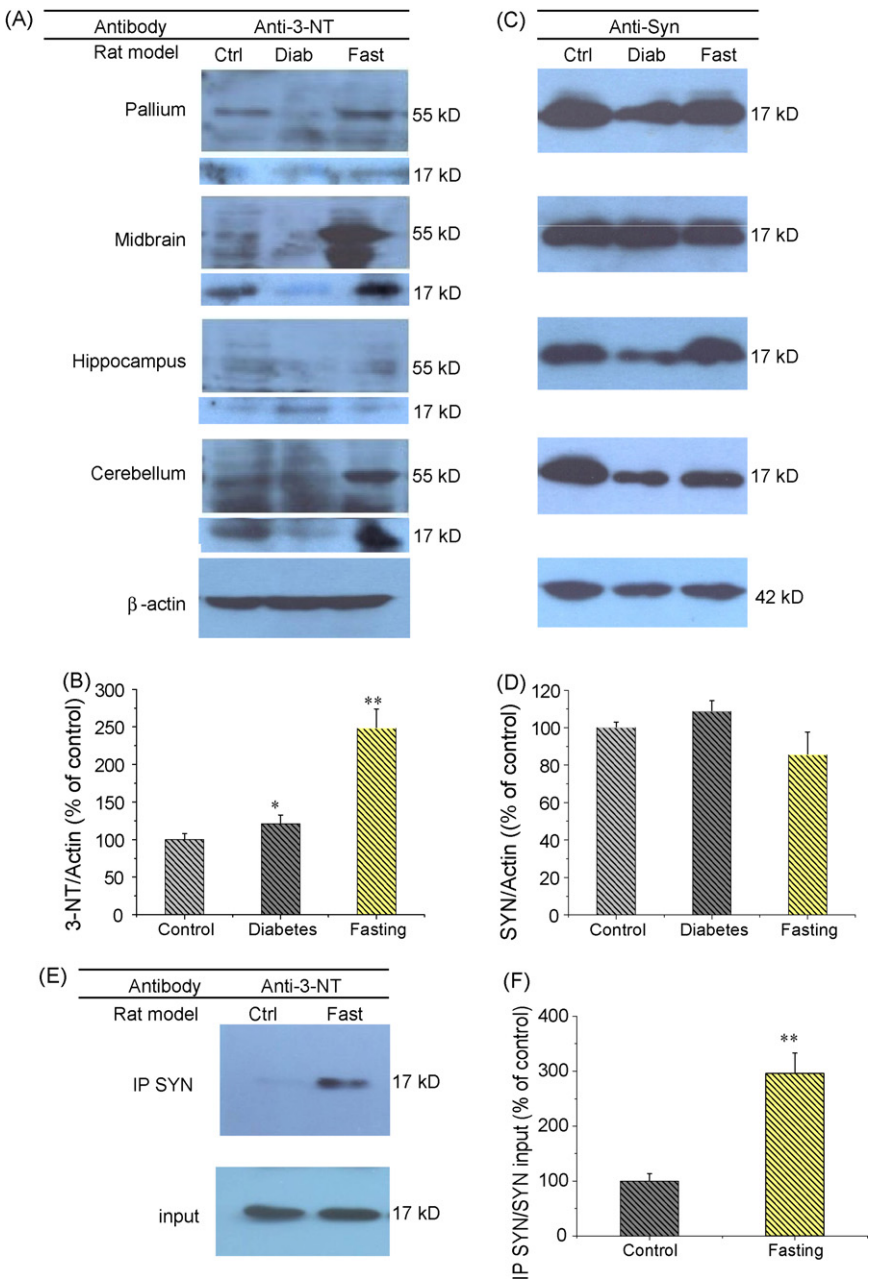

Fig. 2. Abundance of 3-nitrotyrosine and expression of α-synuclein in the brain of SD rats. Western blotting of 3-nitrotyrosine (3-NT)-containing proteins (panel (A)) and α-synuclein (Syn) (panel (C)) are as indicated. Nitrated 55-kDa and 17-kDa proteins were observed in SD rats. β-Actin level was used as a loading control. The results of quantitation are shown in panels (B) and (D) reflect an average of all the brain regions. Immunoprecipitation of nitrated α-synuclein in the brain of SD rats (panel (E)) and its quantitative analysis shown in panel (F). The results are expressed as the mean ± SD ($n = 6$). $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control. *Abbreviations*: Fast, fasting SD rats; Diab, diabetic rats; Ctrl, Control.

Our results suggest that hypoglycemia causes an increase in the nitration of α-synuclein in the rat midbrain. This viewpoint is based on several observations. (1) Under fluorescent confocal microscopy, 3-nitrotyrosine signals were shown to colocalize with α-synuclein signals in the midbrain sections of fasting rats (Fig. 1). (2) A 17-kDa nitrated protein band was found in the midbrain of fasting rats in Western blotting (Fig. 2), suggesting that α-synuclein is nitrated at a higher level. (3) Results from immunoprecipitation confirmed that α-synuclein is nitrated in the brain of fasting rats. (4) The activity of iNOS was significantly increased in the brain and serum of fasting rats compared to control rats (Fig. 3). (5) α-Synuclein has been found to be nitrated under oxidative damage, which is consistent with the previous reports [6,16]. Therefore, we suggest that fasting can induce a high level of protein nitration—especially of α-synuclein—in the rat brain.

In our experiments, the 3-nitrotyrosine level is increased in the brain of diabetic rats, but lower than that of fasting rats (Fig. 1). However, we did not see a marked increase in 3-NT of 55 kDa protein in Western blotting (Fig. 2). This result suggests that the 3-NT level of the other proteins may increase in the midbrain in the diabetic rats. Note that nitrated α-synuclein slightly increases in the hippocampus of diabetic rats. Furthermore, the iNOS activity increases in both fasting and diabetic rats. As described by Deeb et al. [6], NO derived from iNOS is essential for 3-NT modification of a protein. This indicates that fasting promotes the accumulation

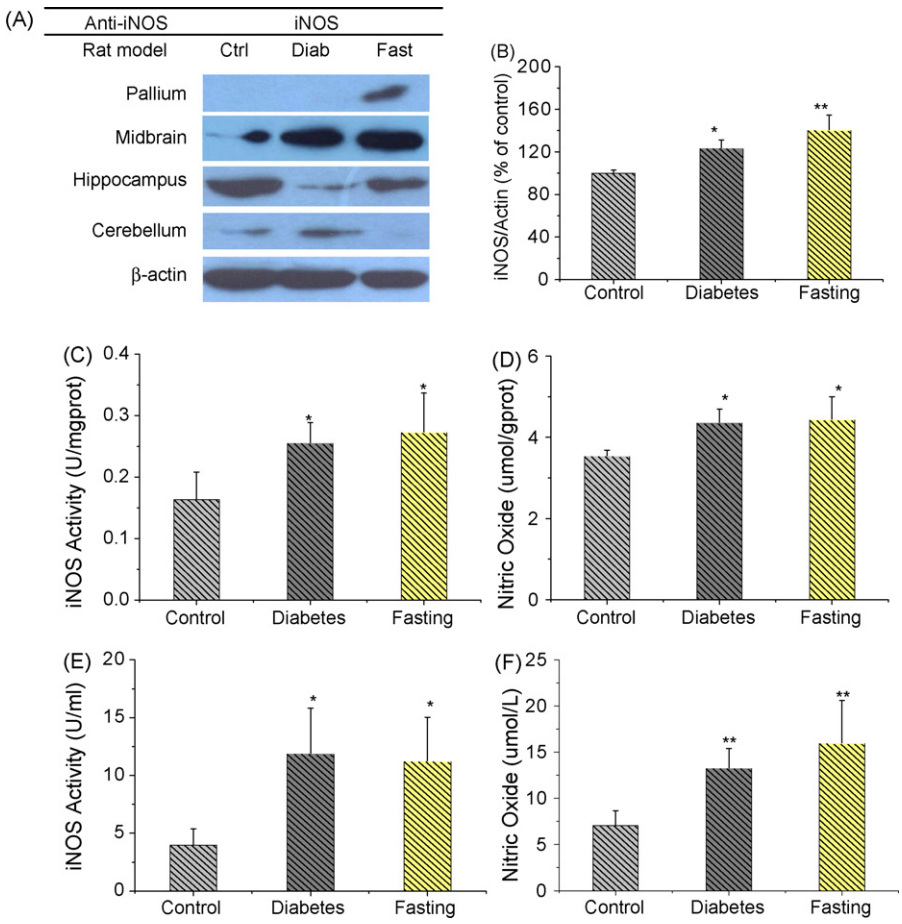

Fig. 3. Inducible NOS activity and NO production. (A) The expression of iNOS was detected by rabbit polyclonal anti-iNOS. (B) Quantitative analysis of iNOS expression in the brain of SD rats. (C and E) iNOS activity in the brain and blood of SD rats. (D and F) Nitric oxide content in the brain and blood of SD rats. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control. *Abbreviations*: Fast, fasting SD rats; Diab, diabetic rats; Ctrl, control.

of nitrotyrosine-containing proteins in rat brain, especially in the midbrain.

It is well established that four tyrosine residues (Tyr 39, Tyr 125, Tyr 133, and Tyr 136) can be nitrated in α-synuclein [8,18]. Post-translational modification of α-synuclein by reactive nitrogen species inhibits fibril formation and results in urea- and SDS-insoluble protease-resistant α-synuclein aggregates that may be responsible for cellular toxicity [11]. Nitration clearly plays an important role in the normal function of α-synuclein and may even be critical to its self-aggregation, but the specific function of α-synuclein nitration is not clear. In the present study, the colocalization of α-synuclein and 3-nitrotyrosine in the midbrain of fasting rats suggests a correlation between hypoglycemia and 3-nitrotyrosine of α-synuclein.

Midbrain plays an important role in reaction with hypoglycemia, fasting and oxidative stress, especially the dopamine energetic neurons [14,23]. Our results show that hypoglycemia causes the expression changes of 3-nitrotyrosine, suggesting that protein nitration may be related to the reaction of midbrain to hypoglycemia. Our unpublished data also show that iNOS activity and nitric oxide levels will increase when SH-SY5Y cells are exposed to nitrated protein. This might be one of the reasons for the increase in activity of iNOS. However, the physiological consequences following these changes need further investigating.

In summary, our observations provide direct evidence for the pathogenic effect of glucose that may be associated with fasting or diabetes in rats. The findings have an important implication for current strategies to elucidate the consequences of fasting in health and disease; and they provide new insight into the underlying mechanisms that lead to protein nitration and toxicity in hypoglycemic animal models.

Acknowledgements

This work was supported by the China Postdoctoral Science Foundation (Nos. 20070410153 and 200801120), NSFC30970695, 973-project-2006CB500703 and CAS-KSCX2-YW-R-119. The authors thank Xudong Zhao and Ruimin Zheng working in the core facility centre of Institute of Biophysics CAS for their technical support.

References

[1] M.A. Abdelmegeed, K.H. Moon, J.P. Hardwick, F.J. Gonzalez, B.J. Song, Role of peroxisome proliferator-activated receptor-alpha in fasting-mediated oxidative stress, Free Radic. Biol. Med. 6 (2009) 767–778.
[2] E.J. Benner, R. Banerjee, A.D. Reynolds, S. Sherman, V.M. Pisarev, V. Tsiperson, C. Nemachek, P. Ciborowski, S. Przedborski, R.L. Mosley, H.E. Gendelman, Nitrated alpha-synuclein immunity accelerates degeneration of nigral dopaminergic neurons, PLoS ONE 3 (2008) e1376.
[3] S. Bo, R. Gambino, S. Guidi, B. Silli, L. Gentile, M. Cassader, G.F. Pagano, Plasma nitrotyrosine levels, antioxidant vitamins and hyperglycaemia, Diabet. Med. 22 (2005) 1185–1189.
[4] J.M Burrin, C.P. Price, Performance of three enzymic methods for filter paper glucose determination, Ann. Clin. Biochem. 21 (Pt 5) (1984) 411–416.
[5] A. Ceriello, L. Quagliaro, B. Catone, R. Pascon, M. Piazzola, B. Bais, G. Marra, L. Tonutti, C. Taboga, E. Motz, Role of hyperglycemia in nitrotyrosine postprandial generation, Diabetes Care 25 (2002) 1439–1443.
[6] R.S. Deeb, H. Shen, C. Gamss, T. Gavrilova, B.D. Summers, R. Kraemer, G. Hao, S.S. Gross, M. Laine, N. Maeda, D.P. Hajjar, R.K. Upmacis, Inducible nitric oxide syn-

thase mediates prostaglandin h2 synthase nitration and suppresses eicosanoid production, Am. J. Pathol. 168 (2006) 349–362.
[7] B.I. Giasson, J.E. Duda, I.V. Murray, Q. Chen, J.M. Souza, H.I. Hurtig, H. Ischiropoulos, J.Q. Trojanowski, V.M. Lee, Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions, Science 290 (2000) 985–989.
[8] R. Hodara, E.H. Norris, B.I. Giasson, A.J. Mishizen-Eberz, D.R. Lynch, V.M. Lee, H. Ischiropoulos, Functional consequences of alpha-synuclein tyrosine nitration: diminished binding to lipid vesicles and increased fibril formation, J. Biol. Chem. 279 (2004) 47746–47753.
[9] E.M. Horvath, R. Magenheim, E. Kugler, G. Vacz, A. Szigethy, F. Levardi, M. Kollai, C. Szabo, Z. Lacza, Nitrative stress and poly(ADP-ribose) polymerase activation in healthy and gestational diabetic pregnancies, Diabetologia 52 (2009) 1935–1947.
[10] H. Ischiropoulos, Biological selectivity and functional aspects of protein tyrosine nitration, Biochem. Biophys. Res. Commun. 305 (2003) 776–783.
[11] H. Ischiropoulos, Oxidative modifications of alpha-synuclein, Ann. N.Y. Acad. Sci. 991 (2003) 93–100.
[12] T. Ishikawa, F. Kohno, R. Kawase, Y. Yamamoto, K. Nakayama, Contribution of nitric oxide produced by inducible nitric oxide synthase to vascular responses of mesenteric arterioles in streptozotocin-diabetic rats, Br. J. Pharmacol. 141 (2004) 269–276.
[13] H.J. Luth, V. Ogunlade, B. Kuhla, R. Kientsch-Engel, P. Stahl, J. Webster, T. Arendt, G. Munch, Age- and stage-dependent accumulation of advanced glycation end products in intracellular deposits in normal and Alzheimer's disease brains, Cereb. Cortex 15 (2005) 211–220.
[14] S. Marinelli, M. Federici, P. Giacomini, G. Bernardi, N.B. Mercuri, Hypoglycemia enhances ionotropic but reduces metabotropic glutamate responses in substantia nigra dopaminergic neurons, J. Neurophysiol. 85 (2001) 1159–1166.
[15] F. Mori, Y.S. Piao, S. Hayashi, H. Fujiwara, M. Hasegawa, M. Yoshimoto, T. Iwatsubo, H. Takahashi, K. Wakabayashi, Alpha-synuclein accumulates in Purkinje cells in Lewy body disease but not in multiple system atrophy, J. Neuropathol. Exp. Neurol. 62 (2003) 812–819.
[16] S.M. Nadtochiy, P.R. Baker, B.A. Freeman, P.S. Brookes, Mitochondrial nitroalkene formation and mild uncoupling in ischaemic preconditioning: implications for cardioprotection, Cardiovasc. Res. 82 (2009) 333–340.
[17] V. Narayanan, S. Scarlata, Membrane binding and self-association of alpha-synucleins, Biochemistry 40 (2001) 9927–9934.
[18] E.H. Norris, B.I. Giasson, H. Ischiropoulos, V.M. Lee, Effects of oxidative and nitrative challenges on alpha-synuclein fibrillogenesis involve distinct mechanisms of protein modifications, J. Biol. Chem. 278 (2003) 27230–27240.
[19] V.B. O'Donnell, J.P. Eiserich, P.H. Chumley, M.J. Jablonsky, N.R. Krishna, M. Kirk, S. Barnes, V.M. Darley-Usmar, B.A. Freeman, Nitration of unsaturated fatty acids by nitric oxide-derived reactive nitrogen species peroxynitrite, nitrous acid, nitrogen dioxide, and nitronium ion, Chem. Res. Toxicol. 12 (1999) 83–92.
[20] E. Paxinou, Q. Chen, M. Weisse, B.I. Giasson, E.H. Norris, S.M. Rueter, J.Q. Trojanowski, V.M. Lee, H. Ischiropoulos, Induction of alpha-synuclein aggregation by intracellular nitrative insult, J. Neurosci. 21 (2001) 8053–8061.
[21] P. Ponnuswamy, E. Ostermeier, A. Schrottle, J. Chen, P.L. Huang, G. Ertl, B. Nieswandt, P.J. Kuhlencordt, Oxidative stress and compartment of gene expression determine proatherosclerotic effects of inducible nitric oxide synthase, Am. J. Pathol. 174 (2009) 2400–2410.
[22] X.Y. Ren, Y.N. Li, J.S. Qi, T. Niu, Peroxynitrite-induced protein nitration contributes to liver mitochondrial damage in diabetic rats, J. Diabetes Complications 22 (2008) 357–364.
[23] V. Singh, M. Carman, J. Roeper, A. Bonci, Brief ischemia causes long-term depression in midbrain dopamine neurons, Eur. J. Neurosci. 26 (2007) 1489–1499.
[24] A. Takeda, M. Mallory, M. Sundsmo, W. Honer, L. Hansen, E. Masliah, Abnormal accumulation of NACP/alpha-synuclein in neurodegenerative disorders, Am. J. Pathol. 152 (1998) 367–372.
[25] H. Tohgi, T. Abe, K. Yamazaki, T. Murata, E. Ishizaki, C. Isobe, Alterations of 3-nitrotyrosine concentration in the cerebrospinal fluid during aging and in patients with Alzheimer's disease, Neurosci. Lett. 269 (1999) 52–54.
[26] X.L. Wang, D.L. Rainwater, A. Leone, M.C. Mahaney, Effects of diabetes on plasma nitrotyrosine levels, Diabet. Med. 21 (2004) 577–580.
[27] C.R. White, R.P. Patel, V. Darley-Usmar, Nitric oxide donor generation from reactions of peroxynitrite, Methods Enzymol. 301 (1999) 288–298.
[28] Z. Ying, T. Kampfrath, G. Thurston, B. Farrar, M. Lippmann, A. Wang, Q. Sun, L.C. Chen, S. Rajagopalan, Ambient particulates alter vascular function through induction of reactive oxygen and nitrogen species, Toxicol. Sci. 111 (2009) 80–88.
[29] X. Zhan, Y. Du, J.S. Crabb, X. Gu, T.S. Kern, J.W. Crabb, Targets of tyrosine nitration in diabetic rat retina, Mol. Cell Proteomics 7 (2008) 864–874.