Activation of sirtuin 1 attenuates cerebral ventricular streptozotocin-induced tau hyperphosphorylation and cognitive injuries in rat hippocampi

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Abstract Patients with diabetes in the aging population are at high risk of Alzheimer's disease (AD), and reduction of sirtuin 1 (SIRT1) activity occurs simultaneously with the accumulation of hyperphosphorylated tau in the AD-affected brain. It is not clear, however, whether SIRT1 is a suitable molecular target for the treatment of AD. Here, we employed a rat model of brain insulin resistance with intracerebroventricular injection of streptozotocin (ICV-STZ; 3 mg/kg, twice with an interval of 48 h). The ICV-STZ-treated rats were administrated with resveratrol (RSV; SIRT1-specific activator) or a vehicle via intraperitoneal injection for 8 weeks (30 mg/kg, once per day). In ICV-STZ-treated rats, the levels of phosphorylated tau and phosphorylated extracellular signal-regulated kinases 1 and 2 (ERK1/2) at the hippocampi were increased significantly, whereas SIRT1 activity was decreased without change of its expression level. The capacity of spatial memory was also significantly lower in ICV-

STZ-treated rats compared with age-matched control. RSV, a specific activator of SIRT1, which reversed the ICV-STZ-induced decrease in SIRT1 activity, increases in ERK1/2 phosphorylation, tau phosphorylation, and impairment of cognitive capability in rats. In conclusion, SIRT1 protects hippocampus neurons from tau hyperphosphorylation and prevents cognitive impairment induced by ICV-STZ brain insulin resistance with decreased hippocampus ERK1/2 activity.

Keywords SIRT1 · Tau phosphorylation · ERK1/2 · Streptozotocin

Introduction

Numerous epidemiological studies have shown that type 2 diabetes mellitus (T2DM) increases the risk of Alzheimer's disease (AD) (Arvanitakis et al. 2004; Stewart and Liolitsa 1999; Sanz et al. 2012). T2DM shares many common features with AD, such as disrupted glucose metabolism, insulin resistance, and cognitive impairment (Arvanitakis et al. 2004; Liu et al. 2011). It is therefore suggested that there is a convergent point between these two diseases. Evidence exists to support that defective brain insulin signaling contributes to the occurrence of AD (Hoyer and Nitsch 1989). Streptozotocin (STZ) has been accepted widely as a drug to induce animal models of both DM and AD. Previous studies have shown that

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intracerebroventricular (ICV) injection of STZ induces brain insulin resistance through the reduction of insulin receptor (IR) expression and causes desensitization of IRs (Plaschke et al. 2010). ICV-STZ treatment causes impairment of brain glucose metabolism leading to oxidative stress, which facilitates the alternation of AD-like pathology, including production of β -amyloid ($A\beta$) and tau hyperphosphorylation and cognitive impairment. The model of ICV-STZ has been considered as a valid experimental model to explore etiology of sporadic Alzheimer's disease (sAD) (Grunblatt et al. 2007; Hoyer and Lannert 2008; Baluchnejadmojarad and Roghani 2006; Hoyer et al. 2000). The mechanisms underlying STZ-induced AD-like pathological changes are still elusive.

Sirtuin 1 (SIRT1) is a highly conserved NAD^+ -dependent protein deacetylase that promotes mitochondrial function and maintains homeostasis of energy metabolism through its function of deacetylation (Braidy et al. 2012; Araki et al. 2004). The activation of SIRT1 attenuates the generation of $A\beta$ peptides by increasing α -secretase activity in vitro (Qin et al. 2006). In double transgenic APP^{swe}/PSEN1^{dE9} mice, production of $A\beta$ and behavioral deficits are mitigated by overexpressing SIRT1 and are exacerbated by SIRT1 knockout. The mechanisms of SIRT1-regulating production of $A\beta$ are done through direct activation on the transcription of the gene-encoding α -secretase (ADAM10) (Donmez et al. 2010), suggesting that SIRT1 is involved in both AD and DM and may serve as a convergent point linking AD and DM.

Hyperphosphorylation and aggregation of tau forms neurofibrillary tangles (NFTs), which are recognized as a hallmark of AD. Hyperphosphorylation of tau is an early sign in the process of AD development. The mechanisms causing tau hyperphosphorylation are not clear, which obstructs the improvement in the prevention and treatment of AD. The pathogenesis of tau pathologies needs to be clarified. Phosphorylation of Jun N-terminal kinase (JNK) and extracellular signal-regulated kinases 1 and 2 (ERK1/2) induced by hyperglycemia exacerbates ischemia-induced brain injuries (Farrokhnia et al. 2005; He et al. 2003; Kurihara et al. 2004; Li et al. 2001), whereas inhibition of ERK1/2 and JNK signaling pathways reduces the ischemic brain damage in normo- or hyperglycemic conditions (Guan et al. 2005; Namura et al. 2001; Zhang et al. 2006). The increase in phosphorylated ERK1/2 is also observed in AD-affected brains.

Studies have shown that the reduction of SIRT1 parallels with the accumulation of tau in Alzheimer's disease, and the upregulation of SIRT1 ameliorates insulin sensitivity in insulin-resistant models in rodents (Roskoski 2012). All these studies imply that SIRT1 may be involved in regulating glucose metabolism or insulin resistance and in the process of AD development. ERK1/2 may be regulated in the process, but the detailed signaling mechanisms need to be clarified. In this study, we have demonstrated that the activation of SIRT1 attenuated brain tau hyperphosphorylation and memory deficits in ICV-STZ-treated rats.

Materials and methods

Antibodies and chemicals

Rabbit polyclonal antibodies (pAb) against tau phosphorylation at Ser396, Thr231, and Thr205 were purchased from Biosource (Camarillo, CA, USA). mAb Tau1 against unphosphorylated tau and mAb PP2Ac were from Millipore (Billerica, MA, USA); mAb Tau5 against total tau was from Lab Vision Corp (Fremont, CA, USA); mAb acetylated lysine, pAb GSK-3, pS9-GSK-3 β , JNK, and p-JNK at Thr83/Tyr185 sites and ERK1/2 and p-ERK1/2 at Thr202/Tyr204 sites were obtained from Cell Signaling Technology (Beverly, MA, USA); pAbs against SIRT1 and p-PP2Ac-Y307 were from Abcam (Cambridge, UK); and mAb DM1A against α -tubulin and resveratrol (RSV) were from Sigma (St Louis, Mo, USA). BCA kit was provided by Pierce (Rockford, IL, USA).

Animals and treatment

Sprague–Dawley (SD) rats (male, weight 250 ± 20 g, 3 months) were obtained from the Experimental Animal Center of Tongji Medical College. All animal experiments were performed according to the “Policies on the Use of Animals and Humans in Neuroscience Research” by the Society for Neuroscience in 1995 and approved by Tongji Medical College Animal Experimental Ethics Committee. All rats were maintained at 22 ± 2 °C on a 12-h light/dark cycle (lights on at 6:00 a.m.), provided with water and food ad libitum, and fasted finally 12 h before the experiment.

All rats were divided randomly into three groups ($n=10$): control, STZ, and STZ+RSV. The rats were

anesthetized with 6 % chloral hydrate (6 ml/kg) via intraperitoneal injection and placed in a stereotaxic instrument (SR-6N; Narishige Scientific Instrument Laboratory, Tokyo, Japan). STZ (3 mg/kg) dissolved in artificial cerebrospinal fluid (CSF) was injected slowly into the bilateral cerebroventricles in the STZ group rats twice at an interval of 48 h using Hamilton® syringe with the following coordinates: 0.8 mm anterior to posterior (AP) bregma, 1.5 mm midline to lateral (ML), and 4.0 mm dorsal to ventral (DV) dura. The rats in the control group underwent the same surgical procedures, and artificial CSF alone was injected in the same volume, respectively. The ICV-STZ-treated rats were administered with resveratrol (SIRT1 agonist, 30 mg/kg dissolved in 1 ml of 0.5 % DMSO) or 0.5 % DMSO alone in a volume of 1 ml/day for 8 weeks by intraperitoneal (ip) injection, respectively, in the STZ+RSV and STZ groups, and the rats in the control group were treated with 0.5 % DMSO in the same volume and times via intraperitoneal injection.

Morris water maze test

The water maze was in a round tank (160 cm in diameter) containing water (temperature at 22–25 °C) mixed with a nontoxic black dye to make it opaque. All trials started at 08:00 a.m., and the rats were placed in the water maze room 1 h before the water maze trial daily. For the hidden platform trial, rats were trained to find a hidden platform (12 cm in diameter) submerged 1.5 cm under the water surface. The training consisted of four trials per day for six consecutive days. In each trial, rats were allowed to search for the platform for 60 s until they land on it or are gently guided to it if they failed to find the platform within the 60 s. After that, rats were allowed to remain on the platform for 30 s before being removed and placed in their home cages. On day 8, the platform was removed from the tank, and a probe test lasting 60 s was conducted. The time to reach the platform (escape latency), path length, swimming speed, and time spent in each quadrant were monitored by a computerized tracking system connected to a video camera above the pool.

Western blotting

Hippocampi were homogenized in a cooled buffer containing 10 mM Tris–HCl (pH 7.6), 50 mM NaF,

1 mM Na_3VO_4 , 1 mM EDTA, 1 mM benzamidine, 1 mM phenylmethylsulfonylfluoride (PMSF), and 10 µg/ml protease inhibitor cocktail (leupeptin, aprotinin, and pepstatin A). The homogenates were mixed with a loading buffer (200 mM Tris–HCl (pH 7.6), 8 % sodium dodecyl sulfate (SDS), 40 % glycerol, 40 mM dithiothreitol (DTT), 4 % β -mercaptoethanol, 0.05 % bromophenol blue), boiled in a water bath for 10 min, and then centrifuged at 12,000×g for 10 min. Supernatants were collected and used for Western blot analysis. The protein concentration was estimated using the BCA kit according to manufacturer's instructions (Pierce, Rockford, IL, USA). For Western blot analysis, equal amounts of protein were fractionated by 10 % SDS-PAGE and transferred to nitrocellulose membrane. The membranes were blocked with 5 % nonfat milk dissolved in Tris-buffered saline (50 mM Tris–HCl, 150 mM NaCl, pH 7.6) for 1 h and probed with primary antibodies overnight at 4 °C. Blots were incubated with IRDye 800CW goat anti-mouse or anti-rabbit secondary antibody (Licor Biosciences, Lincoln, NE, USA) for 1 h at room temperature and visualized using the Odyssey Infrared Imaging System (Licor Biosciences, Lincoln, NE, USA).

Co-immunoprecipitation

Hippocampi were homogenized in a cooled buffer (on ice) (50 mM Tris–HCl, pH 7.4, 250 mM NaCl, 5 mM EDTA, 0.1 % Triton X-100, containing 50 mM NaF, 1 mM PMSF, 10 mg/ml leupeptin, 0.5 mg/ml aprotinin and 0.1 mM Na_3VO_4) for 30 min. The lysates were centrifuged at 10,000×g for 10 min at 4 °C. The supernatants (0.5 mg) were incubated with the indicated antibody at 4 °C overnight with gentle rotation, then mixed (20 µl) with the suspension of protein G Sepharose beads (1:1), and incubated for 2 h at 4 °C with gentle rotation. The beads were collected by centrifugation and washed extensively with lysis buffer. The bound proteins were dissociated by boiling the beads in 2× Laemmli sample buffer and examined by Western blot analysis.

Measurement activity of SIRT1 deacetylase

SIRT1 activity was determined using a SIRT1 Fluorometric Activity Assay Kit (GMS50287.2, GENMED) according to the manufacturer's

instructions. Briefly, lysates were prepared with GENMED lysis buffer. Afterwards, 55 μ l of buffer solution (reagent E) and 5 μ l of substrate (reagent F) were added to a 96-well plate with 20 μ l of replenisher (reagent I) or lysates (10 μ g/ μ l, 200 μ g). The mixtures were then incubated for 60 min at 30 °C, and the reactions were stopped by adding 10 μ l of stop solution (reagent G) followed by 10 μ l of enzymolysis liquid (reagent H). After incubation for 60 min at 30 °C, the fluorescence intensity at 405 nm was recorded, and the mixture was normalized to total protein.

NAD/NADH ratio assay

The assay for NAD/NADH ratio was performed as reported previously (Visser et al. 2004). Briefly, for a 50- μ l sample, NADH was destructed by the addition of 5 μ l of HCl (1 mM), and NAD was destructed by the addition of 5 μ l of KOH (1 mM) and subsequent heating at 60 °C for 5 min. After the destructions, the sample was neutralized by the addition of 5 μ l of either 1 mM KOH or 1 mM HCl. The assay mixture (100 μ l) consisted of 60 μ l of pretreated sample as described above, 15 μ l of ADH solution (9,000 U/ml), and 25 μ l of ethanol solution (including 5-ethylphenazinium ethyl sulfate (PES, 4 mg/ml) and thiazolyl blue (MTT, 5.0 mg/ml)). After 5 min of incubation, the absorbance was measured at 590 nm using the Synergy2 Multi-Mode Microplate Reader (BioTek, USA).

Statistical analysis

All data were presented as mean $\pm$ SEM and analyzed using the SPSS 11.0 statistical software (SPSS, Chicago, IL, USA). Statistical significance was determined by one-way ANOVA followed by Tukey's test for multiple comparisons with 95 % confidence interval and Student's two-tailed t test.

Results

The levels of tau phosphorylation were significantly enhanced with a simultaneous SIRT1 inactivation in ICV-STZ-infused rats

To investigate the mechanisms of ICV-STZ-induced tau phosphorylation in rats, after ICV-STZ treatment

for 4 or 8 weeks, the level of tau phosphorylation and activity and expression of SIRT1 in the hippocampus samples were detected by Western blot analysis or using fluorometric activity assay kit. We found that tau phosphorylation was significantly increased at the Thr205 and Ser396 sites on the eighth week but not on the fourth week after ICV-STZ administration as compared with the control group (Fig. 1a–d). Based on the result, we selected 8 weeks after treatment with ICV-STZ for the following experiments. The previous studies have shown that SIRT1 promotes mitochondrial function and maintains homeostasis of energy metabolism (Rodgers et al. 2005; Ramadori et al. 2011; Gillum et al. 2010). We therefore measured hippocampus SIRT1 expression and activity in ICV-STZ-treated and control rats by Western blot analysis and using fluorometric activity assay kit, respectively. The results showed that activity of SIRT1 decreased to 32 % of control levels in ICV-STZ-treated rats, but the expression levels of SIRT1 were not different between two groups (Fig. 2a–c). To explore the causes of SIRT1 inactivation in ICV-STZ-treated rats, as SIRT1 is a NAD⁺-dependent histone deacetylase, its activity may be regulated by the ratio of NAD/NADH in vivo. We therefore detected the ratio of NAD⁺/NADH in this study. We found that the ratio of NAD/NADH decreased to 31.6 % in the control group in ICV-STZ-treated rats (Fig. 2d), suggesting that decrease in SIRT1 activity was caused by NAD⁺ dependency in ICV-STZ-treated rats.

Activation of SIRT1 attenuated tau phosphorylation in ICV-STZ-treated rats

We speculated that reversing SIRT1 activity could attenuate tau phosphorylation in ICV-STZ-treated rats. To determine whether increasing activity of SIRT1 attenuates ICV-STZ-induced AD-like tau phosphorylation, rats treated with ICV-STZ were administered with or without resveratrol (SIRT1 agonist, 30 mg/kg) by ip injection for 8 weeks (detailed in the “Material and methods” section), and the activity of SIRT1 and tau phosphorylation was measured by fluorometric activity assay and Western blot assay. We observed that RSV restored almost completely the decrease in SIRT1 activity by ICV-STZ treatment (Fig. 3a). Meanwhile, the increase in tau hyperphosphorylation induced by ICV-STZ was attenuated significantly by RSV (Fig. 3b, c). These results indicate that RSV effectively reverses STZ-induced

Fig. 1 ICV-STZ-induced tau hyperphosphorylation in the hippocampus of rats. After rats were treated with ICV-STZ for 4 or 8 weeks, the extracts of rat hippocampus were prepared. The levels of tau phosphorylation were detected by site-specific primary antibodies as indicated on the blots: 4 weeks after ICV-STZ treatment (a), 8 weeks after ICV-STZ treatment (c), and the quantitative analysis was normalized against DM1A and intensity in the control group was taken as 1 unit (b, d). $n=10$; $*P<0.05$, $**P<0.01$ versus the control group

changes of SIRT1 inactivation and tau hyperphosphorylation, suggesting that inactivation of SIRT1 is

related to tau hyperphosphorylation in ICV-STZ-treated rats.

Fig. 2 ICV-STZ-induced downregulation of SIRT1 activity. After rats treated with ICV-STZ for 8 weeks, the levels of SIRT1 were examined in the extracts of rat hippocampus by Western blot analysis (a), and quantitative analysis was performed (b). The activity of SIRT1 and NAD/NADH ratio were detected using the assay kits (c, d) respectively. $n=10$; $*P<0.05$, $**P<0.01$ versus the control group

Fig. 3 Resveratrol reversed ICV-STZ-induced SIRT1 inactivity and tau hyperphosphorylation. The rats treated with ICV-STZ were administrated resveratrol or solvent control ip for 8 weeks. The SIRT1 activity and levels of tau phosphorylation were tested using assay kits or by Western blot analysis of the extracts of rat

hippocampus respectively (**a**, **b**). The quantitative analysis of **b** was performed with 1 unit as that obtained in the control group (normalized against total tau probed by Tau5) (**c**). $n=10$; * $P<0.05$ versus the control group; # $P<0.05$ versus the ICV-STZ-treated group

SIRT1 attenuated tau phosphorylation via decreasing ERK1/2 phosphorylation

SIRT1 is a NAD^+ -dependent protein deacetylase, so it may not directly phosphorylate tau protein. It is well known that an imbalance of protein kinases and protein phosphatase causes tau hyperphosphorylation. The protein kinases related to energy metabolism and tau phosphorylation, such as GSK3 β , JNK, p38, and ERK1/2, are several. In addition, PP2A is the main phosphatase implicated in dephosphorylating the tau proteins. For exploring which protein kinases and/or phosphatase were involved in tau hyperphosphorylation and SIRT1 activation in ICV-STZ-treated rats, the above-mentioned protein kinases and phosphatase were analyzed by Western blot analysis. The results here showed that levels of ERK1/2 phosphorylation were significantly increased and RSV treatment mitigated such change of phosphorylation. There were, however, no changes in the expression of GSK3 β , JNK, and p38 phosphorylation in all treatments, whereas total protein levels of these kinases, the activity-dependent phosphorylation of PP2A catalytic subunit (PP2Ac) at Tyr307 site, and total PP2A showed no difference among the three groups (Fig. 4a, b). These results suggest that the increase in p-ERK1/2 (functional activation) may be responsible for the tau hyperphosphorylation in ICV-STZ-treated rats.

Signaling pathways leading to hippocampus p-ERK1/2 (activation) in ICV-STZ-treated rats are still unknown. To clarify this issue, the levels of ERK1/2 acylation at Lys sites and interaction between ERK1/2

and SIRT1 were measured in the hippocampus homogenate of ICV-STZ-treated rats with co-immunoprecipitation and Western blot analysis. The results showed that acetylation of ERK1/2 at Lys sites was evoked through the interaction between SIRT1 and ERK1/2 in ICV-STZ-treated rats (Fig. 4c, d). It is therefore suggested that ERK1/2 may be acetylated and such modification of acylation may be associated with the action of SIRT1 and ERK1/2 phosphorylation in vivo.

Resveratrol ameliorated ICV-STZ-induced spatial memory deficit in rats

To investigate the effects of SIRT1 activation on the spatial learning ability of ICV-STZ-treated rats, we evaluated the spatial learning capability of rats using the Morris water maze (MWM). The latency of the rat to find the hidden platform dramatically increased, and time of platform quadrant crossing significantly decreased in ICV-STZ-treated (for 8 weeks) rats. Simultaneous application of RSV improved the searching strategy of the ICV-STZ-treated rats, including a shorter latency and significantly increased time of platform quadrant crossing (Fig. 5a, b). To exclude the effects of STZ-induced motion incapability of rats on spatial memory, swimming speed in MWM and body weight of rats were recorded every week, and no significant difference was observed among the three groups of rats (Fig. 5c, d). Such observation suggests that ICV-STZ treatment in this experiment did not significantly impact the body metabolism and motion capacity of rats.

Fig. 4 Resveratrol mitigated ICDV-STZ caused by the increase of p-ERK1/2 via impacting acylation of ERK1/2 in rats. After the ICDV-STZ-treated rats were administrated resveratrol for 8 weeks, the extracts of rat hippocampus were prepared. The levels of GSK3β, ERK1/2, JNK, and PP2Ac were measured by Western blot analysis (**a**), and quantitative analysis of (**a**) was performed with 1 unit as that in the control group (normalized respectively

to the total level of protein) (**b**). The interaction between SIRT1 and ERK1/2 and acylation of ERK1/2 at Lys sites were detected with co-immunoprecipitation; the hippocampus extracts were precipitated with ERK1/2 or SIRT1 antibodies, respectively, and the precipitation was examined by Western blot Analysis using Ac-Lys (**c**) or ERK1/2 (**d**). $n=10$; $*P<0.05$ versus the control group; $\#P<0.05$ versus the ICDV-STZ-treated group

Discussion

The hyperphosphorylated tau, which increases its biological half-life in vivo (Min et al. 2010), alters its microtubule binding and enhances aggregation to form NFTs in AD-affected brains (Cohen et al. 2011). Several epidemiological and experimental studies have demonstrated that diabetes mellitus increases the risk of sporadic AD, suggesting a close linkage between these two disorders (Steen et al. 2005; Li et al. 2007; Akter et al. 2011). In the present study, a rat model that is resistant to brain insulin was made by ICDV-STZ treatment twice at an interval of 48 h. Previous studies demonstrated that the administration of STZ via the intracerebroventricles reduced insulin receptor mRNA and protein expression in the hippocampus of the brain and resulted in brain insulin resistance in ICDV-STZ-treated rodent models (Plaschke et al. 2010). This central STZ treatment reduces insulin signaling in the brain, whereas it avoids intraperitoneal STZ-injection-induced whole body insulin deficiency and islet cell toxicity. This model was therefore selected in this

experiment to study whether SIRT1 attenuated insulin-resistant induced tau hyperphosphorylation and spatial memory deficits and to explore the underlying mechanisms. It was found that tau phosphorylation significantly increased at the Thr205 and Ser396 sites after ICDV-STZ treatment for 8 weeks (Fig. 1a–d). These results are consistent with previous similar studies (Chu and Qian 2005; Grunblatt et al. 2007; Deng et al. 2009), and further underlying mechanisms have been explored in this experiment.

SIRT1 has been reported as a promising therapeutic target for age-related diseases such as type 2 diabetes mellitus and neurodegenerative diseases (Milne et al. 2007; Braidy et al. 2012). A recent report showed that SIRT1 levels were significantly reduced in AD-affected brains, and this reduction paralleled the accumulation of tau (Julien et al. 2009); which raised the possibility that SIRT1 might regulate tau phosphorylation levels in vivo. Accumulated evidence suggested that SIRT1 activity was downregulated in STZ-induced diabetes rodents, and therefore, it was speculated that a decrease in SIRT1 activity was

Fig. 5 Resveratrol ameliorated ICV-STZ-induced spatial memory deficit in rats. After the ICV-STZ-treated rats were treated with or without resveratrol ip for 8 weeks, the rats were trained to remember the hidden platform in the Morris water maze for 6 days and the latency (time to find platform) was recorded (learning process) (a). Representative swim paths and number of platform crossing during the probe test (b). Swimming speed in MWM (c) and body weight of rats (d) were recorded without differences between groups. * $P < 0.05$ versus the control group; # $P < 0.05$ versus the STZ group

involved in tau hyperphosphorylation. The activation of SIRT1 might reverse this tau hyperphosphorylation in ICV-STZ-treated rats. Results in this experiment showed that activity of SIRT1 decreased to 68 % of the control in ICV-STZ-treated rats, but the expression of SIRT1 was not changed by ICV-STZ treatment and the ratio of NAD/NADH was decreased to 31.6 % of the control in ICV-STZ-treated rats (Fig. 2a–d), suggesting that ICV-STZ reduced SIRT1 activity by reducing the ratio of NAD/NADH in the hippocampus of the treated rats. We also demonstrated that stimulation of SIRT1 with its specific activator, RSV, effectively elevated SIRT1 activity in ICV-STZ-treated rats and attenuated ICV-STZ-induced tau hyperphosphorylation in the hippocampi of rats (Fig. 3a–c). Taking these data together, it is suggested that SIRT1 inactivation may be a key element that is responsible for tau hyperphosphorylation in ICV-STZ-treated rats.

ICV-STZ impairs the brain insulin signaling pathways and ultimately induces AD-like tau protein and A β pathology (Salkovic-Petrisic et al. 2006; Grunblatt et al. 2007; Salkovic-Petrisic and Hoyer 2007). The PI3K/GSK3 β and MAPK/ERK are major downstream signals of insulin receptor activation, and these kinases may also phosphorylate tau in vitro and

in vivo (Pei et al. 2002, 2003; Takata et al. 2009). It was observed in this experiment that levels of p-ERK1/2 were increased in ICV-STZ-treated rats compared with that in the control group (Fig. 4a, b). When ICV-STZ-treated rats were infused with RSV at the dose of 3 mM in a volume of 1 ml/day for 8 weeks by intraperitoneal injection, it was found that SIRT1 was significantly activated, and increases in p-tau and p-ERK1/2 were reversed. The activity of ERK1/2 is determined by the phosphorylation of activity-dependent phosphorylation sites, and there is a positive relationship between activity and phosphorylation of ERK1/2 at Thr202/Tyr204 (Roskoski 2012). There were no changes of p-GSK3 β and p-JNK in this study, which is a clear discrepancy with the previous study and may be due to the difference in doses, treatment times, and technical ways of STZ injection (Shonesy et al. 2012). PP2A is the main protein phosphatase to make tau dephosphorylation in the brain and its phosphorylation at Tyr307 (an inactive type) is increased in the AD-affected brain (Liu et al. 2008). The levels of phosphorylation and total PP2A were not significantly alternated among three groups in this study (Fig. 4a, b). Considering all of the above-mentioned data, it is suggested that the activation of SIRT1 with RSV attenuates ICV-STZ-induced tau

hyperphosphorylation through decreasing p-ERK1/2 (active type) and reduces tau abnormal hyperphosphorylation. This view is also supported by high levels of activated ERK1/2 in AD-affected brains (Pei et al. 2002, 2003).

SIRT1 is a cytoplasmic enzyme that mediates NAD⁺-dependent deacetylation of target substrates. SIRT1 actively regulates substrates by reducing the acetylation of target substrates, such as PGC-1 α , P53, and LKB1. In the current study, it was observed that there was an interaction between SIRT1 and ERK1/2. Lysine motif of ERK1/2 in the hippocampus was acetylated in ICV-STZ-treated rats (Fig. 4c, d), suggesting that SIRT1-mediated activity of ERK1/2 via the regulation of its acylation.

Previous studies reported that systemic STZ and ICV-STZ administrations result in learning and memory loss (Biessels et al. 1996a; Gagne et al. 1997; Gardoni et al. 2002; Kamal et al. 2006; Shonesy et al. 2012). Because systemic STZ administration results in systemic toxicity and pancreatic beta-cell death, evidenced by chronic hyperglycemia (Biessels et al. 1996b), hypercorticism (Chandna et al. 2002), and hypoinsulinemia (Tjalve and Castonguay 1983), it is difficult to define a conclusion regarding the mechanisms underlying spatial memory loss. ICV-STZ administration is a much limited drug delivery strategy, causing a reduction of insulin receptor expression and insulin resistance in the brain (Plaschke et al. 2010). Such STZ treatment also caused spatial memory loss (Biessels et al. 1996a; Shonesy et al. 2012). We explored here that SIRT1 activation attenuated ICV-STZ-induced AD-like tau hyperphosphorylation accompanied by impairment of spatial memory in rats. Body weights of rats showed no difference among ICV-STZ-treated and control rats, suggesting that the ICV-STZ-treated rats did not suffer from systemic toxicity induced by STZ. The latency to find the hidden platform dramatically increased, and times of platform quadrant crossing significantly decreased in ICV-STZ-treated rats, whereas simultaneous application of RSV with ICV-STZ for 8 weeks improved the spatial memory of the rats including reduced latency and increased times of platform quadrant crossing. It is suggested that ICV-STZ causes spatial memory impairment by inactivation of SIRT1 in the brain hippocampus, whereas RSV may effectively reverse memory impairment in the ICV-STZ-treated rats.

Evidence has been provided that SIRT1 is required for maintaining cognitive function, synaptic plasticity, and neuronal metabolism homeostasis, and activation of SIRT1 improves energy metabolism balance and cognitive ability (Banks et al. 2008; Purushotham et al. 2012; Kim et al. 2007). Undoubtedly, the current data and the data from previous studies further support the view that SIRT1 is a causative molecule linking insulin resistance and sporadic AD and that RSV-induced activation of SIRT1 mitigates ICV-STZ-induced AD-like tau hyperphosphorylation and memory impairment.

In conclusion, inactivation of SIRT1, tau hyperphosphorylation, and memory impairment occurred in ICV-STZ-treated rats, and activation of SIRT1 by RSV attenuated tau hyperphosphorylation and memory impairment via inhibiting ERK1/2 activity. It is therefore suggested that SIRT1 be a therapeutic target for the treatment of AD with diabetes.

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Conflict of interest There are no actual or potential conflicts of interest.

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