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Genetic Ablation of Tau Mitigates Cognitive Impairment Induced by Type 1 Diabetes

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Patients affected by diabetes show an increased risk of developing Alzheimer disease (AD). Similarly, patients with AD show impaired insulin function and glucose metabolism. However, the underlying molecular mechanisms connecting these two disorders are still not well understood. Herein, we investigated the microtubule-associated protein tau as a new link between AD and diabetes. To determine whether diabetes causes cognitive decline by a tau-dependent mechanism, we treated non-transgenic (Ntg) and tau-knockout mice with streptozotocin, causing type 1 diabetes-like disease (T1D). Interestingly, although induction of T1D in Ntg mice led to cellular and behavioral deficits, it did not do so in tau-knockout mice. Thus, data suggest that tau is a fundamental mediator of the induction of cognitive impairments in T1D. Tau dysregulation, which causes a reduction in synaptic protein levels, may be responsible for the cognitive decline observed in Ntg streptozotocin-treated mice. Concomitantly, we demonstrate the novel finding that depletion of endogenous tau mitigates behavioral impairment and synaptic deficits induced in T1D-like mice. Overall, our data reveal that tau is a key molecular factor responsible for the induction of cognitive deficits observed in T1D and represents a potential therapeutic target for diabetes and patients with AD. *(Am J Pathol 2014, 184: 819–826; http://dx.doi.org/10.1016/j.ajpath.2013.11.021)*

The incidence and prevalence of age-related neurodegenerative and metabolic disorders are growing because of the increasing life expectancy of the human population in industrialized countries. Diabetes, the most common metabolic disorder, is largely characterized by hyperglycemia, but it is also associated with vascular disorders and cognitive impairments.[1–8] More than 176 million people are affected by diabetes in the world, and it is estimated to reach 366 million in 2030.[9] There are two clinical forms of diabetes: type 1 diabetes (insulin-dependent diabetes) and type 2 diabetes (non–insulin-dependent diabetes).

Epidemiological studies show that diabetic patients have a significantly increased risk of developing Alzheimer disease (AD) versus healthy individuals.[1,2,10–14] AD is a neurodegenerative disorder characterized by progressive loss of memory and cognitive skills. Neuropathologically, AD is characterized by the presence of senile plaques of β-amyloid (Aβ) and neurofibrillary tangles composed by hyperphosphorylated forms of tau protein and neuronal and synaptic loss.[15,16] Both AD and diabetes share several

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pathobiochemical features, such as oxidative stress, formation of advanced glycation end products, dysregulated glucose metabolism, and altered insulin signaling.[17] Notably, recent evidence using animal models shows that diabetes can promote aberrant tau modifications.[18–24]

Microtubule-associated protein tau is a cytoskeleton protein that regulates neuronal development and promotes assembly and stability of microtubules, which are critical for vesicle transport.[16] In the central nervous system, almost 20% of tau is phosphorylated under physiological conditions; however, in pathological conditions, such as AD, tau is hyperphosphorylated. This hyperphosphorylation causes a loss of affinity for the microtubules, altering intracellular trafficking and consequently leading to synapse dysfunction, neuronal degeneration, and cognitive decline.[15,25–28] Tau can be phosphorylated by several serine/threonine kinases, including glycogen synthase kinase 3β (GSK3β).[29–31] More important, insulin has a key role in metabolic signaling and regulates the activity of some kinases that are responsible for tau phosphorylation.[32,33] Recent evidence using animal models of diabetes suggests that impaired insulin signaling causes tau hyperphosphorylation through GSK3β activation.[18–24,28]

Herein, we investigated tau as a key molecular factor in type 1 diabetes-like disease (T1D) to induce cognitive impairment. We evaluated the effect of insulin deficiency and hyperglycemia in non-transgenic (Ntg) and tau-knockout (tauKO) mice using streptozotocin (STZ). Our results indicate that STZ treatment causes hyperphosphorylation of tau in Ntg mice through activation of GSK3β. These increments on hyperphosphorylated tau correlate with spatial cognitive dysfunction and changes in synaptic proteins. Notably, tauKO mice treated with STZ show no cognitive or synaptic deficits. Overall, our data indicate that T1D impairs cognition via tau-dependent mechanisms, and removal of tau prevents cognitive deficits.

Materials and Methods

Animals

Four-month-old Ntg and tauKO mice were used ($n = 10$ to 13 males per group). All animal procedures were performed in accordance with NIH and University of California guidelines and Use Committee at the University of California, Irvine.

Diabetes Induction and Blood Glucose and Insulin Measurements

Diabetes was induced as described previously.[9,24,34,35] Briefly, Ntg and tauKO mice received a single injection of STZ (150 mg/kg, i.p.) diluted in 0.1 mol/L citrate buffer (pH 4.5). STZ, a glucosamine-nitrosourea compound, is toxic to the insulin-producing β-cells of the pancreas, and i.p. administration of STZ induces hyperglycemia and insulin deficiency, rendering it a valuable model to study T1D.[27] The onset of diabetes was confirmed by assessing glucose levels (Nipro Diagnostics, Fort Lauderdale, FL) in blood samples collected from the tail vein, 3 days after STZ treatment. Only animals with blood glucose levels ≥200 mg/dL, 3 days after STZ treatment, were used in the experiments.[36] In total, the determination of blood glucose levels (and body weight) was measured 3 and 40 days after STZ injection. In addition, insulin levels were measured 40 days after STZ administration (Millipore, Billerica, MA). STZ-treated mice showed reduced insulin and increased glucose levels. No differences in these measurements were found between Ntg and tauKO mice (Table 1).

Behavioral Test

Hidden Morris water maze (MWM) tests were conducted as described previously.[37] Mice were trained to swim to a 14-cm−diameter circular Plexiglas platform submerged 1.5 cm beneath the surface of the water and invisible to the mice while swimming. The platform was located in a fixed position, equidistant from the center and the wall of the tank. Mice were subjected to four training trials per day. During each trial, mice were placed into the tank at one of four designated start points per day in a pseudorandom order. Mice were trained for as many days as needed to reach the training criterion of 25 seconds (escape latency). If the mice failed to find the platform within 60 seconds, they were manually guided to the platform and allowed to remain there for 5 seconds. The probe trial was assessed 24 hours after the last training session and consisted of a 60-second free swim in the pool without the platform. Performance was monitored with the EthoVision XT video tracking system (Noldus Information Technology, Leesburg, VA).

Tissue Preparation

After deep anesthesia with sodium pentobarbital (60 mg/kg, i.p.), Ntg and tauKO mice were perfused transcardially with

Table 1 Significant Hyperglycemia Levels in Ntg and tauKO Mice 3 Days and 4 Weeks after Streptozotocin Injection

Genotype	*N*	Blood glucose 3 days after STZ injection (mg/dL)	Final blood glucose 40 days after STZ injection (mg/dL)	Insulin levels 40 days after STZ injection (ng/mL)
Ntg	13	114 ± 7	136 ± 6	0.65 ± 0.19
Ntg-STZ	13	292 ± 28*	316 ± 24*	0.15 ± 0.05**
tauKO	10	134 ± 10	152 ± 5	0.45 ± 0.12
tauKO-STZ	10	242 ± 21*	305 ± 33*	0.12 ± 0.08**

The values represent the means ± SEM. Two-way analysis of variances at 3 days: genotype [$F(1,42) = 0.52$], treatment [$F(1,42) = 53.30$, $P < 0.0001$], and interaction [$F(1,42) = 3.09$, $P = 0.08$]; and 4 weeks: genotype [$F(1,42) = 0.01$], treatment [$F(1,42) = 66.09$, $P < 0.0001$], and interaction [$F(1,42) = 0.41$]; and insulin levels: genotype [$F(1,16) = 0.69$], treatment [$F(1,16) = 8.93$, $P < 0.01$], and interaction [$F(1,16) = 0.40$]. Bonferroni's pairwise comparisons were performed.
*$P \leq 0.001$, **$P \leq 0.05$.

0.1 mol/L PBS, pH 7.4. Protein extracts were prepared by homogenizing whole brain hemisphere samples in T-per extraction buffer (Thermo Fisher Scientific, Rockford, IL) complemented with complete miniprotease inhibitor tablets (Roche Diagnostics GmbH, Mannheim, Germany) and phosphatases inhibitors (5 mmol/L; Sigma-Aldrich, St. Louis, MO), followed by centrifugation at 100,000 × *g* for 1 hour. Protein concentration in the supernatant was determined using the Bradford assay.

Immunoblotting

Equal amounts of protein (20 μg) were separated on 4% to 12% Bis-Tris gel (Invitrogen, Carlsbad, CA) and transferred to nitrocellulose membranes. Membranes were blocked for 1 hour in 5% (w/v) suspension of nonfat milk in 0.2% Tween 20 Tris-buffered saline (pH 7.5). After blocking, the membranes were incubated overnight, at 4°C, with one of the following primary antibodies: anti-postsynaptic density protein 95 (PSD95; 1:1000), anti-cAMP response element binding (CREB; 1:1000), anti−*p*-CREB (Ser^{133}; 1:1000), anti−*p*-phosphoinositide 3-kinase (PI3K; p85; 1:1000), anti-PI3K (1:1000), anti−*p*-AKT (Ser^{473}; 1:1000), anti-AKT (1:1000), anti−*p*-GSK3β (Ser^{9}; 1:1000), anti−*p*-p38-mitogen-activated protein kinase (MAPK; 1:1000), anti−p38-MAPK (1:1000; Cell Signaling Technology, Danvers, MA), anti-synaptophysin (1:5000; Sigma-Aldrich), anti-AT8 (1:1000), anti-AT100 (1:1000), anti-AT180 (1:1000), anti-AT270 (1:1000; Thermo Fisher Scientific), anti-p-tau paired helical filament (PHF; 1:1000; Dr. Peter Davies, Albert Einstein College of Medicine, Manhasset, NY), anti-total tau (1:3000; Dako, Carpinteria, CA), anti−*p*-insulin receptor (IR; Tyr^{972}; 1:1000), anti-IR (1:1000), anti-GSK3β (1:1000), anti-Cdk5 (1:1000; Millipore), anti-p35/p25 (1:200), anti−extracellular signal−regulated kinase (ERK) 1/2 (1:500), anti−*p*-ERK 1/2 (1:500), or anti−glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:5000; Santa Cruz Biotechnology, Santa Cruz, CA). The membranes were washed in 0.2% Tween 20 Tris-buffered saline for 20 minutes and incubated at 20°C with the specific secondary antibody at a dilution of 1:10,000 (Thermo Fisher Scientific) for 60 minutes. The blots were developed using Super Signal (Thermo Fisher Scientific).

Statistical Analyses

All immunoblot data were quantitatively analyzed using ImageJ software, version 1.4 (NIH, Bethesda, MD). The data were subsequently analyzed by Student's *t*-test comparison or two-way analysis of variance (treatment versus genotype), followed by Bonferroni's comparisons using Graphpad Prism software version 4.0c (Graphpad Prism Inc., San Diego, CA) and Statview software, version 4.57 (Abacus Concepts, Baltimore, MD). The significance was set at 95% of confidence. All values are presented as means ± SEM.

Results

T1D Causes Hippocampal Cognitive Deficit by Tau-Dependent Mechanisms

To assess the relevance of tau on diabetes-induced cognitive dysfunction, vehicle and STZ-diabetic Ntg and tauKO mice were tested on the hippocampal-dependent behavior test, the MWM. Ntg-STZ−treated mice showed significant impairment in learning during MWM acquisition compared with Ntg-vehicle mice (Figure 1A). Interestingly, no differences in learning were detected between tauKO-vehicle and tauKO-STZ mice. Moreover, Ntg-vehicle mice reached criterion in 3 days, tauKO-vehicle and tauKO-STZ mice required 4 days, and Ntg-STZ mice did not reach the criterion after 5 days of

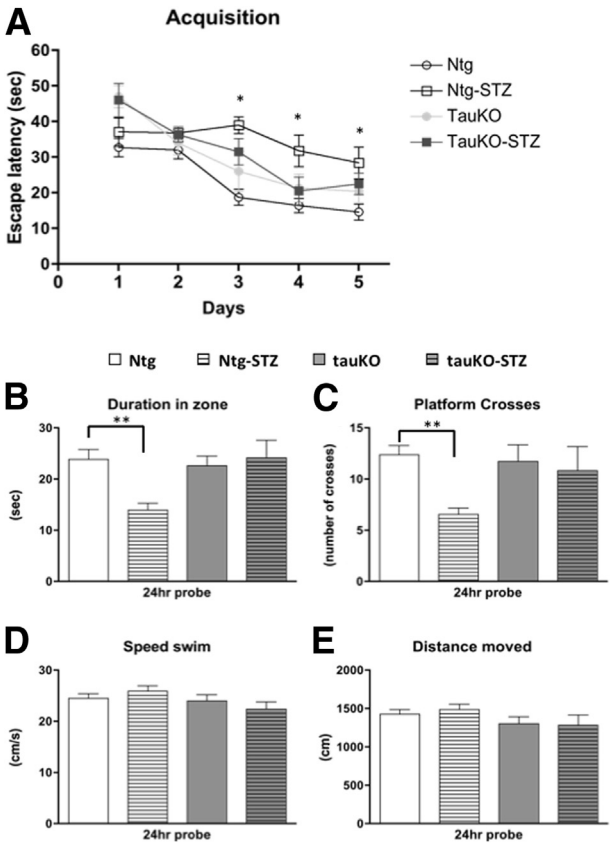

Figure 1 Streptozotocin treatment induces hippocampal cognitive impairment in Ntg mice through a tau-dependent mechanism. Mice were trained on the spatial reference version of the MWM (n = 10 to 12 per group) at 4 months of age. Acquisition curves (**A**) are shown for the 5 days of training on the MWM. *$P < 0.05$. Mixed analysis of variance: trials [$F(4,148) = 27.75$, $P < 0.0001$], treatment [$F(3,37) = 8.84$, $P < 0.001$], and interaction [$F(12,148) = 2.05$, $P < 0.05$]. **B** and **C:** Time spent in the platform quadrant (**B**) and number of crosses (**C**) of NTg, NTg-STZ, tauKO, and tauKO-STZ groups. Time spent in the target zone for NTg-STZ was 58.48% ± 5.75%. Two-way analysis of variance: genotype [$F(1,31) = 3.20$], treatment [$F(1,31) = 3.67$, $P = 0.006$], and interaction [$F(1,31) = 5.95$, $P < 0.05$]. Number of platform crossings for NTg-STZ was 52.89% ± 5.15%. Two-way analysis of variance: genotype [$F(1,28) = 6.50$, $P < 0.05$], treatment [$F(1,28) = 1.90$], and interaction [$F(1,28) = 3.53$, $P = 0.05$]. Pairwise comparisons: **$P < 0.01$ (**B** and **C**). Speed swim (**D**) and traveled distance (**E**). The values represent the means ± SEM.

training. Together, these data indicate that STZ treatment impaired spatial learning only in Ntg mice and removal of tau prevents learning deficits (Figure 1A).

In addition, mice were tested 24 hours after the last training trial to determine any impairment on memory. The results show that Ntg-STZ mice displayed significant impairment on long-term memory compared with Ntg-vehicle, as determined by a significant decrease in the time spent in the platform quadrant and reduced number of crosses (Figure 1, B and C). Interestingly, the genetic deletion of tau prevents memory deficits, because no differences were observed between tauKO-vehicle and tauKO-STZ mice in these two memory tasks. Moreover, the cognitive impairment observed in mice treated with STZ was not attributed to motor deficits, because no statistical differences were noted among groups for swim speed and traveled distance (Figure 1, D and E). Taken together, these data indicate that T1D induced severe hippocampal learning and memory deficits through tau-dependent mechanisms, and tau ablation mitigates these cognitive impairments.

T1D Causes Synaptic Deficits and CREB Dysregulation

To understand the molecular mechanisms by which T1D causes cognitive decline, the levels of memory-related transcriptional factors, CREB, and synaptic proteins (eg, PSD-95 and synaptophysin) were analyzed by Western blot analysis. Changes in these neural networks have been associated with memory impairments in several neurodegenerative disorders.[38,39] Our study revealed a significant decrease in the steady-state levels of the synaptic proteins, PSD95 and synaptophysin, in the Ntg-STZ−treated compared with Ntg-vehicle mice. Interestingly, no differences were observed between both tauKO-vehicle− and tauKO-STZ−treated mice (Figure 2).

In addition, important reductions were observed in the steady-state levels of the phosphorylated form of CREB in Ntg-STZ compared with Ntg-vehicle mice (Figure 2). Furthermore, no differences were detected in tauKO-vehicle− compared with tauKO-STZ−treated mice (Figure 2). These results indicate that memory and learning impairments observed in the MWM test in Ntg-STZ−treated mice are likely due to alterations in synaptic proteins and memory-related transcriptional factors. In addition, our data suggest that tau has an important role mediating these synaptic deficits induced by T1D.

T1D Induces Tau Hyperphosphorylation

The microtubule-associated protein tau is a cytoskeleton protein that contributes to microtubule stability. The capacity of tau to bind to the microtubules is regulated by its phosphorylated state. Thus, hyperphosphorylated tau lacks the affinity for the microtubules and promotes their destabilization.[15,16] In addition, a recent study indicates that hyperphosphorylated tau is localized in the dendrites affecting the postsynaptic compartment.[27,40] Together, these studies indicate that tau hyperphosphorylation is involved in several pathological mechanisms leading to synaptic dysfunction and cognitive impairment.

5herefore, we investigate the effect of STZ treatment on tau phosphorylation to determine whether an increase of hyperphosphorylated state can contribute to the cognitive deficits observed under the T1D. The results showed significant increases in tau phosphorylation levels at residues Ser202/Thr205 (recognized by the AT8 antibody) and Thr 231 (recognized by the AT180 antibody) in the Ntg-STZ−treated compared with Ntg mice (Figure 3). In addition, we observed considerable, but not statistically significant, increases in tau phosphorylation at residues Ser212/Thr214 (recognized by the AT100 antibody), Thr181 (recognized by the AT270 antibody), and Ser396/404 (recognized by the PHF-1 antibody), in Ntg-STZ−treated compared with Ntg mice (Figure 3). TauKO mice did not exhibit any tau hyperphosphorylation because the endogenous tau gene was deleted (data not shown). Thus, the results

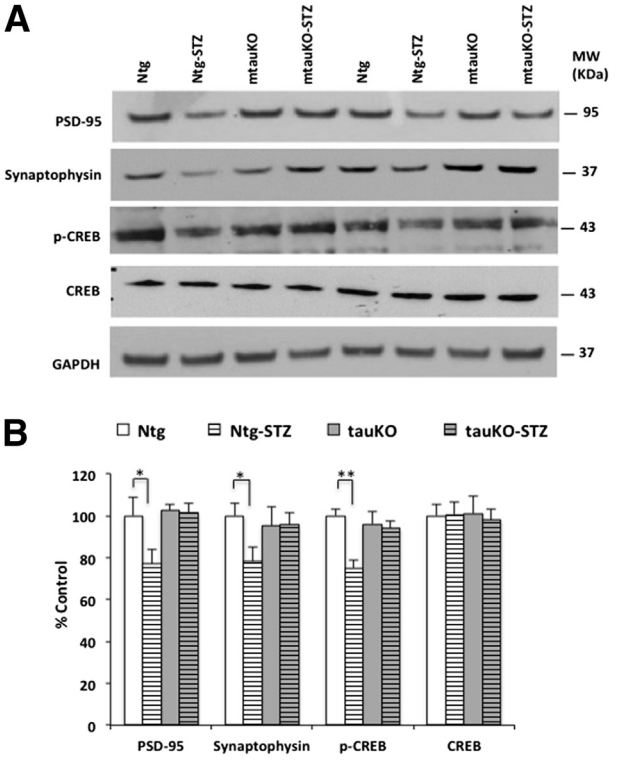

Figure 2 Streptozotocin treatment impairs memory-related intracellular signaling and levels of synaptic-related proteins. **A:** Immunoblot analyses of PSD-95, synaptophysin, *p*-CREB, and CREB of protein extracts from whole-brain homogenates of Ntg, Ntg-STZ, tauKO, and tauKO-STZ at 5 months of age are shown in alternating lanes. **B:** Quantification normalized to GAPDH and expressed as percentage of control. Pairwise comparisons: $*P < 0.05$, $**P < 0.001$ for PSD-95 (29.3% ± 7.4%), genotype [$F(1,11) = 1.85$], treatment [$F(1,11) = 6.53$, $P < 0.05$], interaction [$F(1,11) = 5.53$, $P < 0.05$]; synaptophysin (26.4% ± 8.9%), genotype [$F(1,11) = 0.51$], treatment [$F(1,11) = 1.98$], interaction [$F(1,11) = 7.21$, $P < 0.05$]; and phosphorylated CREB expression (24.9% ± 3.5%), genotype [$F(1,20) = 3.61$], treatment [$F(1,20) = 11.62$, $P < 0.01$], interaction [$F(1,20) = 8.09$, $P < 0.05$]. The values represent the means ± SEM. MW, molecular weight.

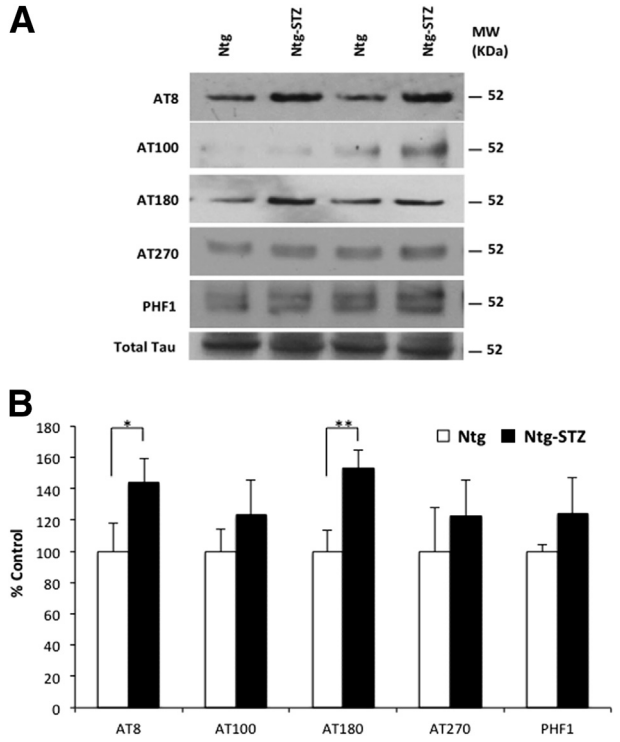

Figure 3 Streptozotocin treatment leads to tau hyperphosphorylation in Ntg mice. **A:** Immunoblot analyses of phospho-tau epitopes, including pSer199/202 tau (AT8), pSer212/Thr214 (AT100), pThr231 (AT180), pThr181 (AT270), and pSer396/404 (PHF-1) of protein extracts from whole-brain homogenates of Ntg and Ntg-STZ mice at 5 months of age, are shown in alternating lanes. **B:** Quantification normalized to GAPDH and expressed as percentage of control. A significant increase was observed in *p*-tau epitopes at pSer199/202 [47.02% ± 11.97%, $*P < 0.05$, unpaired *t*-test (17) = 2.53] and Thr231 [53.37% ± 11.24%, $**P < 0.01$, unpaired *t*-test (20) = 3.03] in Ntg-STZ compared with Ntg-vehicle mice. Furthermore, increases of tau phosphorylation at residues Ser212/Thr214, Thr181, and Ser396/404 were found in Ntg-STZ compared with Ntg-vehicle mice, although they were not statistically significant. The values represent the means ± SEM. MW, molecular weight.

indicate that STZ treatment led to tau hyperphosphorylation at several epitopes, and these increases in tau phosphorylation presumably contribute to cognitive impairment observed in T1D-like Ntg mice.

T1D Impairs IR/PI3K/AKT Pathway, and Increases GSK3β Activity

Deficiency on insulin signaling has been related to increases in tau hyperphosphorylation.[41] Our study shows that STZ induces insulin deficiency (Table 1) and then might contribute to tau hyperphosphorylation. To further investigate the molecular mechanism underlying this effect, we analyzed specific downstream molecules implicated in the insulin pathway.

The IR is the first step in the activation of the insulin pathway. The binding of insulin with the receptor induces the dimerization and autophosphorylation of the IR-β subunit. Therefore, total levels of IR and its phosphorylated form were investigated by Western blot analysis. The results show a severe decrease of steady-state level of phosphorylated IR in both Ntg-STZ and tauKO-STZ mice, compared with respective control mice (Figure 4). In addition, no differences were observed in total IR levels, indicating that the significant decrease of phosphorylated IR induced by STZ treatment is not associated with a change in expression of the IR. Next, we investigated the activities of major downstream kinases involved in the insulin pathway, including PI3K, Akt, GSK3β, and MAPK pathways, by

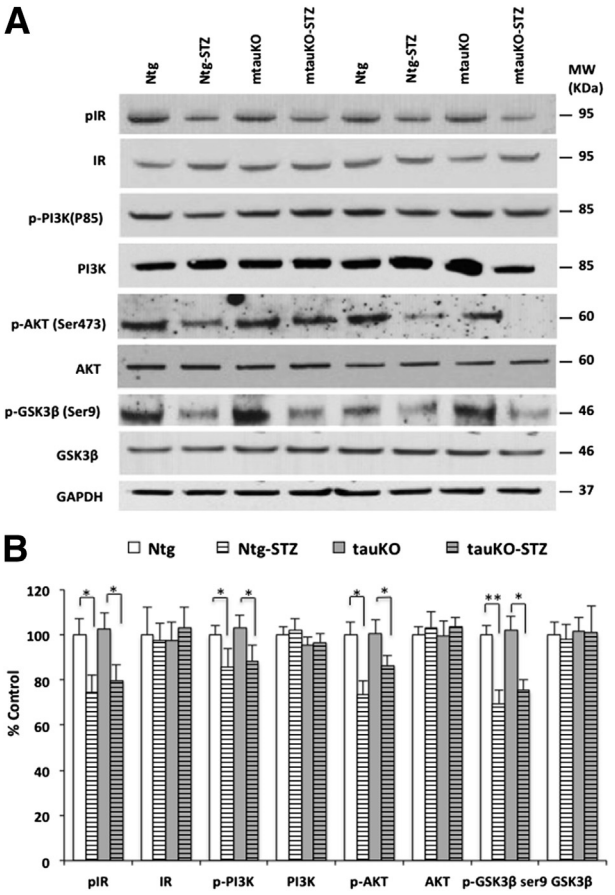

Figure 4 Streptozotocin treatment alters the IR/PI3K/AKT pathway in Ntg and tauKO mice. **A:** Immunoblot analyses of pIR, IR, pPI3k(p85), PI3k, pAKT(Ser473), AKT, GSK3β (Ser9), and GSK3β of protein extracts from whole-brain homogenates of Ntg, Ntg-STZ, tauKO, and tauKO-STZ mice at 5 months of age are shown on alternating lanes. **B:** Quantification normalized to GAPDH and expressed as percentage of control. Pairwise comparisons: $*P < 0.05$, $**P < 0.01$ for phosphorylated IR (Ntg-STZ, 31.8% ± 5.1%; tauKO-STZ, 20.2% ± 7.2%; two-way analysis of variance: genotype [$F(1,10) = 2.23$], treatment [$F(1,10) = 20.03$, $P < 0.01$], interaction [$F(1,10) = 0.43$]) and phosphorylated PI3K (Ntg-STZ, 14.3% ± 8.0%; tauKO-STZ, 18.9% ± 3.0%). Two-way analysis of variance: genotype [$F(1,11) = 0.11$], treatment [$F(1,11) = 9.93$, $P < 0.01$], interaction [$F(1,10) = 0.45$]. Phosphorylated AKT at residue Ser473 (Ntg-STZ, 26.3% ± 6.0%; tauKO-STZ, 13.5% ± 4.5%; two-way analysis of variance: genotype [$F(1,10) = 2.78$], treatment [$F(1,10) = 17.12$, $P < 0.01$], interaction [$F(1,10) = 0.42$]) and GSK3β-Ser9 (NTg-STZ, 30.4% ± 6.0%; tauKO-STZ, 24.7% ± 5.0%; two-way analysis of variance: genotype [$F(1,12) = 0.53$], treatment [$F(1,12) = 27.83$, $P < 0.001$], interaction [$F(1,12) = 0.10$]). The values represent the means ± SEM. MW, molecular weight.

measuring their site-specific phosphorylation, which is known to determine their activation.[18,42] In this regard, we first analyzed PI3K and the regulatory subunit p85. The steady-state level analysis showed a significant reduction of the regulatory subunit p85 in both Ntg-STZ and tauKO-STZ mice compared with respective control mice. In addition, no changes were observed in total PI3K level (Figure 4). In correlation with PI3K data, similar significant decreases in the steady-state level of AKT phosphorylated at residue Ser473 were detected in Ntg and tauKO STZ-treated compared with vehicle mice. Furthermore, these data were correlated with reduction of the phosphorylated form of GSK3β at residue Ser9 in Ntg and tauKO mice STZ treated compared with respective controls. Moreover, no changes were observed in total AKT and GSK3β levels (Figure 4). In addition, no statistical differences were observed in CDK5, ERK, and p38 kinase levels, or their respective phosphorylated forms, in mice treated with STZ compared with respective controls (Figure 5). Together, our data indicate that the T1D-like state down-regulates key enzymes in the insulin signaling pathway in both Ntg and tauKO mice, and tau hyperphosphorylation, observed in Ntg-STZ mice, is related to increases in the active form of GSK3β, as evidenced by reduction in phosphorylation at residue Ser9, which inhibits GSK3β activity.

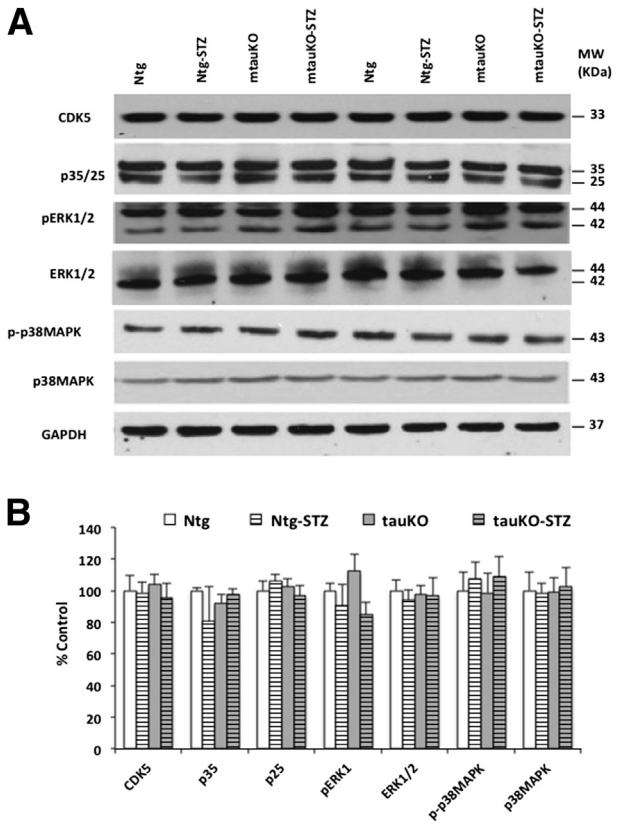

Figure 5 Streptozotocin treatment does not alter Cdk5, ERK, and p38-MAPK kinases. **A:** Immunoblot analyses of cdk5, p25/p35, ERK 1/2, phosphorylated ERK 1/2, p38-MAPK, and phosphorylated p38-MAPK of protein extracts from whole-brain homogenates of Ntg, Ntg-STZ, tauKO, and tauKO-STZ mice at 5 months of age are shown in alternating lanes. **B:** Quantification normalized to GAPDH and expressed as percentage of control. The values represent the means ± SEM. MW, molecular weight.

Discussion

Epidemiological and clinical evidence suggests that diabetes is a risk factor that contributes to AD pathological progression.[10–14] AD and diabetes share several clinical and biochemical features, suggesting a common molecular pathway underlying these two diseases[17]; however, such a mechanism is not well known. In the present study, we provide functional and molecular evidence that tau is a critical molecular factor for T1D to mediate cognitive impairment. Notably, genetic deletion of endogenous tau gene prevents the synaptic degeneration and cognitive impairment. Hence, our results indicate that tau is a critical factor mediating cognitive impairment induced by T1D.

Tau is a cytoskeleton protein that has a key role in the assembly and stability of microtubules. In pathological conditions, hyperphosphorylation of tau causes disruption of the microtubules, alters the postsynaptic physiological features, and leads to synaptic dysfunction with consequent cognitive deficits.[15,16,40] Recent findings in humans and animal models show that diabetes promotes aberrant tau modifications through insulin signaling.[18–24,43] In addition, insulin knockout mice showed cellular ultrastructural alterations induced by hyperphosphorylation in tau and neurofilaments.[44] Although these studies suggest that tau is involved in diabetes pathological features, the impact of tau hyperphosphorylation on the disease progression is still unknown. In our study, we demonstrated the novel finding that T1D promotes impairment in spatial learning and memory, as determined by MWM in NTg mice, whereas tauKO mice show no deficit. These data suggest that tau deletion prevents the cognitive impairment, and tau hyperphosphorylation is fundamental to induce cognitive decline. In parallel with our data, several important studies in AD models showed that reducing tau levels ameliorates neuronal dysfunction and axonal transport defects.[33,45] In addition, a recent study suggests that tau depletion prevents neuronal loss by overactivation of GSK3β.[46] Consistent with these data, we observed that tauKO mice prevent the reduction of synaptic markers, such as synaptophysin and PSD-95, and the decrease of phosphorylated-CREB protein in Ntg-STZ mice. Hence, our findings indicate that tau reduction prevents cognitive impairments and synaptic deficits induced by T1D.

Interestingly, despite tauKO mice being null for the tau gene, these mice did not show behavioral and synaptic deficits compared with the Ntg mice in our study. These results are in agreement with previous findings: they showed that several tauKO mouse lines did not exhibit altered phenotypes or malformations.[47] These studies reveal that the lack of tau was associated with a significant increase in the microtubule-associated protein 1A (MAP1A) and that it

might compensate the loss of tau by MAP1A up-regulation.[47] However, only in aged mice (approximately 12 months of age), in which MAP1A is not increased, tauKO mice develop behavioral impairments and motor deficits. Therefore, our young tauKO mice did not show synaptic and cognitive deficits as the result of a possible increase in the level of MAP1A that compensates for the lack of tau.

Impaired insulin signaling has a robust impact on the central nervous system and is associated with impaired learning, memory, and mental flexibility.[2,48,49] The IRs are widely expressed in the brain of rodents and humans.[50] When stimulated by insulin, IRs trigger several signaling pathways, including MAPK and PI3K pathways.[20,51,52] Our results showed that a significant reduction in the activation of IRs due to insulin deficiency correlates with a decrease in the activation of downstream kinases. In particular, we found in both Ntg and tauKO mice, significant decreases in *p*-PI3K and *p*-AKT, and consequently activation on GSK3β, as evidenced by the reduction in phosphorylation at residue Ser9.[18,42] Thus, these results together indicate that T1D alters the insulin pathway, leading the activation of GSK3β. Ultimately, this increase on GSK3β contributes to the hyperphosphorylation of tau observed in our mice. Notably, although it is well known that insulin signaling plays a key role in cognitive processes and synaptic plasticity, our results clearly reveal that, despite similar alterations of insulin signaling in both genotypes after T1D induction, only the Ntg-STZ group, not tauKO-STZ mice, exhibited cognitive impairments. Hence, these data indicate, for the first time to our knowledge, that tau proteins are crucial downstream targets of the insulin pathway and mediator of cognitive deficits in a condition of insulin deficiency.

Overall, our results show the novel finding that inducing T1D impairs cognition via a tau-dependent mechanism and removing tau prevents the cognitive deficits. Therefore, our results provide new insights into the mechanisms underlying the interaction of these two diseases, indicating that tau is a key molecular target for the development of future drugs for treating and/or preventing AD in patients with diabetes.

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