Bilateral injection of isoproterenol into hippocampus induces Alzheimer-like hyperphosphorylation of tau and spatial memory deficit in rat

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Abstract The abnormal hyperphosphorylation of tau protein is one of the hallmarks of Alzheimer disease and other tauopathies; as yet the exact role of various tau kinases in this pathology is not fully understood. Here, we show that injection of isoproterenol, an activator of cAMP-dependent kinase (PKA), into rat hippocampus bilaterally results in the activation of PKA, calcium/calmodulin-dependent kinase II and cyclin-dependent kinase-5, inhibition of protein phosphatase-2A, hyperphosphorylation of tau at several Alzheimer-like epitopes and a disturbance of spatial memory retention 48 h after the drug injection. These findings suggest the involvement of PKA and PKA-mediated signaling pathway in the Alzheimer-like tau hyperphosphorylation and memory impairment.

Keywords: Alzheimer disease; Tau hyperphosphorylation; cAMP-dependent protein kinase; Calcium/calmodulin-dependent protein kinase II; Isoproterenol; Protein phosphatase-2A

1. Introduction

Abnormal hyperphosphorylation of the microtubule associated protein tau is one of the histopathological hallmarks of Alzheimer’s disease (AD) and related tauopathies [1,2]. The cosegregation of certain mutations in tau gene with the disease in familial frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17), a tauopathy, has demonstrated that abnormalities in this protein can be a primary cause of neurodegeneration [3,4]. An imbalance of the activities of tau kinases and tau phosphatases in affected neurons might be responsible for hyperphosphorylation of tau protein. Recently, a number of studies have focused on phosphatases related to tau phosphorylation [5–9]. However, the in vivo information on the identity and the exact role of protein kinases participating in the regulation of tau phosphorylation, to date, is limited.

Among several neuronal kinases that have been shown to hyperphosphorylate tau in vitro are glycogen synthase kinase-3 (GSK-3), cyclin-dependent kinase-5 (cdk-5), mitogen-activated protein kinase (MAPK), calcium/calmodulin-dependent kinase II (CaMKII), and cAMP-dependent kinase (PKA) (for review, see [10–12]). However, which of these kinases are involved in the phosphorylation of tau in vivo is still not understood. PKA is one of the major microtubule-associated protein kinases abundant in adult brain, thus being strategically positioned to influence tau phosphorylation [13,14]. PKA is a heterotrimer and comprises two regulatory subunits and two catalytic units. After activation of adenylyl cyclase via extracellular signaling events or by specific activators such as isoproterenol (IP), ATP is converted into cAMP. The CAMP then binds to the regulatory subunits releasing the now-activated catalytic subunits of PKA, which in turn phosphorylates substrate proteins. Intracellular CAMP levels and the subcellular localization of the enzyme determine PKA function. Analysis of immunohistochemical colocalization demonstrates that the \( \beta \) catalytic subunit of PKA (C\( \beta \)), the \( \beta II \) regulatory subunit of PKA (R\( \beta II \)), and the 79 kDa A-kinase-anchoring-protein (AKAP79) are tightly associated with the neurofibrillary pathology, positioning PKA to participate directly in the pathological process of tangle formation seen in AD [15].

The present study shows that the injection of IP into rat hippocampus bilaterally activates not only PKA but also cdk-5 and CaMKII. Furthermore, it inhibits protein phosphatase (PP)-2A activity and induces AD-like hyperphosphorylation of tau at several sites and spatial memory retention deficit in rats.
2. Materials and methods

2.1. Materials

Primary antibodies to tau are listed in Table 1. Secondary antibodies for Western blot were from Amersham Pharmacia Biotech (Little Chalfont, Buckinghamshire, England). Detection kit (Histostain-SP) for immunohistochemistry was from Zymed Laboratory (South San Francisco, CA). IP obtained from Sigma (St. Louis, MO) was dissolved in normal saline (NS) at a stock concentration of 400 mM and stored at −20 °C till used.

2.2. Hippocampus injection

Sprague-Dawley rats (n = 64), male, 3–5 months old, 250–450 g, were deeply anesthetized intraperitoneally with nembutal or 5% chloral hydrate and placed in a stereotaxic instrument. 2 μl of IP (10 mM) or NS was slowly injected into the right hippocampus in the CA1–CA2 area with a 5 μl microsyringe within 30 min using coordinates from the Paxinos atlas as follows [16]: 4.8 mm anterior to posterior (AP) Bregma, 2.2 mm mid to lateral (ML), and 3.0 mm dorsal to ventral (DV) dura. The needle was left in place 5 min after the injection before being withdrawn. A second injection was given at the same coordinates into the opposite, i.e., the left hippocampus. After behavioral tests, rats were either anesthetized and transcardially perfused (see below) for immunohistochemical studies or their hippocampi were dissected and homogenized at 4 °C for biochemical analysis (see below). Animals employed for biochemical studies at 3, 24, and 144 h were not subjected to behavioral tests. Rat hippocampi of animals containing 50 mM Tris–HCl, pH 7.0, 10 mM β-mercaptoethanol, 1.0 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, and 2.0 μg/ml each of aprotinin, leupeptin, and pepstatin A [6]. The homogenate was centrifuged at 15 000 rpm for 3 min at 4 °C. The resulting supernatant was immediately mixed with 1.0 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, and 2.0 μg/ml each of aprotinin, leupeptin, and pepstatin A [6]. The homogenate was centrifuged at 15 000 rpm for 3 min at 4 °C and the supernatant was used for protein kinase and PP assays (see below). For Western blot (see below), the resulting supernatant was immediately mixed with the same volume of a cocktail of phosphatase inhibitors containing 20 mM β-glycerophosphate, 2.0 mM Na 3VO 4, and 100 mM NaF; pH 7.0.

2.3. Immunohistochemistry

Isoproterenol-injected (n = 5) and NS-injected (n = 5) rats were deeply anesthetized and transcardially perfused with 100 ml of 0.01 M phosphate-buffered saline (PBS), pH 7.4, and then either perfused with 4% paraformaldehyde solution or right half of the brain saved for biochemical studies and the left half was immersion fixed in the parafomaldehyde solution. The brain was dissected out and chopped into a 5 × 3 × 3 mm block containing hippocampus. Each tissue block was postfixed in the same 4% paraformaldehyde solution for 10 h before it was coronally sliced into 40 μm sections with a Vibratome (LANCER, S100, TPI). Free floating sections were blocked in 3% H 2O 2 in absolute methanol for 20 min and non-specific sites were blocked with fetal calf serum for 40 min at 37 °C. Sections were then incubated overnight at room temperature with antibodies listed in Table 1. Immunoreaction was visualized by using biotinylated secondary antibodies (1:200) and avidin-peroxidase con-

Table 1

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Dilution</th>
<th>Typea</th>
<th>Specificityb</th>
<th>Phosphorylation sites c</th>
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<tbody>
<tr>
<td>Tau-1</td>
<td>1:30 000</td>
<td>Mono-</td>
<td>P</td>
<td>Ser-198/Ser-199/ Ser-202</td>
</tr>
<tr>
<td>PHF-1</td>
<td>1:500</td>
<td>Mono-</td>
<td>P</td>
<td>Ser-396/Ser-404</td>
</tr>
<tr>
<td>12e8</td>
<td>1:500</td>
<td>Mono-</td>
<td>P</td>
<td>Ser-262/Ser-356</td>
</tr>
<tr>
<td>M4</td>
<td>1:500</td>
<td>Mono-</td>
<td>P</td>
<td>Thr-231/Ser-235</td>
</tr>
<tr>
<td>92c</td>
<td>1:500</td>
<td>Poly-</td>
<td>P + unP</td>
<td></td>
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Other antibodies used: P199, P214, and P262 are all phosphorylation dependent antibodies, binding to phosphorylated tau at the sites indicated (1:1000); DM1A (tubulin), 1:2000.

2.4. Western blots

The phosphorylation of tau was analyzed by Western blots using 10% SDS-PAGE as described originally by Laemmli [17]. The separated proteins were transferred on Immobilon-P membrane (Millipore, Bedford, MA) and probed with several specific tau antibodies as summarized in Table 1. The blots were developed with peroxidase-conjugated secondary antibodies and DAB system.

2.5. Protein kinase activity assays

The activities of cdk-5, GSK-3, protein kinase C (PKC) and CaMKII were assayed in 25 μl of reaction mixture containing 50 mM HEPES, pH 7.5, 10 mM MgCl 2, 0.06 mg/ml hippocampus extract, 200 μM [γ- 32P]ATP, and 500 μM cd2 peptide (Upstate Biotechnol-ogy) for cdk-5, 50 μM GSK-3 peptide (Upstate Biotechnology, Ithaca, NY) for Gsk-3, or 20 μM sync (Sigma) for CaMKII. In the case of CaMKII, 1 mM CaCl 2 as well as 2 μM calmodulin was also included [8,9]. The activity of PKA was determined as above except that the reaction mixture contained 70 mM Na 2HPO 4/NaH 2PO 4, pH 6.8, 14 mM MgCl 2, 1.4 mM EDTA, 30 μM maltantide (Sigma), 200 μM [γ- 32P]ATP and 0.06 mg/ml hippocampal extract. The reaction was initiated by adding [γ- 32P]ATP. After incubation for 10 min at 30 °C, 10 μl reactive mixture was removed and spotted onto phosphocellulose membrane. The membrane was then washed five times in 1% phospho-meric acid to remove non-protein incorporated 32P, dried and counted by Cerenkov radiation. One unit of activity was defined as the amount of enzyme that catalyzed the incorporation of 1 nmol 32P/min at 30 °C into the specific substrate.

2.6. PP-2A and PP-1 assays

Phosphorylase-b (2 mg/ml) (Sigma) was phosphorylated into phos-
ylase-a by incubating it for 10 min at 30 °C in 40 mM Tris–HCl, pH 8.5, 20 mM β-mercaptoethanol, 0.2 mM CaCl 2, 15 mM MgCl 2, 0.5 mM [γ- 32P]ATP and 10 μg/ml phosphorylase kinase (Sigma). The product of 32P-phosphorylase was separated from free ATP on a Sephadex G-50 column and the protein containing fractions were collected. The activities of PP-2A and PP-1 towards [γ- 32P]phosphoryl-
lase-a were assayed by the release of 32P as described previously [6,18]. The reaction was carried out in 20 μl reaction mixture containing 50 mM Tris, pH 7.0, 10 mM β-mercaptoethanol, 0.1 mM EDTA, 7.5 mM caffeine, 0.2 mg/ml [γ- 32P]-phosphorylase-a and 0.06 mg/ml hippocampal extract. The reaction was started by adding 32P-phos-
ylase-a. After incubation for 30 min at 30 °C, 7 μl of the reaction mixture was spotted on a chromatography paper already spotted with 10 μl stop solution (4 mM cold ATP in 20% trichloro-
acetic acid [TCA]). The released 32P was separated from the substrate by ascending chromatography in 5% TCA in 0.2 M NaCl, and the radioactivity was measured by Cerenkov counting. A PP-1 specific inhibitor, inhibitor-1, was included in the assay for PP-2A activity. PP-1 activity was calculated by subtracting the PP-2A activity from the total phosphatase activity (PP-1 plus PP-2A) assayed in the ab-

2.7. Morris water maze test

The water maze test was conducted as described previously [19,20]. Rats tested in the water maze were extensively handled (2 min every day for 15 days) prior to the test. Two hours before the test, the ani-
mals were brought to the outer room and kept in cages on shelves to acclimatize to the environment and to eliminate directional olfactory and auditory cues. The swimming pool was 60 cm high, 1.2 m in diameter and was made of stainless steel. The water was heated to keep the temperature at 26 ± 2 °C that was also the room temperature maintained for the study. The water in the pool was made opa-
que with milk to hide the escape platform. The Plexiglas platform was 40 cm high, 10 cm in diameter, and its surface was scarred to help the rats climb on it. The water surface was 18 cm from the rim of the pool and the inner wall was always carefully wiped to eliminate any local cues. The rim of the pool was 1.0 m from the nearest visual cue of red and blue marks. A camera was fixed to the ceiling of the room, 1.5 m from the water surface. The camera...
was connected to a digital-tracking device. An IBM computer with the water maze software was employed to process the tracking information.

To exclude the possibility of influence of surgery itself on spatial memory retention, we added a normal control (NC) group without any surgery throughout the test. The submerged platform 2 cm under water was located at a fixed position throughout training and retention sessions. A training session consisted of 4 trials altogether (one trial per quadrant) with a 30 s inter-trial interval, lasting for seven days. On each trial, rats started from one of the middle of the four quadrants facing the wall of the pool and ended when they climbed the platform. The rats were not allowed to search for it more than 60 s, after which they were guided to the platform. On the eighth day, rats were trained as usual and their escape latency and swimming path were recorded as primary protocols. Then, rats were allowed a day’s rest, before IP or NS was injected. The retention test was conducted 48 h after surgery, and all rats were tested and their escape latency and swimming pathways were recorded in the same way. No surgical procedure was performed on NC group and the test results of rats in this group were labeled as NC1 and NC2 as controls to the pre-surgery and post-surgery in IP and NS groups.

2.8. Statistical analysis

One-way ANOVA and Post hoc Dunnett’s t tests were employed to compare means of behavioral tests among different groups. For quantification of Western blots, densitometric scanning was performed and analyzed with a computer analysis package (NIH Image 1.52 image analysis software). The phosphatase and kinase activities of the IP-treated samples were expressed as the percentage of the activities of the control samples of each set. The student’s t test was used for analyses of the results of Western blots and enzyme activities. All data were presented as means ± S.D. of 3–5 independent experiments and analyzed with the SPSS package 10.0. P values <0.05 were considered statistically significant.

3. Results

In order to activate PKA, we injected IP bilaterally in the hippocampi of adult rats. The control animals were similarly treated with NS, the vehicle used for the IP injections. As expected, the activity of PKA was markedly stimulated in the hippocampus and was maximum at 48 h post-injection with IP (n = 5, P < 0.05, Fig. 1). The IP-induced increase in the PKA activity was seen 24 h but not 3 h post-injection. At 24 and 144 h post-injection, the levels of PKA activity were similar in the hippocampi of the drug injected animals. Because of the involvement of PKA in other signaling pathways, we also assayed in the drug-treated and control animals the activities of cdk-5, GSK-3, PKC, CaMKII, PP-2A and PP-1, whose activities have been strongly implicated in the abnormal hyperphosphorylation of tau. We found the activation of cdk-5 at 48 and 144 h, and of CaMKII at 48 h post IP injection. Furthermore, the activity of PP-2A was found to be decreased at both 48 and 144 h post-injection with IP (Fig. 1). In contrast, we were unable to detect any significant changes in the activities of GSK-3β, PKC and PP-1 (data not shown).

The effect of IP injection on the phosphorylation of tau was examined by Western blots of the hippocampal homogenates from IP injected (n = 5 for each time period) and control (n = 5 for each time period) animals developed with various phospho-dependent tau antibodies. The blots were then scanned and analyzed by computer-assisted densitometry for quantification. Any changes in the total tau were examined by developing the Western blots with a phospho-independent rabbit antibody 92e that recognizes total tau, and normalizing these tau levels by the level of tubulin seen by Western blots developed with tubulin antibody DM1A. An increase in the abnormal hyperphosphorylation at Ser-214 (P214 sites, P < 0.001), Ser-262/356 (12E8 sites, P < 0.001), and Ser-396/404 (PHF-1 sites, P < 0.05) of tau in the IP injected animals was observed 24 and 48 h but not 3 h after the drug injection (Fig. 2). Phosphorylation of tau at Ser-199 (P199 sites, P < 0.05) decreased 48 h post IP injection. No significant change in the level of total tau was detected.

To examine the topography of the abnormal hyperphosphorylation of tau in the hippocampus of the IP injected animals, immunohistochemical staining of the drug-injected and the saline-injected control hippocampi was carried out with several phospho-dependent antibodies. The results showed an increase

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**Fig. 1.** Effect of IP injection on activities of various kinases and phosphatases. Rat hippocampi were dissected and homogenized into 10% homogenates at 3, 24, 48, and 144 h after IP (n = 5 for each time period) or vehicle injection (n = 5 for each time period). The activities of PKA, cdk-5, CaMKII and PP-2A were assayed as described in Section 2. The phosphatase and kinase activities of the IP-treated samples were expressed as the percentage of the activities of the control samples of each set. The activities of PKA, cdk-5 and CaMKII were increased, while that of PP-2A was decreased. Bars represent means ± S.D. of 3 independent assays ( P < 0.05).
in the phosphorylation of tau at Ser-262/356 (12E8 sites), Ser-396/404 (PHF-1 sites), Thr-231/Ser-235 (M4 sites) and Ser-198/199/202 (Tau-1 sites). An especially intense staining was observed with tau antibody 12E8 to Ser-262/356 in animals, which were sacrificed 48 h post IP injection (Fig. 3). The pyramidal neurons of CA3/CA4 areas of the IP injected animals were particularly immunostained. The hyperphosphorylated tau distributed mainly in the soma of hippocampal pyramidal neurons, an abnormal distribution of tau similar to that seen in AD brain. Hyperphosphorylation of tau at the above sites at 48 h decreased and almost disappeared at day 6 and day 14 after injection (data not shown). In addition, the immunostaining with antibodies to pSer-214 showed an overall increase in the intensity of staining but without differential staining of any subregion of the hippocampus (data not shown). The immunohistochemical staining of tau is known to depend on its conformational state [1,2]. Thus, in some cases, immunohistochemical staining might not completely parallel with the corresponding Western blots data.

Spatial learning and memory is a hippocampus-dependent process and almost all AD patients develop a spatial learning and memory deficit. To examine whether the IP-induced activation of PKA and the abnormal hyperphosphorylation of tau would associate with a dysfunction of spatial memory, we next employed the well-established Morris water maze to investigate whether memory retention was altered in the IP-injected rats. To study positive behavioral alterations accurately and to study the association of behavioral abnormalities with tau phosphorylation, we employed the post-training test method. That is, the retention of spatial memory was determined at 48 h post-injection in rats pre-trained for a week before IP injection, and the ability of the animals to remember the location of the hidden platform in the water maze guided by distal cues was measured and recorded. Each group of 10 rats was tested. The IP injected rats were found to have difficulty in picking up the distal cues (Fig. 4A) and their escape latency to find the hidden platform was remarkably prolonged in comparison with that observed pre-injection (19.59 ± 6.16 s vs. 8.24 ± 1.68 s, \( P < 0.01 \)). In contrast, the escape latency of NS and NC groups did not change significantly (NS, post-injection: 12.32 ± 4.64 s vs. pre-injection: 10.05 ± 2.56 s, \( P = 0.19 \); NC, NC2: 9.83 ± 3.10 s vs. NC1: 8.97 ± 1.90 s, \( P = 0.48 \)). The escape latency of NS group fell more in post-injection than NC group but did not reach a statistical significance, implicating that surgical procedure itself had little effect on rats’ performance in water maze. Furthermore, significant differences in

![Western blots](image-url)
escape latency of IP group to that of NC\textsubscript{2} \((P < 0.01)\) and NS \((P < 0.01)\) group were observed (Fig. 4A). Recordings of the swimming paths of pre-injection and post-injection of the same rat from each group, i.e., IP and NS, and as non-injection control, the NC animals revealed that the IP-injected animals swam randomly rather than directly in search of the hidden platform (Fig. 4B). This unsystematical search strategy was usual in IP group. On the contrary, NS (Fig. 4Ba and Bb) and NC (Fig. 4Ba and Bb) rats did not change their swimming path as much. They navigated in a comparatively direct line to find the hidden platform although sometimes thigmotaxical search strategy was observed. These behavioral studies revealed that the injection of IP into the hippocampus impaired the retention of spatial memory and spatial recognition in rats. Notably, in agreement with the time pattern of tau phosphorylation, memory retention deficits disappeared at day 6 after IP injection (data not shown), suggesting a link between tau phosphorylation and behavioral abnormalities in this condition.

4. Discussion

In vitro studies have shown that PKA is not only able to phosphorylate tau at several abnormal sites as seen in AD brain by itself but also enhances or stimulates the phosphorylation of tau subsequently by other kinases, such as GSK-3\textbeta\ and cdk-5 [13,21,22]. By using metabolically competent rat brain slices, PKA has been reported to regulate tau phosphorylation in situ [23,24]. In AD brain, PKA-dependent phosphorylation of tau at Ser-214 and Ser-409 was coincident with the initial appearance of filamentous aggregates of tau, suggesting that phosphorylation of tau by
PKA plays a key role in the early stage of NFT formation [14]. However, neither the exact role of PKA in phosphorylation of tau in vivo nor the effect of PKA activation on spatial memory was previously known. IP is an epinephrine analog that stimulates β-adrenergic receptors and increases the intracellular cAMP levels. cAMP then binds to the regulatory subunits and activates PKA, which in turn phosphorylates substrate proteins including tau. In the present study, by injecting IP into rat hippocampus bilaterally we have discovered that IP in vivo not only activates PKA but also cdk-5 and CaMKII. Furthermore, it inhibits PP-2A activity and produces abnormal hyperphosphorylation of tau, especially at Ser-262, and impairs spatial memory retention. These findings show that activation of PKA might lead to changes in the cdk-5, CaMKII and PP-2A signaling and the abnormal hyperphosphorylation of tau. Thus, it is tempting to speculate that PKA activation might be involved in AD type neurofibrillary degeneration and the associated deficits in memory retention. The IP-injected rats might serve as a useful pharmacological animal model to study the effects of anti-neurofibrillary degeneration drugs for AD and other tauopathies.

Apart from specific phosphorylation of tau directly by PKA, this kinase might co-operate with other neuronal kinases or/and phosphatases building up a regulatory network of tau phosphorylation. A particularly important PKA target protein is DARPP-32, a dopamine and cAMP regulated phosphoprotein with molecular weight of 32 kDa. Phosphorylation of threonine-34 by PKA allows DARPP-32 to bind to and inhibit the activity of PP-1 powerfully, which results in a diverse group of PP-1 target proteins remaining phosphorylated [25]. Such targets include ion channels (some of which are also direct PKA targets) and transcription factors, for example, cAMP-responsive element binding protein (CREB). So the DARPP-32-mediated inhibition of PP-1 acts as an “accomplice” of PKA-induced phosphorylation unless phosphate groups are removed from Thr-34 in DARPP-32, particularly by PP-2B, counteracting the effects of PKA. To verify if this pathway is also involved in our experimental system, we determined both the expression and activity of PP-1. To our surprise, we did not detect any significant change in PP-1 activity although an enhancement in the expression of this phosphatase was detected by immunohistochemistry and Western blots (data not shown). Glutamate ligand-gated calcium ion channels and voltage-gated calcium ion channels are PKA target proteins too. They are activated when phosphorylated by PKA, thus leading to an influx of calcium. The elevated calcium intracellularly triggers the activation of a handful of calcium/calmodulin-dependent protein kinases like CaMKII and protein phosphatases involved in tau phosphorylation. CaMKII is another possible key kinase related to the regulation of cytoskeletal protein phosphorylation, and it is well documented that this enzyme participates in hippocampus-dependent learning and memory [26,27]. Very well fitted to this pathway, we found a significantly elevated CaMKII activity during IP-stimulated PKA activation. Previous studies have shown that dephosphorylation of CaMKII by PP-2A at Thr286 inhibits the kinase activity [9,28]. Alternatively, stimulation of the CaMKII activity observed in this study might thus be related to the downregulation of PP-2A. PP-2B is a calcium/calmodulin-dependent phosphatase, whose main function is counteracting the effects of PKA phosphorylation in order to regulate and fine-tune the phosphorylation stature of PP-1 target protein [29]. Whether PP-2B activation compensated the IP-induced PKA activation to dephosphorylate hyperphosphorylated tau and resulted in a significant restoration of memory retrieval when rats were tested in the water maze at 6 day post-injection in our study (data not shown) remains an exciting question.

The fact that hyperphosphorylation of tau at Ser-198/199/202, Ser-214, Thr-231/Ser-235, Ser-262/Ser-356, and Ser-396/Ser-404 sites was associated with behavioral abnormalities upon PKA activation in adult rats in the present study suggested that tau hyperphosphorylation at these sites was probably a requisite for impairment in spatial memory retention. Spatial learning and memory in the water maze is hippocampus-dependent that needs not only the activation of preexisting proteins but also protein synthesis and mRNA transcriptions [30,31]. A variety of evidence suggests that PKA and CREB are necessary participants in the formation of long-term potentiation (LTP), which is considered a potential cellular mechanism underlying hippocampus-dependent learning and memory in normal rats [32–34]. Phosphorylation of Ser-133 by PKA pushes CREB functioning in LTP. In accordance with a previous study by Taylor et al. [35] who have shown that PKA activation in prefrontal cortex impairs working memory performance in rats, the activation of PKA by IP in rat hippocampi in the present study caused disturbance in spatial memory retention, implicating that inappropriate activation of PKA might be detrimental to memory in vivo. In addition, due to the complex network in signaling pathways, other kinases and phosphatases activated by IP might also participate in the regulation of behavioral changes in vivo. Indeed, the roles of cdk-5 and CaMKII activation and PP-2A inhibition in associative-learning and memory retention have been suggested in rodents recently [36–38]. Thus, imbalanced activity among kinases and/or phosphatases after β-adrenergic receptor activation [39] might collectively disrupt spatial memory. Of course, other mechanisms except for tau hyperphosphorylation, including the glutamate-mediated excitotoxic neurotransmission and imperturbation of calcium homeostasis, might also be involved. The causal role of PKA upon IP injection in abnormal tau phosphorylation and memory retention will require studies employing inhibitors of PKA or gene manipulation related to PKA in vivo. In an independent study, we have found that specific inhibition of PKA activity by Rp-adenosine 3',5'-cyclic monophosphorothioate triethyl [40] or injection of melatonin [41] inhibits the abnormal hyperphosphorylation of tau at Ser214, PHF-1 and Tau-1 sites.

In conclusion, the present study shows that IP induces, in addition to PKA, the activation of CaMKII and cdk-5. Furthermore, it inhibits the activity of PP-2A, and induces abnormal hyperphosphorylation of tau at Ser-198/199/202, Thr-231/Ser-235, Ser-214, Ser-262 and Ser-396/404 and impairment in spatial memory in adult rats. These findings might suggest a pivotal upstream role of PKA in a signal transduction cascade that leads to Alzheimer-like abnormal hyperphosphorylation of tau and a consequent memory impairment. Thus, PKA is a promising pharmacological therapeutic target for AD and other tauopathies that are characterized by the abnormal hyperphosphorylation of tau.
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