Tyrosine hydroxylase (TH) catalyzes the rate-limiting step in the biosynthetic pathway of the catecholamines dopamine (DA), norepinephrine, and epinephrine. The enzyme exists as a tetramer, with each subunit composed of N-terminal regulatory domain and a C-terminal catalytic domain. TH activity is required for embryonic development and survival in mammals; TH knock-out mice die before or at birth (1, 2). The expression of TH is exquisitely regulated at the levels of gene transcription and protein turnover of the phosphorylation-defective mutant TH S31A was not altered by cdk5 activity. Taken together, these data suggest that cdk5 phosphorylation of TH is an important regulator of TH activity through stabilization of TH protein levels.

Tyrosine hydroxylase (TH) catalyzes the rate-limiting step in the biosynthetic pathway of the catecholamines dopamine (DA), norepinephrine, and epinephrine. The enzyme exists as a tetramer, with each subunit composed of an N-terminal regulatory domain and a C-terminal catalytic domain. TH activity is required for embryonic development and survival in mammals; TH knock-out mice die before or at birth (1, 2). The regulation of TH levels and its enzymatic activity are essential in modulating the concentrations of catecholamines. TH expression is exquisitely regulated at the levels of gene transcription, RNA processing, and RNA and enzyme stability (3). Short-term regulation of TH enzymatic activity is achieved through direct modulation of the enzyme by catecholamine feedback inhibition, allosteric regulation and phosphorylation by kinases. Four phosphorylation sites in the N-terminal region of TH (serines 8, 19, 31, and 40) have been identified in vitro studies, and some of the protein kinases that phosphorylate these sites have been identified (3, 4).

cdk5 is a proline-directed kinase that phosphorylates serine and threonine residues. The kinase activity of cdk5 is highest in postmitotic neurons of the central nervous system and is contingent on the expression patterns of the regulatory subunit, p35 or p39, with which cdk5 associates (5). The most recognized role for cdk5 is the regulation of neuronal migration and positioning in the developing cortex. The substrates of cdk5 have been implicated in diverse neuronal functions, including cell adhesion, cytoskeletal regulation, and synaptic plasticity (5). There is a role for cdk5 in DA-mediated postsynaptic signaling events through the DA and cyclic AMP-regulated phosphoprotein, DARPP-32; cdk5-dependent phosphorylation at Thr-75 of DARPP-32 alters the efficacy of DA signaling (6). Thus, cdk5 is an important regulator of postsynaptic function mediated by DA signaling. However, a role for cdk5 in presynaptic components of DA signaling has not been examined.

In the present study, we investigated the possibility that phosphorylation of TH by cdk5 affects its enzymatic function. We found that TH contains several proline-directed serine/threonine residues, which comprise the minimal cdk5 phosphorylation consensus sequence (7). Through site-directed mutagenesis of these sites, we found that phosphorylation by cdk5 positively regulates TH activity and protein levels, implicating cdk5 as an important regulator of catecholamine synthesis.

The abbreviations used are: TH, tyrosine hydroxylase; DA, dopamine; cdk5, cyclin-dependent kinase 5; GST, glutathione S-transferase; MEK, mitogen activated protein kinase kinase; WT, wild-type; pS31, phospho-Ser-31; MAPK, mitogen-activated protein kinase; PD, Parkinson’s disease.

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generated by PCR using complementary primers containing the mutations. Full-length TH and full-length point mutants were subcloned into the BamHI and EcoRI sites pGEX4T2 or pcDNA3 (Invitrogen). All constructs were confirmed by sequencing. The expression constructs for Cdk5 and p35 have been described previously (8).

**Kinase Assay**—5 μg of purified recombinant GST fusion proteins were incubated in kinase buffer (30 mM HEPES, pH 7.2, 10 mM MgCl₂, 5 mM MnCl₂, and 1 mM dithiothreitol) containing 0.3 μg of p35/cdk5 produced in baculovirus, 100 μM cold ATP and 2.5 μCi of [γ-³²P]ATP in a final volume of 50 μl for 30 min at room temperature. The reaction was stopped with 50 μl of 2X SDS-PAGE sample buffer. Samples were separated by SDS-PAGE, and [γ-³²P]ATP incorporation was determined by autoradiography.

**PC-12 Cell Culture**—Cells in culture were maintained in RPMI 1640 medium supplemented with 10% horse serum, 5% fetal calf serum, and 100 μg/ml penicillin/streptomycin. For differentiation, cells were plated at a density of 1 × 10⁵ cells/well in a collagen-coated 12-well dish in medium containing RPMI 1640 medium, penicillin/streptomycin, and nerve growth factor (100 ng/ml) for 7–10 days. Culture medium and nerve growth factor were replaced every 2–3 days. Culture medium and nerve growth factor were replaced every 2–3 days. Differentiated cells were treated with cdk5 and/or mitogen-activated protein kinase kinase (MEK) inhibitors for 9 h. Cells were rinsed once in phosphate-buffered saline and lysed in radioimmunoprecipitation assay buffer (150 mM NaCl, 50 mM Tris, pH 8.0, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS) containing protease and phosphatase inhibitors. Samples were analyzed by Western blot.

**Immunohistochemical Studies**—Mice were anesthetized with Avertin and transcardially perfused with phosphate-buffered saline, followed by 4% paraformaldehyde. Comparable 6-μm paraffin sections were deparaffinized and rehydrated. Antigen retrieval of consecutive coronal sections was performed by microwave irradiation. Sections were then incubated with primary antibodies overnight at 4 °C, followed by streptavidin-biotin peroxidase conjugated secondary antibody (Vector Laboratories, Burlingame, CA). Polyclonal TH antibody (1:1000) and polyclonal phospho-specific Ser-31 TH antibody (1:100) were used.

**COS-7 Cell Transfections**—COS-7 cells were grown to 95% confluence in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and 100 μg/ml penicillin/streptomycin. Medium lacking antibiotics was replaced before transfection with Lipofectamine 2000 (Invitrogen). For TH activity measurements, 2 μg of pcDNA3-TH or pcDNA3-THS31A in the presence or absence of 1 μg of pcDNA3-p35 and 1 μg of CMV-cdk5-HA were transfected in 100-mm Petri dishes overnight. Cells were rinsed once in phosphate-buffered saline containing protease (Roche) and phosphatase inhibitors (including 10 mM NaF, 2 mM Na₂VO₃, 50 mM β-glycerophosphate, and 2 mM sodium pyrophosphate) and lysed in 10 mM NaHPO₄, pH 7.0, containing protease and phosphatase inhibitors, with a Dounce homogenizer. The samples were centrifuged at 11,300 × g for 15 min at 4 °C, and the supernatants were analyzed for TH activity. A aliquot of the homogenate was taken for Western blot analysis. For cycloheximide treatment, 1 μg of THS31A in the presence or absence of 0.5 μg of pcDNA3-p35 and 0.5 μg of CMV-cdk5-HA were transfected in 6-well dishes overnight. Fresh

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![Diagram A](image1.png)

**A**. GST-TH domain fragments are shown. reg, regulatory domain; cat, catalytic domain.

**B**. In vitro kinase assays were performed using purified recombinant p25/cdk5. GST-TH fragments and GST full-length wild-type TH and the mutants T3A, S8A, S31A, and S126A were analyzed. Histone H1 was used as a positive control.

**C**. Alignment of mouse, rat, human, and chicken TH sequences. Residues with black shading indicate the conserved proline-directed Ser-31 consensus site.

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**FIG. 1.** Cdk5 phosphorylates TH at Ser-31 in vitro. A, GST-TH domain fragments are shown. reg, regulatory domain; cat, catalytic domain. B, in vitro kinase assays were performed using purified recombinant p25/cdk5. GST-TH fragments and GST full-length wild-type TH and the mutants T3A, S8A, S31A, and S126A were analyzed. Histone H1 was used as a positive control. C, alignment of mouse, rat, human, and chicken TH sequences. Residues with black shading indicate the conserved proline-directed Ser-31 consensus site. Gray shading indicates identical residues.
medium was replaced containing 20 μg/ml cycloheximide. Cells were rinsed once in phosphate-buffered saline and lysed in radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors at 0, 8, 16, and 24 h after treatment. Equal amounts of lysates were subjected to SDS-PAGE and Western blot analysis.

TH Activity Assay—TH activity was measured by quantifying tritiated water production from the hydroxylation of 3,5-[3H]-L-tyrosine to 3,4-dihydroxyphenylalanine as described previously (9). Cell lysates (40 μl) were added to 50 μl of assay mixture to yield a final reaction mixture containing 50 mM HEPES, 50 μM L-tyrosine, 100 μM FeSO₄, 1200 units of catalase, 5 mM dithiothreitol, and 0.5 μCi of [3H]-tyrosine. After the addition of 1 mM (6R)-5,6,7,8-tetrahydro-L-biopterin dihydrochloride (Schirks Laboratories, Jona, Switzerland), the reaction was incubated at 37 °C for 20 min and stopped by the addition of 1 ml of 7.5% activated charcoal in 1N HCl. The samples were centrifuged and 5 ml of Safescint (American Bioanalytical; Natick, MA) was added to the supernatant. Radioactivity was quantified in a liquid scintillation counter. A blank value obtained from samples that did not receive (6R)-5,6,7,8-tetrahydro-L-biopterin dihydrochloride was subtracted from all values.

Preparation of Brain Lysates and Western Blot Analysis—Mouse brains were homogenized in radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors (see above). Lysates were either used fresh or aliquoted, frozen in liquid nitrogen, and stored at −80 °C until use. Equal amounts of brain lysates were subjected to SDS-PAGE and Western blot analysis.

Mesencephalic Cultures—Mesencephalon from embryonic day 13 cdk5 heterozygous matings were dissected, trypsinized, and mechanically dissociated. The cells were resuspended in neurobasal medium supplemented with 10% horse serum. The dissociated cells were plated into 12-well trays previously coated with poly-D-lysine and laminin. The medium was replaced with neurobasal medium supplemented with B27, 1 mM L-glutamine, and 100 μg/ml penicillin/streptomycin 4 h later. The cultures were maintained at 37 °C in a 95% air/5% CO₂ atmosphere with 100% relative humidity for 5 days. Samples were lysed in radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors and subjected to SDS-PAGE and Western blot analysis.

Densitometric Analysis—The Western blot images on X-ray film were scanned and quantified using Image Gauge (version 3.4; Fuji Photo Film Co.). Data were analyzed by Prism (version 3.02; GraphPad Software, San Diego, CA). Differences were considered significant at p < 0.05.

RESULTS

Cdk5 Phosphorylates TH at Ser-31 in Vitro—Mouse TH contains seven proline-directed serines and threonines that are candidates for phosphorylation by cdk5, four sites within the N-terminal regulatory domain and three in the C-terminal catalytic domain. To determine whether cdk5 phosphorylates TH, we tested GST fragments of the regulatory and catalytic domains of TH (Fig. 1A). In our initial studies, we observed robust phosphorylation of the GST-TH regulatory fragment.
(31–164) by cdk5 in vitro, whereas phosphorylation of the GST–TH catalytic domain was negligible (Fig. 1B, left, lanes 3–4) with equimolar amounts of protein loaded (Fig. 1B, right, lanes 3–4). Therefore, we made phosphorylation defective single alanine mutants of all four sites within the N-terminal regulatory region in the context of full-length TH to map the site(s) of phosphorylation. Analysis of these recombinant proteins revealed that phosphorylation of the S31A mutant was notably reduced compared with wild-type (WT) TH, whereas phosphorylation of the T3A, S8A, and S126A mutants were unaffected (Fig. 1, left, lanes 5–9). These data indicate that TH is an in vitro substrate of cdk5 and that Ser-31 in the N-terminal regulatory domain is the primary cdk5 phosphorylation site. Furthermore, this site is conserved in several vertebrate species (Fig. 1C), suggesting that cdk5-mediated phosphorylation of TH might be an important regulator of TH function.


cdk5 Activity Mediates Ser-31 Phosphorylation of TH in PC-12 Cells—To evaluate whether a direct link exists between Cdk5 activity and Ser-31 phosphorylation, the cdk5 inhibitors roscovitine and olomoucine were used in differentiated PC-12 cells that express high levels of endogenous TH. This cell line is suitable to determine the effect of cdk5 on TH because PC-12 cells exhibit increased p35 and cdk5 protein levels and cdk5 activity upon differentiation with nerve growth factor treatment (10). We used a TH phosphorylation-specific antibody for phospho-Ser-31 (pS31) that recognizes a ~55-kDa band in vehicle-treated cells (Fig. 2A). Treatment with the cdk5 inhibitors roscovitine and olomoucine significantly reduced the degree of basal TH phosphorylation at this site to ~70 and 60% of vehicle-treated controls, respectively (Fig. 2, A and B). The administration of either MEK inhibitor, U0126 or PD98059, also reduced the level of pS31-TH (Fig. 2, A and B; data not shown), consistent with the observation that mitogen-activated protein kinases (MAPKs) phosphorylate this site (11). Short-term treatment with the inhibitors did not alter total levels of TH, MAPK, or cdk5 immunoreactivity (Fig. 2A). Importantly, both U0126 and PD98059 effectively reduced basal levels of phospho-MAPK immunoreactivity (Fig. 2A and data not shown, respectively), whereas roscovitine and olomoucine did not affect phospho-MAPK levels. These data suggest that the decrease in pS31-TH levels after treatment with the cdk5 inhibitors is not caused by a nonspecific action of the compounds on MEK. It is noteworthy that cotreatment with the cdk5 and MAPK inhibitors significantly reduced the pS31 signal more than either inhibitor alone (Fig. 2, A and B). These data indicate that both cdk5 and MAPK phosphorylate Ser-31 in TH.

Increased pS31-TH Immunoreactivity in Mice with Increased Cdk5 Activity—We used a mouse model with increased cdk5 activity to evaluate whether TH is phosphorylated by cdk5 in vivo. p25, the proteolytic cleavage product of p35, causes prolonged activation of cdk5 (12), and an increase in expression in inducible mice results in enhanced phosphorylation of certain cdk5 substrates (13). The inducible mice are bitransgenic and overexpress p25 in the forebrain in the absence of doxycycline, a tetracycline derivative. We analyzed WT and p25 mice that have been induced for 5 weeks and display ~2.5-fold increases in cdk5 activity in the forebrain. Immunohistochemical analysis using pS31-TH revealed enhanced immunoreactivity in the perikaryon of neurons in the substantia nigra of p25 transgenic mice compared with WT mice (Fig. 2, G–I), consistent with the localization of p25 to somatodendritic compartments by staining (13). TH immunoreactivity in adjacent sections was comparable between WT and p25 transgenic mice, indicating that there were no gross morphological changes to this brain region at this point in the transgenic animal (Fig. 2, C–F). These data indicate that cdk5 phosphorylates TH at Ser-31 in vivo.

Cdk5 Activity Stabilizes TH Protein Levels and Increases Total TH Activity—Phosphorylation of Ser-31 has been reported to increase TH activity using purified TH and MAPK proteins (11, 14). Increased TH activity was also associated with Ser-31 phosphorylation in a neuroblastoma cell line (15). To determine whether phosphorylation of TH at Ser-31 by cdk5 affects the enzymatic activity of TH, we transfected full-length WT or the phosphorylation-defective S31A mutant constructs in the presence or absence of p35/cdk5. Cells were harvested and lysates were analyzed for TH activity using an in vitro radiometric assay. *, statistically significant p < 0.01 from TH transfected alone, unpaired two-tailed Student’s t test (n = 4). B, transfected cell lysates from A were analyzed by Western blot for pS31-TH, TH, p35, cdk5 (upper band, HA-tagged cdk5; lower band, endogenous cdk5) and actin.
whereas there was little to no signal with the alanine mutant with or without p35/cdk5 (Fig. 3B). It was intriguing that total TH levels were also increased in the presence of cdk5 activity with WT TH but not with the S31A mutant of TH. These data indicate that phosphorylation of TH on Ser-31 results in an increase in total TH protein levels, thereby increasing total TH activity in the cell.

Ser-31 Phosphorylation of TH by Cdk5 Decreases the Turnover of TH—In light of our observations of increased TH protein levels in the presence of cdk5, we hypothesized that overexpression of cdk5 increases the stability of TH. To test whether phosphorylation at Ser-31 affects the turnover of TH protein levels, COS-7 cells that coexpress TH and p35/cdk5 were treated with cycloheximide and harvested at 0, 8, 16, and 24 h after treatment. p35 levels were dramatically reduced within 8 h of cycloheximide treatment and persisted after 24 h (Fig. 4A), consistent with previous findings that indicate a short half-life for p35 protein (8). The more stable cdk5 protein remains unaffected even at the 24-h time point (Fig. 4A). Total TH protein levels in cells transfected with TH alone declined more rapidly than in cells expressing TH in the presence of p35/cdk5 (Fig. 4, A and B). After 24 h, ~75% of total TH levels remained in cells cotransfected with p35/cdk5 compared with 50% in cells transfected with TH and empty vector (Fig. 4, A and B). The presence of p35/cdk5 did not affect the stability of the S31A mutant, and the turnover of the S31A TH protein seemed to parallel that of WT TH alone. These data indicate that phosphorylation of TH at Ser-31 by cdk5 increases the stability of the protein.

TH Protein Levels are Reduced in Cdk5 Knock-out Mice—To examine whether cdk5 activity affects the stability of TH in vivo, we analyzed samples from WT and cdk5 knock-out mice, which die before or at birth (16). We dissected striata, a brain region enriched with TH, and analyzed TH protein levels. It is noteworthy that the total levels of TH from cdk5 knock-out striatal lysates were reduced to ~50% of WT at embryonic days 16 and 18 (Fig. 5A). Levels of pS31-TH in embryonic tissue were beyond the levels of detection by Western blot in that the amount of TH protein was less than 10% of adult tissue (data not shown). Similar results were consistently observed with total TH levels from whole brain lysates at late embryonic stages (Fig. 5B), suggesting that loss of cdk5 activity renders the TH protein more labile. To evaluate whether developmental defects in the cdk5 knock-out mice contribute to our observations in brain lysates, we cultured embryonic day 13 mesencephalic neurons containing TH-positive cells from littermates of cdk5 heterozygous matings. The number of cells obtained from these tissues was not different between cdk5 knock-out and wild-type or heterozygous mice (data not shown). The dissociated neurons in these cultures seemed healthy and were analyzed at 5 days in vitro for TH levels. In support of our above findings, TH levels in cdk5 knock-out cultures were significantly reduced to ~45% of littermate controls (Fig. 5, C and D), but tubulin levels were unchanged. These results
Samples were also analyzed for p35, cdk5, and b-III tubulin. E16, embryonic day 16. B, TH protein levels in whole brain lysates are reduced in late embryonic stages of cdk5 knock-out mice compared with wild-type littermate control mice. Samples were also analyzed for p35, cdk5, and b-III tubulin. C, representative Western blot shows decreased levels of TH in 5-day in vitro lysates from embryonic day 13 mesencephalic cultures of cdk5 knock-out mice compared with wild-type littermate control mice. Samples were also analyzed for p35, cdk5, and b-III tubulin. D, quantification of immunoreactive bands for TH from three sets of embryonic day 13 mesencephalic cultures derived from cdk5 heterozygous matings. * TH levels are significantly reduced, p < 0.05 in cdk5 knock-out (KO) lysates compare with WT or heterozygous (HET) lysates by an unpaired two-tailed Student’s t test (n = 19 for WT/HET; n = 5 for KO).

suggest that the effects of loss of cdk5 on TH protein stability are most probably not caused by developmental abnormalities or cell loss per se. It is noteworthy that levels of activated MAPK were unaffected in knock-out cultures (Fig. 5C). Taken together, these data indicate that cdk5 activity positively regulates TH levels through a MAPK-independent mechanism.

**DISCUSSION**

The regulation of TH activity is a critical determinant of catecholaminergic levels and function. Phosphorylation of TH at multiple sites by various protein kinases differentially affects the enzymatic activity of TH (3). Herein, we identify cdk5 as a novel kinase that phosphorylates TH at Ser-31 in vitro and in vivo. We show that total TH protein levels and activity increase after phosphorylation by cdk5 and that these parameters are unaffected with the S31A mutant of TH. In addition, we observe an increase in the stability of TH protein levels in the presence of cdk5 activity. Together, these data indicate that Ser-31 phosphorylation of TH by cdk5 is an important modulator of TH protein stability and catecholaminergic synthesis.

Post-translational modification of TH by phosphorylation plays a critical role in regulating TH function. Four serine residues (8, 19, 31, 40) in the regulatory domain of rat TH have been shown to be phosphorylated both in vitro and in vivo by at least seven different kinases (4). Phosphorylation of Ser-31 in TH was reported to be mediated by MAPK1 and MAPK2 in PC-12 cells (11). Our data support these findings, in that MEK inhibition reduced Ser-31 phosphorylation in PC-12 cells. In addition, we show that this site is also phosphorylated by cdk5. However, activation of the MAPK pathway by nerve growth factor treatment in PC-12 cells has been shown to induce sustained expression of p35 and to increase cdk5 activity (10). It is thus possible that the MAPK-mediated effects on Ser-31 phosphorylation are a result of activation of the cdk5 pathway. It is unclear whether the phosphorylation of Ser-31 by MAPKs in this system is a direct or indirect effect. Reciprocally, cdk5 activity has been shown to up-regulate MAPKs in p25 transgenic mice (13). Thus, we cannot rule out the possibility that the increased immunoreactivity we observed by staining in TH positive neurons in p25 transgenic may be an effect of MAPKs. These examples of “cross-talk” between the two systems make it difficult to unequivocally demonstrate an independent contribution by either kinase alone. Nevertheless, our data from mesencephalic cultures indicate that cdk5 can regulate TH levels independent of MAPK.

We also demonstrate that there are decreased TH levels in cdk5 knock-out mice, indicating that phosphorylation by cdk5 increases the stability of TH. However, the levels of TH do not seem to be altered in PC-12 cells that have been treated with cdk5 inhibitors despite decreases in Ser-31 phosphorylation. One explanation for this discrepancy is that the degree of kinase inhibition may be different between short-term in vitro inhibitor treatment compared with long-term deficiency in cdk5 knock-out tissue. Thus, the extent and duration of decreased cdk5 activity in the treated PC-12 cells may not be sufficient to mediate changes in TH protein levels. This premise is further supported by the half-life (~17 h) of endogenous TH protein in PC-12 cells (17); changes to TH protein levels may not be detectable in our experiments because of the relatively short timeframe (9 h). On the other hand, the fraction of Ser-31 phosphorylated TH in PC-12 cells is unclear. It is possible that the amount of pS31 TH is small compared to the total levels of TH. If this is the case, then significant changes to a small pool of protein might not be sufficient to influence total TH levels.
Tyrosine Hydroxylase Phosphorylation by Cdk5

Some of the phosphorylation events in the N-terminal regulatory domain have been associated with increases in enzymatic activity (4). For example, MAPK1- and MAPK2-mediated phosphorylation of Ser-31 has been shown to enhance TH activity up to 2-fold (11, 14, 18). Our findings support a role for Ser-31 phosphorylation in the up-regulation of TH activity through the novel mechanism of increased stabilization of TH protein. Reduced protein turnover is potentially important and may have relevance to overall catecholaminergic function. Alterations in TH levels and its enzymatic activity could result in significant changes in catecholamine levels. For example, after treatment of primary mesencephalic neurons with glial cell line-derived neurotrophic factor, MAPK-mediated TH phosphorylation of Ser-31 was associated with a 3-fold enhancement of DA synthesis (15). Therefore, considerable augmentation of DA production could result in profound changes in neurotransmitter regulation and release, leading to altered synaptic or behavioral outcomes.

As such, disruptions in TH enzymatic activity may contribute to a number of neuropathological conditions. For example, point mutations in the coding sequence of hTH have been linked to juvenile parkinsonism, L-DOPA responsive dystonia, and Segawa’s syndrome (19). In addition, aberrantly high levels of DA synthesis can also be neurotoxic because the metabolism of DA leads to the formation of several cytotoxic molecules, including superoxide anions, DA quinones, and hydroxyl radicals (20). DA-containing brain regions, such as the substantia nigra, are thought to be particularly susceptible to oxidative stress because of the highly oxidative environment. As such, accumulating evidence supports a role for oxidative stress and DA toxicity in the pathogenesis of Parkinson’s disease (PD), a neurodegenerative disorder that is primarily characterized by the loss of nigrostriatal dopaminergic neurons (21). Severe nigral cell loss and the presence of cytoplasmic inclusions (called Lewy bodies) in the substantia nigra are generally considered the hallmarks of idiopathic PD (22). The colocalization of cdk5 with Lewy bodies in PD brains, as revealed by postmortem examination, suggests a link between cdk5 and PD pathology (23, 24). Our results demonstrate a direct connection between cdk5 activity and TH regulation. In the future, it will be important to address the relevance of this connection in neurodegenerative diseases such as PD.

In summary, we have identified a novel substrate of cdk5 that has important implications for presynaptic regulation of catecholamines. Phosphorylation of TH at Ser-31 plays a critical role in TH protein stability and positively impacts total TH activity. Thus, in the absence of cdk5 activity, the protein is unstable and thus more susceptible to degradation. Taken together, our data strongly indicate that cdk5 activity is an important regulator of TH levels. Further studies will be required to investigate the relevance of this regulation in physiology and disease.

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REFERENCES