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Molecules and Cells

Expression of p25, an Aberrant Cyclin-Dependent Kinase 5 Activator, Stimulates Basal Secretion in PC12 Cells

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Although alterations in the functions of neurotransmitter systems have been implicated in the pathology of Alzheimer's disease (AD), the mechanisms that give rise to these alterations are not well understood. The amount of p25, an aberrant cleavage product of p35 that activates cyclin-dependent kinase 5 (Cdk5), is elevated in AD brains. The role of Cdk5 in neurotransmitter release has been well established. In this study, we examined whether p25 was linked to altered neurotransmitter release in AD. Transient or stable expression of p25 significantly increased basal secretion of human growth hormone (hGH) or neurotransmitter in PC12 cells. Expression of a p25 phosphorylation-deficient mutant, T138A, inhibited basal hGH secretion relative to the p25 wild type, suggesting the involvement of Thr138 phosphorylation in secretion. The expression and activity of β-site amyloid precursor protein cleaving enzyme 1 (BACE1), a key protease in the generation of β-amyloid, are increased in AD brains. Our previous studies indicated that overexpression of BACE1 enhanced basal secretion of hGH in PC12 cells. Transient coexpression of p25 and BACE1 further stimulated spontaneous basal secretion. These results indicate a novel role for p25 in the secretory pathway and suggest that elevated levels of p25 and BACE1 in AD brains may contribute to altered neurotransmitter pathology of AD through enhancing spontaneous basal secretion.

INTRODUCTION

Alzheimer's disease (AD), an irreversible, age-related, progressive brain disorder characterized by dementia, is prevalent among individuals over the age of 65. The brains of people with AD exhibit two pathological hallmarks: amyloid plaques and neurofibrillary tangles, which are insoluble deposits of β-amyloid (Aβ) and hyperphosphorylated tau protein, respectively. AD is also characterized by alterations in the function of the neurotransmitter systems that release acetylcholine, glutamate, norepinephrine, serotonin, and other chemical messengers (Lanari et al., 2006; Selkoe, 2002). Pharmacological intervention targeting these neurotransmitter systems forms the basis of current AD treatments. The concentration of norepinephrine and glutamate in cerebrospinal fluid (CSF), which is likely an estimate of neurotransmitter concentrations at synapses, are significantly higher in patients with AD than in control subjects (Csernansky et al., 1996; Elrod et al., 1997; Jimenez-Jimenez et al., 1998; Smith et al., 1985) although conflicting results have been reported (Jimenez-Jimenez et al., 1998). Together, these findings suggest that altered neurotransmitter release may be responsible for the pathogenesis of AD.

p25 is an aberrant cleavage product of p35, a protein that activates cyclin-dependent kinase 5 (Cdk5); further, p25/Cdk5 has been suggested to be involved in the formation of neurofibrillary tangles and Aβ in AD (Cruz et al., 2003; 2006). p25 expression and Cdk5 activity increase in sporadic AD brains (Patrick et al., 1999; Swatton et al., 2004; Tseng et al., 2002), although the increase in p25 is controversial (Tandon et al., 2003). Accumulating evidence indicates that Cdk5 controls neurotransmitter release through phosphorylation of the various substrates such as Munc18-1 and the P/Q-type Ca^{2+} channel (Barclay et al., 2004; Chung, 2008; Tomizawa et al., 2002). Considering the key role of Cdk5 in neurotransmitter release, we hypothesized that disturbances in the activity of Cdk5 due to p25 contributed to abnormal regulation of neurotransmitter release in presynaptic terminals.

In this study, we investigated the role of p25 in exocytosis in PC12 cells by using labeled ^{3}H-norepinephrine or human growth hormone (hGH). The hGH system has been widely used to study the effects of co-transfected proteins on regulated secretion in secretory cells such as PC12 and bovine chromaffin cells (Chung et al., 1999; Lee et al., 2007; Sugita et al., 1999). The Ca^{2+}-dependent secretion of dense core vesicles in PC12 cells is similar to synaptic vesicle exocytosis in neurons, making PC12 cells a practical model system in which to analyze regulated exocytosis in neurons (Choi et al., 2007; Sugita et al., 1999). Here, we show that the transient or stable expression of p25 significantly increases spontaneous basal secretion in PC12 cells.

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MATERIALS AND METHODS

Plasmids

Complementary DNAs (cDNAs) encoding human p25, Cdk5, and BACE1 were cloned from a human brain cDNA library by using the reverse transcription-polymerase chain reaction (RT-PCR) method, and were then subcloned into the pcDNA-myc/his expression vector (Invitrogen, USA). cDNA encoding the phosphorylation-defective p25T138A mutant (Thr138 → Ala138 as per the numbering for p35) was generated by *Dpn*I-mediated site-directed mutagenesis; the sequences of the resulting clones were then verified.

Cell culture, transfection, and secretion assay

The PC12 cell preparation, transient transfection, and hGH secretion assay were performed as previously described (Lee et al., 2007). For hGH secretion experiments, PC12 cells in 12-well plates were transfected with plasmids encoding hGH and the protein of interest using Lipofectamine 2000 (Invitrogen). Two days after transfection, hGH secretion was measured by using an enzyme-linked immunosorbent assay (ELISA) kit (Roche, Switzerland). To measure constitutive secretion, we determined the amount of hGH secreted into the culture media during 2 days of incubation (normalized to the amount of hGH retained in the transfected cells). In the secretion experiments, transfected cells were incubated in a control physiological salt solution [PSS; 145 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5.6 mM glucose, 15 mM HEPES (pH 7.4), 0.5 mM ascorbate, and 0.5% bovine serum albumin (BSA)] or in a depolarizing PSS buffer containing 100 mM KCl and 50.6 mM NaCl, and the amount of hGH secreted into the buffer and retained in cells at different time points was measured. The amount of secreted hGH was expressed as the percentage of total hGH.

Neurotransmitter uptake and release assay

The neurotransmitter uptake and release assay was performed as previously described (Chung et al., 1998; Lee et al., 2007). PC12 cells were plated on 48-well plates at 3×10^5 cells/ml. PC12 cells were pre-incubated for 3 h in culture medium containing ^{3}H-norepinephrine [1-(7,8-^{3}H)-norepinephrine, 37 MBq/ml, 1.0 mCi/ml] and 0.5 mM ascorbate. Cells were rinsed with control PSS buffer containing 0.5 mM ascorbate. Secretion was subsequently determined during 20 min incubation in PSS buffer containing 5.6 mM KCl or 100 mM KCl. The incubation solution was removed, and the cells were lysed with 1% Triton X-100. The radioactivity in the incubation solution and that in the cell was determined by liquid scintillation counting. Unless otherwise stated, data are represented as means ± SEM, with three samples per group.

PC12 Tet-Off cells stably expressing p25

PC12 Tet-Off cells (Clontech, USA) were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% fetal bovine serum (FBS), 5% horse serum, and 100 μg/ml of G418, in 5% CO_2 at 37°C. To construct a cell line expressing p25 under the regulation of the Tet-Off system, p25 cDNA was subcloned into the pTRE2pur vector (Clontech) to obtain pTRE2puro-p25 cDNA. PC12 Tet-Off cells were transfected with pTRE2puro-p25 cDNA using Lipofectamine 2000 (Invitrogen) following the manufacturer's protocol. At 48 h after transfection, the medium was replaced with RPMI 1640 medium containing 100 μg/ml of G418, 1 μg/ml of doxycycline, and 3 μg/ml of puromycin, and the medium was replaced every 4 days. Dead cells were separated from living cells at 5-7 days. After several washings with RPMI 1640 medium, only living cells were cultured on a 150-mm dish and an isolated single cell colony was transferred serially onto 96-, 24-, 12-, and 6-well plates in order. For protein expression, the clone was further cultured in RPMI 1640 medium lacking doxycycline for 3 days. Stable cell lines were selected by determining p25 expression by Western blotting.

Western blot analysis and immunocytochemistry

For protein expression, PC12 cells transfected with the indicated plasmids or stable PC12 Tet-Off cells were lysed in radioimmunoprecipitation assay (RIPA) buffer [50 mM Tris (pH 8.0), 150 mM NaCl, 1% NP-40, 0.1% sodium dodecyl sulfate, 0.5% deoxycholic acid] containing 1 mM phenylmethylsulphonyl fluoride (PMSF) and a protease inhibitor cocktail. Equal amounts of lysates were subjected to western blot analysis. Antibodies to p25/p35 (C19) and Cdk5 (J3) were obtained from Santa Cruz Biotechnology (USA). For immunocytochemistry, PC12 cells were fixed and permeabilized 2 days after transfection. Cells were incubated with primary antibodies [rabbit anti-p25/p35 antibody (Santa Cruz Biotechnology) or mouse anti-hGH antibody (Zymed, USA) at 1:200 dilutions] for 2 h, and were then incubated with secondary antibodies (Cy3 anti-rabbit antibody and Oregon Green 488 anti-mouse antibody at 1:500 and 1:100 dilutions, respectively) for 1 h. They were then examined under a confocal laser scanning microscope (LSM 510; Carl Zeiss, Germany).

RESULTS

Transient expression of p25 increases basal secretion in PC12 cells

To determine the effect of p25 on hGH secretion, expression plasmids encoding human p25 and hGH (pXGH5) were co-transfected into PC12 cells. As a negative control, PC12 cells were also co-transfected with a control expression vector (pcDNAmyc/his) and pXGH5. Overexpression of p25 in PC12 cells resulted in enhanced basal secretion of hGH relative to that of control cells within the first 2 min of incubation in PSS, and this enhancement continued for the next 13 min (Fig. 1A). The effect was observed in 19 independent experiments, with an average basal secretion enhancement of 46% ± 7% ($p < 0.0001$ versus control) during incubation for 15 min (Fig. 1B). Expression of the transfected proteins was determined by double immunocytochemistry and immunoblotting performed using PC12 cells (Figs. 1C and 3B). Consistent with previous reports (Chung et al., 1999; Khvotchev et al., 2003; Lee et al., 2007; 2008), most transfected cells expressed both p25 and hGH. Immunofluorescent localization of hGH revealed an extensive, fine punctuate pattern throughout the cell and widely dispersed p25 staining throughout the cells (Fig. 1C). In the merged image, hGH and p25 were partially co-localized (Fig. 1C).

We then determined whether the increase in basal secretion was correlated with an increase in constitutive hGH secretion. Transient expression of p25 had no effect on the amounts of hGH secreted into the culture media during 2 days (normalized to the amount of hGH retained in the transfected cells; Fig. 1B, constitutive secretion). These results indicated that spontaneous basal secretion in the absence of a stimulus may arise from secretion from the regulated pathway as previously reported (Lee et al., 2007; 2008; Matsuuchi and Kelly, 1991; Varro et al., 1996). Furthermore, in 19 independent experiments, transient expression of p25 slightly inhibited stimulus-dependent secretion by 13% ± 2% relative to the control cells ($p < 0.0001$ versus control) during 15 min of incubation (Fig. 1B), supporting a role

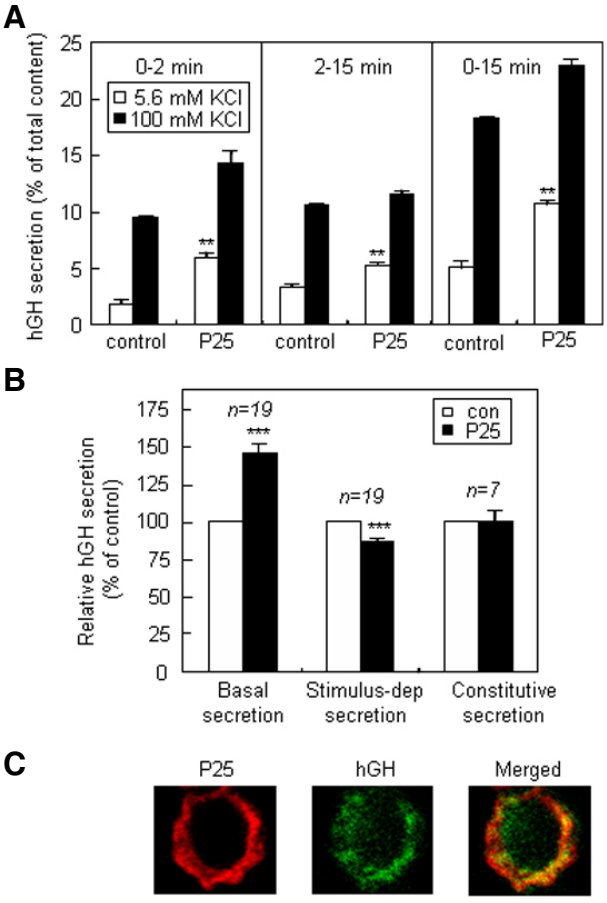

Fig. 1. Overexpression of p25 increases basal secretion in PC12 cells. (A) PC12 cells were co-transfected with an expression plasmid encoding hGH and a control plasmid (pcDNAmyc/his) or an expression plasmid encoding p25. Two days after transfection, cells were incubated in either control PSS buffer (5.6 mM KCl) or depolarizing PSS buffer (100 mM KCl), and hGH secretion was measured after the first 2 min (0-2 min) of incubation and then for the next 13 min (2-15 min) of incubation. Secretion samples from each of the two time points were also combined, and hGH secretion was assessed (0-15 min). The amount of hGH released into the incubation medium and retained in the cells was determined by ELISA. The amount of hGH secretion was expressed as the percentage of total cellular hGH. Three wells of cells were used per group, and the data are represented as the means ± SEM. ** $p < 0.01$ versus basal control secretion (Student's *t*-test). (B) Secretion from p25-transfected cells is plotted as the percentage of hGH secretion from control cells transfected with pcDNAmyc/his. Secretion was measured from cells that had been incubated for 15 min in control PSS buffer containing 5.6 mM KCl (basal secretion). The difference in the secretion from cells that had been incubated in 5.6 mM and 100 mM KCl-containing PSS buffer for 15 min was also measured (stimulus-dependent secretion). The amount of hGH secreted into the culture medium during 2 days of incubation was measured and normalized to the amount of hGH contained in the transfected cells (constitutive secretion). Data are represented as the mean ± SEM of numerous independent experiments as indicated (*n*); each experiment was performed in triplicate. *** $p < 0.001$ versus control (Student's *t*-test). (C) PC12 cells were co-transfected with expression plasmids encoding hGH and p25 and processed for immunofluorescence. Rabbit anti-p25/p35 and mouse anti-hGH antibodies were used to detect p25, hGH, and p25 + hGH (merged).

for p25 in regulating exocytosis in PC12 cells.

Stable expression of p25 also increased basal secretion in PC12 cells

A previous study with chromaffin cells showed that inhibition of Cdk5 with olomoucine decreased norepinephrine secretion (Fletcher et al., 1999); we also observed that norepinephrine secretion from PC12 cells was inhibited by olomoucine (Fig. 2A). However, transient expression of p25 in chromaffin cells has been reported to increase nicotinic agonist-induced secretion without affecting basal hGH secretion (Fletcher et al., 1999). To resolve this seemingly inconsistent finding on hGH secretion in chromaffin cells and PC12 cells transiently transfected with p25 and to use a different experimental design to measure norepinephrine release instead of hGH release, we generated PC12 cells that could stably express p25. To avoid potential toxicity from p25 overexpression (Patrick et al., 1999), exogenous p25 expression was regulated by a Tet-Off system in stably transfected PC12 cells. ^{3}H-norepinephrine secretion was measured from two stable PC12 Tet-Off cells, p25-3 and p25-5, which clearly overexpressed p25 when cultured in a medium lacking doxycyclin (Fig. 2B). Basal secretion of ^{3}H-norepinephrine was enhanced, while stimulus-dependent secretion of ^{3}H-norepinephrine was either inhibited or not affected in PC12 Tet-Off cells stably expressing p25 as compared to control PC12 Tet-Off cells (Fig. 2C). Some difference in secretion may exist between PC12 and chromaffin cells because PC12 cells contain far fewer granules than adrenal chromaffin cells, and many are closely associated with the plasma membrane (Holz, 1999).

Effect of Thr^{138} phosphorylation on basal and stimulus-dependent secretion

The Thr^{138} site (according to the numbering for p35) of p25 is autophosphorylated by Cdk5 (Kamei et al., 2007). To assess the role of Thr^{138} phosphorylation on the ability of p25 to modify secretion, the effect of transfection with expression plasmids encoding the p25 wild type or its phosphorylation-defective mutant (T138A) on hGH secretion was compared in PC12 cells. p25-induced basal hGH secretion tended to be reduced when Thr^{138} in p25 was mutated to Ala, while expression of p25T138A tended to restore stimulus-dependent secretion inhibited by the p25 wild type (Fig. 3A). The amount of exogenously expressed p25 in the transfected cells was similar for p25 and its T138A mutant (Fig. 3B). From nine determinations, the expression of the p25T138A mutant reduced basal secretion by 39% ± 6% ($p < 0.001$) and restored stimulus-dependent secretion inhibited by the p25 wild type by 68% ± 15% ($p < 0.01$) (Fig. 3C). This result suggests the importance of Thr^{138} phosphorylation in secretion in PC12 cells.

Co-expression of p25 and BACE1 further increased basal secretion in PC12 cells

The amounts of BACE1 and p25 are elevated in sporadic AD brains (Fukumoto et al., 2002; Patrick et al., 1999; Swatton et al., 2004; Tseng et al., 2002; Yang et al., 2003). Recently, we showed that overexpression of BACE1 stimulated basal secretion in PC12 cells, suggesting that elevated BACE1 levels in sporadic AD brains may contribute to altered neurotransmitter pathology (Lee et al., 2007). To recapitulate the condition in AD brains and to determine the association between p25 and BACE1 in enhanced basal secretion, plasmid encoding BACE1 was co-transfected with pXGH5 into PC12 cells in the presence or absence of a p25 expression plasmid. Consistent with a previous report (Wen et al., 2008), expression of p25 increased

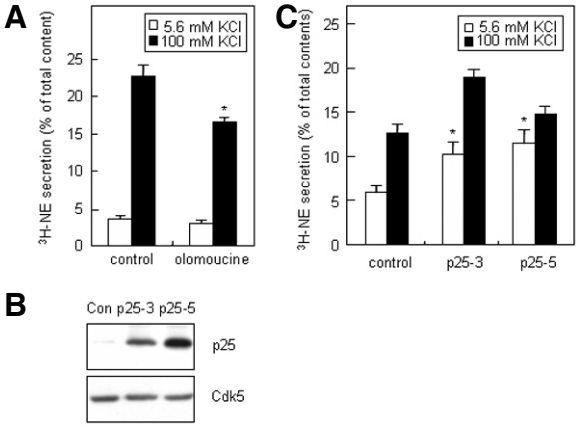

Fig. 2. p25-induced elevation in basal secretion of [^{3}H]-norepinephrine in p25 stably transfected PC12 Tet-Off cells. (A) Effect of olomoucine, a Cdk5 inhibitor, on secretion in PC12 cells. PC12 cells in a 48-well plate were incubated with [^{3}H]-norepinephrine with and without 150 μM of olomoucine for 16 h. The results shown here are representative of two independent experiments. Three wells of cells were used per group, and the data are represented as means ± SEM. * $p < 0.05$ versus stimulus-dependent secretion in the control group (Student's *t*-test). (B) The extracts from PC12 Tet-Off cells (Con) and p25 stably transfected PC12 Tet-Off cell lines (p25-3 and p25-5) were subjected to immunoblotting with anti-p25/p35 and Cdk5 antibodies. Cdk5 was used as a loading control. (C) Secretion from [^{3}H]-norepinephrine (NE)-labeled cells was subsequently determined during a 20-min incubation in control medium containing 5.6 mM KCl or depolarizing medium containing 100 mM KCl. There were 3 wells per group. Data are represented as means ± SEM. * $p < 0.05$ versus control basal secretion (Student's *t*-test).

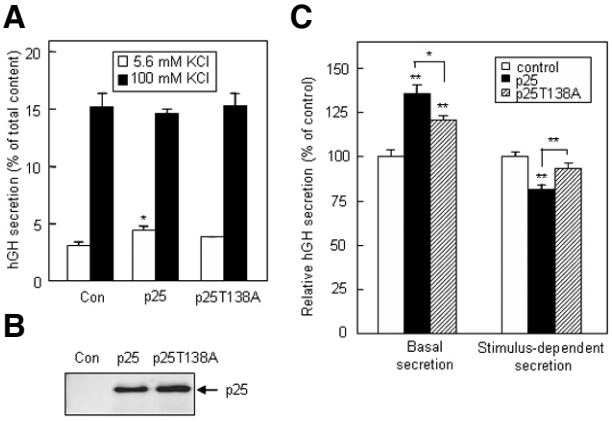

Fig. 3. Effect of phosphorylation of Thr^{138} in p25 on hGH secretion in PC12 cells. (A) PC12 cells were co-transfected with an expression plasmid encoding hGH and either a control plasmid or an expression plasmid encoding the p25 wild type or p25 phosphorylation-site mutant T138A. Two days after transfection, secretion of hGH was measured from cells that had been incubated for 15 min in a control medium (5.6 mM KCl) or a depolarizing medium (100 mM KCl). The results shown here are representative of three independent experiments. (B) Equal amounts of extracts from PC12 cells transfected with the indicated plasmid were subjected to immunoblotting with an anti-p25/p35 antibody. (C) Basal secretion and stimulus-dependent secretion from p25 or p25T138A-transfected cells are plotted as a percentage of the secretion from cells transfected with the control plasmid. Data are represented as means ± SEM of nine determinations (n = 9). * $p < 0.05$, ** $p < 0.01$ versus control group or as indicated above the graph (Student's *t*-test).

levels of BACE protein (Fig. 4A). The BACE1 expression vector (pcDNAmyc/his) used in this study may not contain a p25/cdk5 responsive region identified in the BACE1 endogenous promoter (van den Pol and Ghosh, 1998; Wen et al., 2008); thus, there is a possibility that p25 has an effect on post-transcriptional levels of BACE1. The transfection with expression plasmids encoding BACE1 or p25 increased basal hGH secretion as shown in Fig. 1A and in a previous report (Lee et al., 2007). When BACE1 and p25 were co-transfected, basal hGH secretion was further stimulated (Fig. 4B). In five independent experiments, BACE1 and p25 individually increased basal hGH secretion by 59% ± 18% ($p < 0.05$ versus control) and 43% ± 11% ($p < 0.05$ versus control), respectively. When BACE1 and p25 were co-transfected, the increase in basal secretion was 120% ± 28% ($p < 0.05$ versus control; Fig. 4C). The combination of BACE1 and p25 had an additive effect on basal hGH secretion, suggesting that these proteins may act in concert to stimulate basal secretion and that p25/Cdk5 possibly coordinates the action of the multiple factors involved in basal secretion.

DISCUSSION

In this study, we found that transient or stable expression of p25 caused significant increases in basal secretion in PC12 cells. Stimulus-dependent secretion in PC12 cells was also inhibited to a small extent by expression of p25, suggesting the involvement of p25 in regulated secretion. The study with a phosphorylation-deficient p25 mutant suggests that Cdk5-mediated autophosphorylation of p25 is important for its ability to regulate secretion in PC12 cells. Moreover, co-expression of BACE1 and p25 stimulated basal secretion in an additive manner. This study provides evidence for a novel role of p25 in the secretory pathway.

Increasing evidence suggests that Cdk5 is involved in neurotransmitter release via regulation of various substrates. Munc18-1, synapsin 1, and P/Q subtype voltage-dependent calcium channels are known Cdk5 substrates that are associated with synaptic vesicle exocytosis (Barclay et al., 2004; Chung, 2008; Rosales and Lee, 2006; Tomizawa et al., 2002). However, it is not clear whether Cdk5 acts as a positive or negative regulator of neurotransmitter release, which may occur due to the involvement of Cdk5 at numerous points in synaptic vesicle cycling. Several studies on adrenal chromaffin cells and pancreatic β-cells suggest that Cdk5 functions as a positive regulator of exocytosis via phosphorylation of substrates such as Munc 18-1 (Fletcher et al., 1999; Lee et al., 2004; Lilja et al., 2001; 2004; Rosales et al., 2004; Xin et al., 2004). Evidence also suggests that Cdk5 decreases neurotransmitter release by reducing vesicle fusion pore conductance and quantal size without altering pore opening times (Barclay et al., 2004). Cdk5$^{-/-}$ mice show an increase in the frequency of both miniature endplate potentials (MEPPs), small depolarizations in the resting potential of postsynaptic cells relative to control mice, suggesting that the spontaneous release of neurotransmitter from presynaptic terminals and synaptic transmission may be enhanced in the mutant mice (Fu et al., 2005). This result indicates that the absence of Cdk5 promotes transmitter release at peripheral synapses *in vivo*, suggesting an inhibitory role for Cdk5 in neurotransmitter release. Taking into consideration the

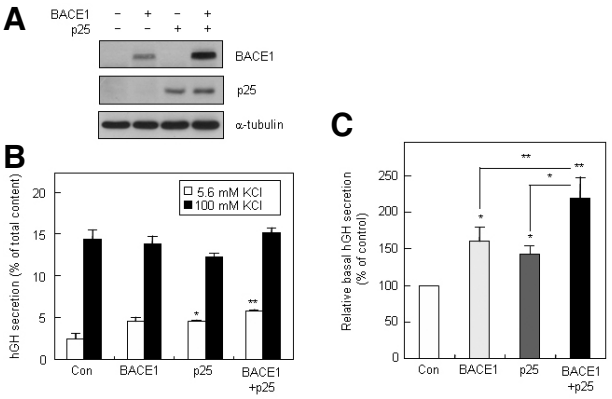

Fig. 4. Co-expression of p25 and BACE1 further increases basal secretion in PC12 cells. (A) Representative Western blots of PC12 cells transfected with plasmids encoding the indicated proteins. (B) PC12 cells were co-transfected with plasmids encoding hGH and the indicated plasmid(s). Secretion was subsequently determined after a 15-min incubation in control medium containing 5.6 mM KCl or depolarizing medium containing 100 mM KCl. The results shown here are representative of five independent experiments. Data are represented as means ± SEM. * $p < 0.05$, ** $p < 0.01$ versus control basal secretion (Student's *t*-test). (C) The secretion from cells transfected with indicated plasmid(s) is plotted as the percentage of basal hGH secretion from control cells transfected with pcDNA-myc/his. Data are represented as means ± SEM of five independent experiments. * $p < 0.05$, ** $p < 0.01$ versus control or as indicated (Student's *t*-test).

critical role of Cdk5 in neurotransmitter release, it is important to understand how p25-induced disturbance in the activity of Cdk5 regulates neurotransmitter release during the progression of neurodegenerative disorders such as AD. The elevated and spontaneous release of hormones or neurotransmitters in the absence of a stimulus induced by expression of p25 observed in this study may be of considerable pathophysiological importance. The spontaneous basal secretion of hGH by p25 in PC12 cells may be the result of slow (basal) activation of regulated release from storage granules (Graham et al., 1997) because expression of p25 had no effect on constitutive secretion (Fig. 1B). However, very few studies have addressed the topic of basal secretion in secretory cells (Matsuuchi and Kelly, 1991; Varro et al., 1996).

Residues Ser^{8} and Thr^{138} of p35 were identified as the major sites of phosphorylation by Cdk5 (Kamei et al., 2007). It has been reported that phosphorylation of p35 at the Thr^{138} residue inhibits the calpain-dependent cleavage of p35 to p25 and that the Thr^{138} site is dephosphorylated in adult rats; however, both Thr^{138} and Ser^{8} were phosphorylated in prenatal brains, suggesting that dephosphorylation of Thr^{138} may be an important determinant of Cdk5/p25-induced cell death associated with neurodegeneration (Kamei et al., 2007; Saito et al., 2003). Another study showed that the phosphorylation of p35 at Thr^{138} and other sites by Cdk5 facilitated the proteasomal degradation of p35 (Patrick et al., 1998). In this study, we assessed the effect of Thr^{138} phosphorylation on the ability of p25 to modify secretion. The results obtained with the phosphorylation-deficient p25 mutant (T138A; Fig. 3) suggest that Cdk5 participates in the phosphorylation of p25 to stimulate basal secretion and to inhibit stimulus-dependent secretion. However, whether the calpain-cleaved p25 is indeed phosphorylated by Cdk5 and whether Thr^{138} phosphorylation has an effect on the role of p25 in the pathological states of neurodegenerative diseases, including AD, remain to be addressed in future studies.

Our findings on the upregulated basal release of hGH by expression of p25 and BACE1 predict that neurotransmitters may be excessively released under normal resting conditions in AD brains. In fact, patients with AD have higher glutamate levels in CSF than controls (Csernansky et al., 1996; Jimenez-Jimenez et al., 1998; Pomara et al., 1992; Smith et al., 1985). The free glutamate concentration in CSF is likely to give a better approximation of the glutamate concentration at synapses as compared to the plasma glutamate concentration (Palmada and Centelles, 1998). In addition, an aging-associated elevation in the norepinephrine concentration in the CSF is found in the earlier stages of AD. The norepinephrine concentration increases further as the disease progresses, despite the loss of locus ceruleus neurons in AD, suggesting that increased brain noradrenergic activity may contribute to the agitated behavior or cognitive deficits of patients with advanced AD (Elrod et al., 1997). Conflicting results of CSF glutamate and norepinephrine levels in AD have been reported, which may be attributable to the technical difficulties involved in determining these levels and/or to the selection of patients (Jimenez-Jimenez et al., 1998). Thus, excessive release of glutamate and norepinephrine in AD is likely to be linked to increased p25 and BACE1 in sporadic AD brains (Fukumoto et al., 2002; Patrick et al., 1999; Swatton et al., 2004; Tseng et al., 2002; Yang et al., 2003). p25/Cdk5 possibly coordinates the action of BACE1 as previously reported (Wen et al., 2008) in addition to deregulating the function of critical Cdk5 substrates implicated in neurotransmitter release (Chung, 2008). Further investigation will be required to determine whether and how the spontaneous basal release of neurotransmitters is altered in the brains of p25 transgenic mice and AD patients.

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