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Cadherins and synaptic plasticity: activity-dependent cyclin-dependent kinase 5 regulation of synaptic β-catenin–cadherin interactions

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Cyclin-dependent kinase 5 (Cdk5)/p35 kinase activity is known to decrease the affinity of β-catenin for cadherin in developing cortical neurons. Our recent work demonstrated that depolarization causes an increased affinity between β-catenin and cadherin. Here, we examine whether Cdk5/p35 regulates β-catenin–cadherin affinity in response to neural activity. In hippocampal neurons depolarization caused a significant decrease in Cdk5 kinase activity, without changing the protein levels of either Cdk5 or p35, suggesting that the proteasome pathway is not involved. Decreasing Cdk5 kinase activity with the inhibitor roscovitine increased the amount of β-catenin that was co-immunoprecipitated with cadherin. Inhibiting Cdk5 activity also resulted in a redistribution of EGFP–β-catenin from the dendritic shaft to the spines, where cadherins are highly concentrated. The redistribution of β-catenin induced by roscovitine is similar to that induced by depolarization. Interestingly, the redistribution induced by the Cdk5 inhibitor was completely blocked by either a tyrosine phosphatase inhibitor, orthovanadate or by point mutations of β-catenin Tyr-654 to Glu or Phe. Immunoprecipitation studies further revealed that roscovitine increases the affinity of the wild-type, but not mutated, EGFP–β-catenin for cadherin. These results suggest that Cdk5 activity regulates the affinity of β-catenin for cadherin by changing the phosphorylation level of β-catenin Tyr-654.

Keywords: cadherin; β-catenin; cyclin-dependent kinase 5; tyrosine phosphorylation; depolarization; synaptic remodelling

1. INTRODUCTION

Synapses can undergo dynamic changes in their strength that last from minutes to hours to days. Activity-induced changes in synaptic proteins probably underlie long-term synaptic plasticity in the brain. Although cell adhesion molecules are thought to participate in synaptic remodelling, the links between neural activity and subsequent structural modifications are unknown. In addition to serving as recognition markers for synaptogenesis, the presence of adhesion molecules in or near the synaptic cleft raises the possibility that they may participate in initiating and maintaining synaptic changes (Murase & Schuman 1999). Cadherins are a family of proteins that mediate Ca^{2+}-dependent homophilic cell adhesion (Takeichi 1990). Cadherins are located at synaptic sites (Fannon & Colman 1996; Uchida *et al.* 1996; Tang *et al.* 1998) and biochemical studies have demonstrated that cadherins associate with core synaptic proteins both pre- and postsynaptically (Husi *et al.* 2000; Phillips *et al.* 2001), suggesting that cadherins regulate synaptic function.

Several recent studies support a role for the classic cadherins in synaptic plasticity (Tang *et al.* 1998; Benson & Tanaka 1998; Tanaka *et al.* 2000; Bozdagi *et al.* 2000). We previously reported (Tang *et al.* 1998) that either cadherin function-blocking antibodies or inhibitory peptides blocked LTP in area CA1 of rat hippocampal slices. Using a different stimulation protocol designed to elicit L-LTP, Benson and colleagues showed that an antibody to N-cadherin blocked early-phase but not L-LTP. The same study documented an increase in the number of cadherin-positive synaptic sites during L-LTP (Bozdagi *et al.* 2000). These observations suggest that cadherins may initiate and/or maintain the synaptic changes that occur during LTP. In contrast, another study found that the magnitude of LTP was enhanced in hippocampal slices prepared from mice that express a truncated version of a specific cadherin, cadherin-11 (Manabe *et al.* 2000), suggesting that, under some conditions, cadherins may also limit the formation of plasticity. A recent study also demonstrated that expression of a dominant negative cadherin perturbed spine morphology in developing neurons (Togashi *et al.* 2002).

The cytoplasmic domain of cadherin interacts with F-actin via proteins called catenins: β-catenin binds directly to the C-terminal part of cadherin, and also binds to α-catenin, which in turn interacts with F-actin (Ozawa *et al.* 1990; Hirano *et al.* 1992). The interaction with actin filaments is required for the adhesive activity of cadherin (Nagafuchi *et al.* 1994). Thus, changes in β-catenin–cadherin affinity may regulate cadherin adhesion. Phosphorylation of β-catenin Tyr-654 significantly decreases the affinity for cadherin (Roura *et al.* 1999). Several

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tyrosine kinases (Matsuyoshi *et al.* 1992; Behrens *et al.* 1993; Hamaguchi *et al.* 1993; Hazan & Norton 1998; Roura *et al.* 1999; Bonvini *et al.* 2001) and tyrosine phosphatases (Balsamo *et al.* 1996; Fuchs *et al.* 1996; Kypta *et al.* 1996; Balsamo *et al.* 1998; Müller *et al.* 1999) are known to regulate β-catenin tyrosine phosphorylation levels. High levels of β-catenin Tyr phosphorylation result in a loss of cadherin adhesion (Ozawa & Kemler 1998), promoting cell migration (Sommers *et al.* 1994; Müller *et al.* 1999) and neurite outgrowth (Pathre *et al.* 2001).

We recently demonstrated that, in adult hippocampal neurons, synaptic activity increases the affinity of β-catenin for cadherin in an NMDAR-dependent manner (Murase *et al.* 2002). Synaptic activity also increases the concentration of β-catenin at dendritic spines where cadherin mediates synaptic connections (Murase *et al.* 2002). The redistribution of β-catenin is probably driven by decreased levels of Tyr phosphorylation. Point mutations of β-catenin Tyr-654 to Glu or Phe cause dramatic alterations in both synaptic structure and function (Murase *et al.* 2002). These results suggest that phosphorylation of β-catenin Tyr-654 plays an important role in activity-induced synaptic remodelling.

Cdk5/p35 is a neuron-specific Ser/Thr kinase whose activity regulates important developmental events such as neural migration (Chae *et al.* 1997) and neurite outgrowth (Nikolic *et al.* 1996). Cdk5 interacts with β-catenin through its regulatory subunit, p35, and regulates the affinity of β-catenin for cadherin in developing cortical neurons (Kwon *et al.* 2000). Although β-catenin can be a substrate for Cdk5 (Kesavapany *et al.* 2001), it is unknown whether β-catenin Ser/Thr phosphorylation affects the affinity for cadherin. Here, we show in adult hippocampal neurons that depolarization inhibits Cdk5 activity, which increases the affinity of β-catenin for cadherin. Inhibition of Cdk5 by roscovitine results in a redistribution of β-catenin from dendritic shaft to spines. A point mutation of Tyr-654 completely blocks the redistribution effect of roscovitine, suggesting that Cdk5 activity regulates the affinity of β-catenin by altering the phosphorylation level of Tyr-654.

2. MATERIAL AND METHODS

(a) *Generation of sindbis virus expressing wild-type and point mutant EGFP-β-catenin*

The details that follow were described previously in Murase *et al.* (2002). Briefly, full length chicken β-catenin cDNA was subcloned into BspEI–EcoRI sites of the pEGFPC1 vector (Clontech). Point mutations were created from EGFP-β-catenin using a QuickChange site-directed mutagenesis kit (Stratagene). The NheI–ApaI fragment was then subcloned into XbaI–ApaI sites of pSindRep5. The recombinants of sindbis virus were prepared by using a sindbis expression system (Invitrogen). The plasmid was linearized by PacI digestion, and was used to perform *in vitro* transcription. The virus was generated in BHK cells. All results were confirmed by DNA sequencing.

(b) *Cultured neurons*

Dissociated hippocampal neurons from postnatal 2 day rat pups were plated at a density of 15 000–45 000 cells cm^{-2} onto polylysine and laminin-coated cover-slips. Cultures were maintained in growth medium (Neurobasal-A supplemented with B27 and GlutaMax-1) for 18–28 days before use. Recombinant DNA was introduced by sindbis virus infection in the growth medium 18–24 hours before imaging. To block protein synthesis, 40 μM anisomycin was added to the growth medium 1–2 hours before imaging.

(c) *Imaging*

HBS containing 110 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$, 10 mM D-glucose and 10 mM HEPES-NaOH (pH 7.4) (osmolarity adjusted to 290 mOsmol with sucrose) was used for imaging. Roscovitine, U0126 (Calbiochem) and sodium orthovanadate were used at final concentrations of 50 μM, 50 μM and 1 mM, respectively. It was confirmed that incubation in 0.2% DMSO from stock solutions did not affect the EGFP–β-catenin distribution in neurons. No cell death was observed after incubation for 3 hours with 50 μM roscovitine as assessed by propidium iodide staining ($n = 9$). To evaluate the effect of orthovanadate, the neurons were incubated with 40 μM anisomycin for more than 1 hour before $t = 0$ to arrest protein synthesis. Images were acquired by an Olympus AX70 CCD microscope with a water emersion objective lens at room temperature (magnification, ×63). A mercury lamp was used for excitation, excitation filter: 480 ± 40 nm; emission filter: 535 ± 50 nm (Chroma 41001). Each image was taken with a 2 s exposure.

(d) *Immunoprecipitation*

Hippocampal slices (300 μm) from 5- to 6-week-old male Sprague–Dawley rats were recovered at room temperature for 1.5 hours on filter paper placed over a tissue culture dish containing oxygenated ACSF (119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, 26.2 mM $NaHCO_3$ and 11.0 mM D-glucose). The slices were incubated in oxygenated ACSF, in some cases containing 50 μM roscovitine, for 3 hours. For the point mutation analysis, the sindbis virus was microinjected with quartz micropipettes prepared from microfilaments (1 mm OD, 0.7 mm OD, 10 cm length, Sutter Instrument) into hippocampal slices (300 μm) from 9-day-old rat pups. The slices were then cultured for 24–36 hours (Stoppini *et al.* 1991). The slices were homogenized in 300 μl lysis buffer (150 mM NaCl, 1% NP-40 and 50 mM Tris–HCl (pH 8.0)) containing a protease inhibitor cocktail (Roche) on ice, and centrifuged at 12 000*g* for 10 min at 4 °C. Monoclonal anti-cadherin, CH-19 (pancadherin) antibody (Sigma), was used for immunoprecipitation. For EGFP–β-catenins, monoclonal anti-GFP antibody (Clontech) was used. Western blot analysis was performed with monoclonal anti-β-catenin antibody, 15B8 (Sigma) or pancadherin CH-19 antibody as primary antibodies, and with peroxidase labelled anti-mouse IgG antibody (Amersham Pharmacia Biotech) as a secondary antibody.

(e) *Kinase assay for Cdk5*

Dissociated hippocampal neuron cultures were used for the kinase assay as described by Nickolic *et al.* (1998). After washing three times with HBS, the neurons were incubated with high KCl–HBS for 5–7 min. The neurons were then recovered in HBS for 30 min before incubating in lysis buffer (ELB), containing 250 mM NaCl, 0.1% NP-40, 50 mM HEPES–KOH (pH 7.0), 5 mM EDTA, protease inhibitor cocktail, on ice for 20 min. The lysate was collected and centrifuged at 12 000*g* for 10 min at 4 °C. Rabbit polyclonal anti-Cdk5 antibody, C-8 (Santa Cruz) was used for immunoprecipitation. The Cdk5 kinase assay was done in the presence of 1 mM ATP, 2 μ Ciγ^{32}P-

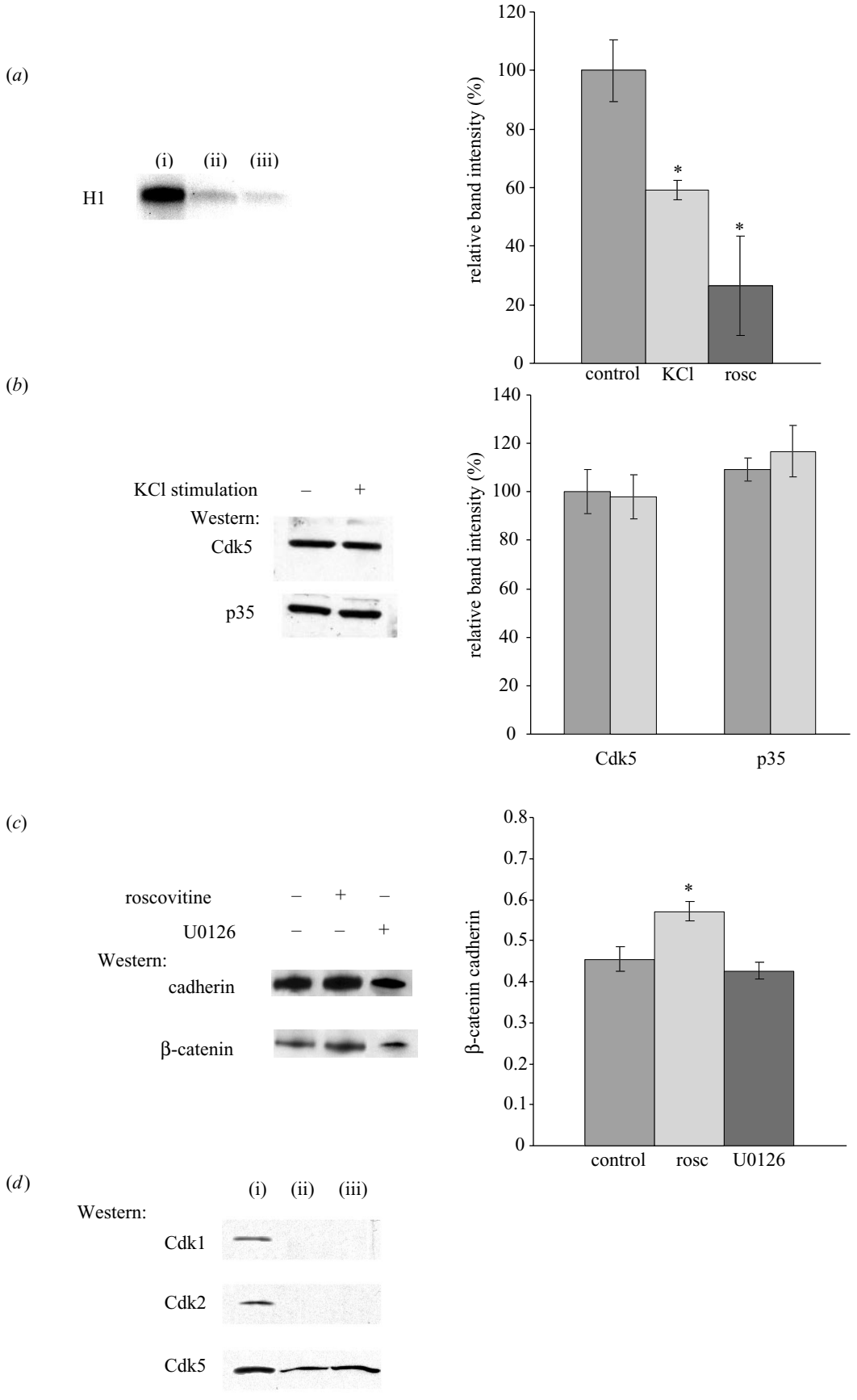

Figure 1. (*Caption overleaf.*)

ATP, 0.5 mg histone H1 (Calbiochem) and kinase buffer containing 50 mM HEPES (pH 7.5) and 10 mM $MgCl_2$ (Nikolic *et al.* 1998). For Western blot, monoclonal anti-Cdc2 p34 (17), anti-Cdk2 (D-12) and anti-Cdk5 (DC 17) antibodies and rabbit polyclonal anti-p35 antibody, C-19 (Santa Cruz) were used as primary antibodies, and peroxidase labelled anti-mouse or anti-

Figure 1. Depolarization causes inhibition of Cdk5. (*a*) γ^{32}P-ATP: ^{32}P incorporation to histone H1 protein. Compared with the control (lane (i)), the Cdk5 kinase activity of KCl-stimulated neurons (lane (ii)) is significantly reduced ($59.3 \pm 3.2\%$ of control, $n = 3$, $p < 0.05$). Roscovitine, a specific Cdk5 inhibitor largely inhibits ^{32}P incorporation (lane (iii)), $16.7 \pm 10.8\%$ of control, $n = 3$, $p < 0.05$). Abbreviation: rosc, roscovitine. (*b*) Cell lysate: total amounts of Cdk5 and p35 from control and depolarized neurons. The inhibition of Cdk5 by depolarization is not caused by degradation of p35 (Cdk5: $98.1 \pm 9.1\%$ of control, $n = 3$, n.s.; p35: $107.0 \pm 10.7\%$, $n = 3$, n.s.). Dark shading, control; light shading, KCl stimulated. (*c*) IP with anti-cadherin: inhibition of Cdk5 increases the affinity between β-catenin and cadherin in adult hippocampal neurons. Cell lysate from control and roscovitine-treated neurons was used for immunoprecipitation with an anti-cadherin antibody. The amount of β-catenin co-immunoprecipitated with anti-cadherin antibody from roscovitine-treated neurons was significantly larger than control ($p < 0.05$, $n = 3$). Abbreviation: rosc, roscovitine. (*d*) Western blot analysis with anti-Cdk1, anti-Cdk2 and anti-Cdk5 antibodies. Lane (i), cell lysate from PC12 cells; lane (ii), cell lysate from DIV18 cultured hippocampal neurons; lane (iii), cell lysate from P10 hippocampal neurons. Both DIV18 and P10 hippocampal neurons showed an abundant expression of Cdk5, but not Cdk1 or Cdk2.

rabbit IgG antibodies (Amersham Pharmacia Biotech) as secondary antibodies. The bands were visualized using ECL system (Amersham Pharmacia Biotech). Statistical significance was assessed with the Student's *t*-test. p values of 0.05 were considered significant and higher values are designated as not significant (n.s.).

(f) *Time-lapse image analysis*

The details were described previously in Murase *et al.* (2002). Briefly, areas containing dendrites of *ca.* 50 μm in length were selected for analysis with Image J software. The m.p.v. of each area was used to calculate the total fluorescence intensity. Every dendritic spine within the area (typically 10–20 spines) was analysed by measuring the m.p.v. within a circle (*ca.* 1.4 μm diameter) surrounding it. The m.p.v. of the dendritic shaft was also measured to calculate the fluorescence intensity of the shaft region. The background was subtracted from each measurement. The photobleaching effect was removed by normalizing with a photobleaching curve (Murase *et al.* 2002).

3. RESULTS

(a) *Cdk5 activity is inhibited by neural activity*

Both Cdk5 and its activator protein, p35, are enriched at synaptic sites in hippocampal neurons (Tomizawa *et al.* 2002). To investigate whether Cdk5 is regulated by neural activity, the phosphorylation of histone H1 protein, a substrate for Cdk5, was measured with immunoprecipitates from dissociated cultured hippocampal neurons. When the neuronal cell lysate was immunoprecipitated with an anti-Cdk5 antibody, an abundant amount of ^{32}P was incorporated into H1, indicating high Cdk5 activity (figure 1*a*). The activity level of Cdk5 was, however, significantly reduced when the neurons were briefly depolarized by the application of high KCl–HBS (figure 1*a*). Although the degradation of p35 is known to regulate the activity of Cdk5 (Patrick *et al.* 1998), the protein levels of Cdk5 and p35 were not changed by KCl stimulation (figure 1*b*). These results indicate that neural activity causes an inhibition of Cdk5 activity by a mechanism that does not involve the degradation of p35.

In developing cortical neurons, the affinity of β-catenin for cadherin is regulated by Cdk5 activity (Kwon *et al.* 2000). Therefore, we next examined whether Cdk5 also regulates the affinity between β-catenin and cadherin in adult hippocampal neurons. When hippocampal slices were incubated with the Cdk inhibitor roscovitine, the amount of β-catenin co-immunoprecipitated with an anti-cadherin antibody was significantly increased compared with that co-immunoprecipitated from the control slices (figure 1*c*). Although roscovitine is also known to inhibit MAPKs (Meijer *et al.* 1997), no significant change was observed in the amount of β-catenin when the slices were incubated with an MEK inhibitor, U0126, indicating that the roscovitine-induced affinity increase is not due to the inhibition of MAPKs (figure 1*c*). Although roscovitine is known to inhibit Cdk1 and Cdk2, as well as Cdk5 (Rudolph *et al.* 1996; Meijer *et al.* 1997), the expression of neither Cdk1 nor Cdk2 was detected from hippocampal cell lysates by Western blot analysis (figure 1*d*), suggesting the effect of roscovitine on cadherin–β-catenin affinity is specifically due to the inhibition of Cdk5. Taken together, these results suggest that the downregulation of β-catenin–cadherin association mediated by Cdk5 is inhibited by neural activity.

(b) *Cdk5 regulates β-catenin distribution*

Our recent study (Murase *et al.* 2002) demonstrated that depolarization increased the affinity between neuronal β-catenin and cadherin, and caused a redistribution of β-catenin from dendritic shafts to spines in a tyrosine-phosphorylation-dependent manner (Murase *et al.* 2002). To investigate whether Cdk5 is involved in this redistribution, time-lapse images were taken of neurons expressing EGFP–β-catenin. As shown in figure 2*a*,*b*(i), the application of roscovitine induced a redistribution of EGFP–β-catenin from dendritic shafts to spines, similar to that induced by KCl stimulation (Murase *et al.* 2002). To test if the redistribution induced by these two treatments shares similar mechanisms, we performed occlusion experiments. We first examined whether a single depolarization event produces a saturated level of redistribution. We depolarized neurons and then 90 min later depolarized them again. No further redistribution was evoked by the second depolarization (mean per cent fluorescence ± s.e.m. at 90 min after the second depolarization: total, $100.0 \pm 5.9\%$; spine, $103.5 \pm 8.1\%$; shaft, $97.9 \pm 2.3\%$, $n = 4$), indicating that the redistribution induced by the first depolarization is maximal and saturated. As shown in figure 2*a*,*b*(ii), a prior depolarization completely blocked the redistribution induced by a subsequent application of roscovitine. By contrast, neurons pre-incubated with roscovitine exhibited additional signficant redistribution by depolarization (figure 2*a*,*b*(iii)). These results suggest that Cdk5 inhibition contributes to depolarization-induced β-

catenin redistribution, but depolarization can also invoke Cdk5-independent pathways to bring about redistribution.

(c) *Point mutations of β-catenin Tyr-654 block regulation of cadherin–β-catenin affinity by Cdk5*

The redistribution of β-catenin induced by depolarization is completely prevented by the general tyrosine phosphatase inhibitor, orthovanadate (Murase *et al.* 2002). Although, orthovanadate has other enzymatic inhibitory effects (Cantley *et al.* 1977; Sargeant & Stinson 1979), an increased level of phosphorylated β-catenin is observed after incubation with orthovanadate (Ozawa & Kemler 1998), suggesting a direct effect on β-catenin phosphotyrosine levels. To determine initially whether the regulation of tyrosine phosphorylation is also involved in roscovitine-induced redistribution, we again used orthovanadate. The effect of roscovitine was completely blocked by orthovanadate, suggesting Tyr phosphorylation is critical for roscovitine-induced redistribution (figure 2*c*,*d*(i)). Because the phosphorylation of β-catenin Tyr-654 is known to regulate the affinity between β-catenin and cadherin (Roura *et al.* 1999), we tested the effect of two different point mutations of Tyr-654, one in which phosphorylation is mimicked (Y654E) and another in which phosphorylation is prevented (Y654F). Neither Y654E– nor Y654F–β-catenin–GFP mutants were redistributed after the application of roscovitine (figure 2*c*,*d*(ii,iii)). Together, these results strongly suggest that decreasing the phosphorylation level of Tyr-654 is a necessary step in the redistribution of EGFP–β-catenin caused by inhibiting Cdk5.

To test if the mutation of β-catenin Tyr-654 blocks the roscovitine-induced affinity increase between cadherin and β-catenin, we microinjected sindbis virus containing cDNA of either the wild-type, Y654E or Y654F mutant β-catenin into organotypic hippocampal slices. After 24–36 hours, the slices exhibited abundant expression of EGFP–β-catenin (figure 3*a*). The cell lysate from the infected slices was immunoprecipitated with an anti-GFP antibody, and blotted with either anti-β-catenin or anti-cadherin antibodies (figure 3*b*). No β-catenin or cadherin bands were observed from lysate prepared from non-infected slices (figure 3*b*, first lane). As expected, both the EGFP–β-catenin fusion proteins showed higher molecular weights (*ca.* 115 kDa) than the endogenous β-catenin (90 kDa). The amount of EGFP–β-catenin immunoprecipitated with anti-GFP was not affected by roscovitine treatment (figure 3*b*). In the case of the wild-type EGFP–β-catenin, the cadherin/EGFP–β-catenin ratio was significantly increased by roscovitine treatment (figure 3*b*,*c*). As expected, the Y654F mutant showed a higher basal affinity for cadherin when compared with the wild-type (compare the control bars in figure 3*c*). The affinity of the Y654F mutant was not further increased by the inhibition of Cdk5 (figure 3*b*,*c*). Alternatively, the Y654E mutant showed a lower basal affinity for cadherin (compare the control bars in figure 3*c*), which was also unaffected by roscovitine (figure 3*b*,*c*). These results are consistent with the redistribution studies and suggest that the action of the Cdk5 occurs upstream of the β-catenin Tyr-654 regulation.

Figure 2. (*a*,*b*) The application of roscovitine causes a movement of β-catenin from shafts to spines. (*a*) Time-lapse images of EGFP-β-catenin pre- and post-treatment. (i) EGFP–β-catenin treated with roscovitine; (ii) pre-depolarized then treated with roscovitine; and (iii) pre-treated with roscovitine then depolarized. Neurons were treated immediately after $t = 0$. Scale bar, 10 μm. (*b*) Relative fluorescence intensities. Spine region (violet), shaft region (orange) and total (blue). (i) Roscovitine ($n = 3$); (ii) roscovitine (pre-depolarized, $n = 5$); and (iii) depolarization (pre-treated with roscovitine, $n = 6$). (*c*,*d*) Point mutations at Tyr-654 block roscovitine-induced EGFP−β-catenin redistribution. (*c*) Time-lapse images of EGFP–β-catenin pre- and post-treatment. (i) EGFP–β-catenin treated with roscovitine in the presence of sodium orthovanadate; (ii) EGFP–Y654E–β-catenin treated with roscovitine; and (iii) EGFP–Y654F–β-catenin treated with roscovitine. Neurons were treated immediately after $t = 0$. Scale bar, 10 μm. (*d*) Relative fluorescence intensities. Spine region (violet), shaft region (orange), and total (blue). (i) EGFP–β-catenin treated with roscovitine in the presence of sodium orthovanadate, $n = 5$; (ii) EGFP–Y654E–β-catenin treated with roscovitine, $n = 4$; and (iii) EGFP–Y654F–β-catenin treated with roscovitine, $n = 4$.

4. DISCUSSION

(a) *Cdk5 regulates the affinity between cadherin and β-catenin in an activity-dependent manner*

Our results indicate that Cdk5 activity is significantly inhibited by synaptic activity. The mechanism by which neural activity regulates Cdk5 remains to be investigated. Here, we show that the degradation of the Cdk5 activator is not involved. It is known that phosphorylation of Cdk5 by a Tyr kinase, Abl, can increase Cdk5 activity (Zukerberg *et al.* 2000). However, very low levels of tyrosine phosphorylation of Cdk5 were detected in hippocampal neurons incubated with Tyr phosphatase inhibitor, and the amount of phosphorylation was not changed by depolarization (data not shown). A recent study has shown the interaction of an inhibitor protein with the Cdk5/p35 complex (Ching *et al.* 2002). One can speculate that such a protein is a synaptically regulated target.

Incubation of hippocampal slices with roscovitine caused a significant increase in β-catenin's affinity for cadherin. The association of β-catenin with cadherin is important for cadherin to achieve high adhesive activity. Therefore, our result suggests a role for Cdk5 in the regulation of cadherin activity in adult hippocampal neurons. Our recent study demonstrated that changes in affinity between β-catenin and cadherin can alter synaptic structure and function (Murase *et al.* 2002). Together with the inhibition of Cdk5 by depolarization, our results suggest an important role for Cdk5 in neural activity-induced synaptic modulation or synaptic plasticity. In fact, a recent study has shown that a transient increase of Cdk5 activity is required for associative learning (Fischer *et al.* 2002).

(b) *Phosphorylation of β-catenin Tyr-654 is a potential target of the affinity regulation by Cdk5*

Cdk5 is known to phosphorylate β-catenin Ser/Thr and regulate the association of β-catenin with presenilin 1

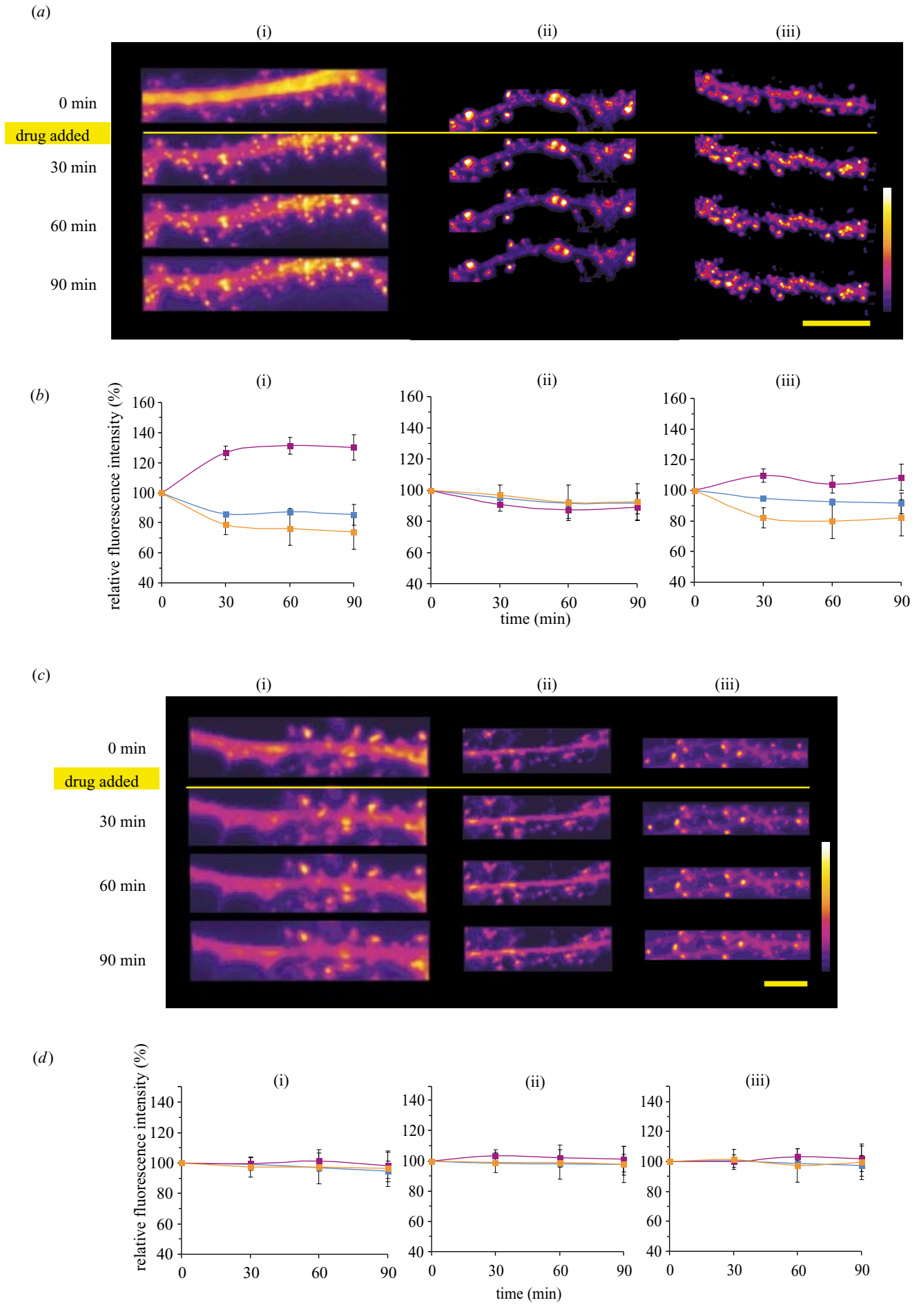

Figure 2. (*Caption on page 753.*)

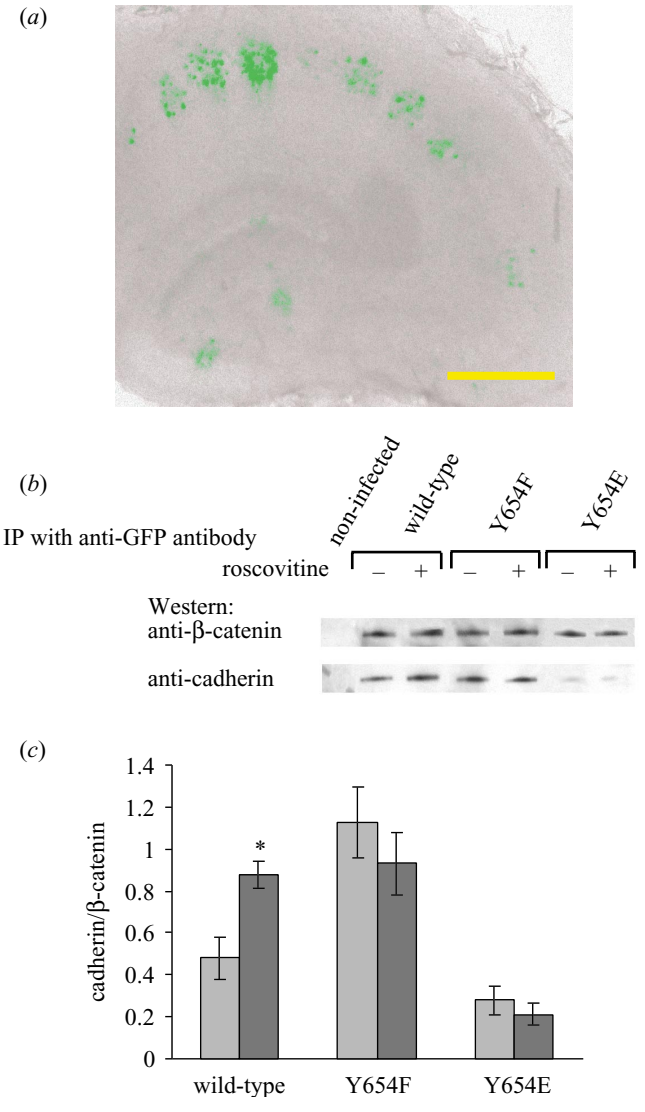

Figure 3. Point mutation at Tyr-654 block roscovitine-induced affinity increase for cadherin. (*a*) An organotypic hippocampal slice expressing EGFP–β-catenin. The DIC and fluorescence images were overlaid. EGFP–β-catenin was introduced via the microinjection of sindbis virus. Scale bar, 500 μm. (*b*) Immunoprecipitation analysis of roscovitine effect on cadherin–β-catenin affinity. Cell lysates from either the wild-type, Y654F- or Y654E-expressing slices were immunoprecipitated with an anti-GFP antibody, and blotted with either anti-β-catenin or anti-cadherin antibodies. Slices were pretreated for 3 hours with either the control medium or medium containing roscovitine. (*c*) Ratios of cadherin : β-catenin band intensities. The wild-type β-catenin showed a significantly increased ratio after roscovitine treatment, whereas Tyr-654 mutants do not (mean ± s.e.m., $n = 3$, wild-type; $^{*}p < 0.05$, Y654F and Y654E; n.s.). Dark shading, control; light shading, roscovitine.

(Kesavapany *et al.* 2001). However, phosphorylation of β-catenin Ser/Thr itself does not appear to change cadherin–β-catenin affinity, because the roscovitine-induced redistribution can be blocked by orthovanadate in the absence of any Ser/Thr phosphatase inhibitors. It is more likely that Cdk5 activity promotes β-catenin Tyr phosphorylation. Because the effect of roscovitine is blocked by mutations of Tyr-654, it is possible that Cdk5 regulates cadherin–β-catenin affinity through the activity of a Tyr kinase or phosphatase, whose target is β-catenin Tyr-654. The detailed molecular mechanism for how Cdk5 modulates phosphorylation of β-catenin Tyr-654 remains to be investigated. It is possible that phosphorylation of β-catenin Ser/Thr by Cdk5 induces the association of Tyr kinases to β-catenin (or the dissociation of Tyr phosphatases from β-catenin). Taken together, our results suggest that the depolarization-induced enhancement of cadherin–β-catenin affinity is at least partly achieved by Cdk5 inhibition, which results in the decreased phosphorylation level of β-catenin Tyr-654. Future studies will determine the contribution of β-catenin's localization to synaptic plasticity in hippocampal slices.

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GLOSSARY

ACSF: artificial cerebral spinal fluid
Cdk5: cyclin-dependent kinase 5
DIC: differential interference contrast
EGFP: enhanced green fluorescent protein
GFP: green fluorescent protein
HBS: HEPES buffered saline
L-LTP: late-phase long-term potentiation
LTP: long-term potentiation
m.p.v.: mean pixel value