Regulation of the Fusion Pore Conductance during Exocytosis by Cyclin-dependent Kinase 5*

Cyclin-dependent kinase 5 (Cdk5) is a serine/threonine kinase involved in synaptogenesis and brain development, and its enzymatic activity is essential for slow forms of synaptic vesicle endocytosis. Recent work also has implicated Cdk5 in exocytosis and synaptic plasticity. Pharmacological inhibition of Cdk5 modifies secretion in neuroendocrine cells, synaptosomes, and brain slices; however, the specific mechanisms involved remain unclear. Here we demonstrate that dominant-negative inhibition of Cdk5 increases quantal size and broadens the kinetics of individual exocytotic events measured by amperometry in adrenal chromaffin cells. Conversely, Cdk5 overexpression narrows the kinetics of fusion, consistent with an increase in the extent of kiss-and-run exocytosis. Cdk5 inhibition also increases the total charge and current of catecholamine released during the amperometric foot, representing a modification of the conductance of the initial fusion pore connecting the granule and plasma membrane. We suggest that these effects are not attributable to an alteration in catecholamine content of secretory granules and therefore represent an effect on the fusion mechanism itself. Finally, mutational silencing of the Cdk5 phosphorylation site in Munc18, an essential protein of the late stages of vesicle fusion, has identical effects on amperometric spikes as dominant-negative Cdk5 but does not affect the amperometric foot. Cells expressing Munc18 T574A have increased quantal size and broader kinetics of fusion. These results suggest that Cdk5 could, in part, control the kinetics of exocytosis through phosphorylation of Munc18, but Cdk5 also must have Munc18-independent effects that modify fusion pore conductance, which may underlie a role of Cdk5 in synaptic plasticity.

Exocytosis, the process of vesicle fusion and neurotransmitter release, is tightly regulated and requires inherent cellular mechanisms both to monitor and to adjust to changing secretory needs. Exocytosis proceeds through two alternative mechanisms: 1) full fusion, where the initial fusion pore expands, eventually collapsing into the target membrane and releasing all vesicular content, and 2) kiss-and-run exocytosis, where the fusion pore reverses, vesicle integrity is reformed, and partial release of content is achieved (1–5). Fine control over the choice of fusion mechanism may underlie presynaptic mechanisms of short term synaptic plasticity. Endocytosis also alternates between discrete modes of full membrane retrieval and kiss-and-run (6, 7), and it is likely that cells employ common regulatory pathways in the selection of vesicle-trafficking mode. Protein phosphorylation has been identified as a regulator of exocytosis (8, 9); activation of protein kinase C promotes kiss-and-run (10) through phosphorylation of Munc18, which inhibits its syntaxin binding affinity (11). The mode of endocytosis also is regulated by protein phosphorylation (12, 13); however, regulatory kinases common to both pathways have not been identified.

Cyclin-dependent kinase 5 (Cdk5) is a serine/threonine kinase that is ubiquitously expressed in its active form in neural tissue of the central nervous system (14). Although a member of the Cdk family, Cdk5 uniquely is not activated by cyclins and plays no obvious role in cell cycle pathways. Expression of active Cdk5 in the adult nervous system (15) has suggested a critical role in neuronal function. Substantial recent work has identified multiple diverse functions for Cdk5 including synaptogenesis (16, 17) and axonal targeting (18, 19), development of neurodegenerative diseases (20–22), neuronal cytoskeletal dynamics (23, 24), and endocytosis (13). Cdk5 is essential for promoting the slow mode of compensatory endocytosis by phosphorylating endocytotic proteins such as dynamin (25, 26). Cdk5 is a potential candidate as a common regulator of the mode of endo- and exocytosis because it also is implicated in exocytosis. The total amount of secretion is promoted by Cdk5 in neuroendocrine cells (27) and in pancreatic β-cells (28, 29). Cdk5, however, has the opposite effect in neurons. Pharmacological inhibition increases secretion in synaptosomes (30) and striatal slices (31). It is unknown whether Cdk5 can regulate late events in exocytosis such as a switch to kiss-and-run.

We investigated whether Cdk5 controls the mode of exocytosis by measuring the late stages of exocytosis using carbon fiber amperometry in adrenal chromaffin cells. Inhibition of Cdk5 by overexpression of a dominant-negative form (dnCdk5) increased quantal size and temporal parameters of individual amperometric spikes, indicative of a preferential switch toward full fusion events. Expression of dnCdk5 also enhanced flux through the initial fusion pore. Cdk5 overexpression shortened the kinetics of individual amperometric spikes, functionally analogous to that seen with protein kinase C phosphorylation.
of Munc18. Finally, overexpression of a Munc18 mutant (T574A) unphosphorylatable by Cdk5 mimicked the effects of dnCdk5 on amperometric spikes but not amperometric feet, supporting the hypothesis that Cdk5 regulation of exocytosis is not exclusively via phosphorylation of Munc18.

MATERIALS AND METHODS

Cell Culture and Transfection of Chromaffin Cells—Freshly isolated bovine adrenal chromaffin cells (32) were plated on non-tissue culture-treated 10-cm petri dishes and left overnight at 37 °C. Non-attached cells were resuspended in growth medium at a density of 1 × 10^7 cells/ml. Plasmids (encoding enhanced green fluorescent protein and one of Cdk5, dnCdk5, or T574A Munc18) were mixed and added at 20 μg/ml, and cells were electroporated using a Bio-Rad Gene Pulser II. Cells were immediately diluted to 1 × 10^6 cells/ml with fresh growth medium and maintained in culture for 3–5 days. The cDNAs for Cdk5 and P35 were gifts from Dr. J. H. Wang (Hong Kong University of Science and Technology) and were subcloned into a mammalian expression vector (pcDNA3.1). dnCdk5 (D144N) (28) was produced by site-directed mutagenesis to produce T574E and T574A mutants.

Expression of Plasmids in HeLa Cells—HeLa cells were transfected with 1 μg of pcDNA3.1 (controls) or the indicated plasmid using FuGENE transfection reagent (Roche Applied Science). After 72 h, the cells were lysed in 200 μl of SDS dissociation buffer. Samples were separated by SDS-PAGE, transferred to nitrocellulose, and probed with a monoclonal antibody against Cdk5 (Oncogene Research Products) or Munc18 (Oncogene Research Products) and subsequent figures, values shown are mean ± S.E. or as a box plot of median values. Significance was determined by using ANOVA using a Molecular Devices plate reader. Extent of binding was normalized to maximal binding.

Amperometric Recording—Electrophysiological recording conditions were as described previously (11, 34, 35). Briefly, cells were incubated in both buffer (139 mM potassium glutamate, 0.2 mM EGTA, 20 mM PIPES, 2 mM ATP, and 2 mM MgCl₂, pH 6.5) and a 5 μM–diameter carbon fiber was positioned in contact with a target cell. Exocytosis in cells was stimulated with a permeabilization/stimulation buffer (139 mM potassium glutamate, 20 mM PIPES, 5 mM EGTA, 2 mM ATP, 2 mM MgCl₂, 20 μM digitonin, and 10 μM free Ca²⁺, pH 6.5) pressure-induced fusion events from a glass pipette on the opposite side of the cell. Amperometric responses were monitored with a VA-10 amplifier (NPI Electronic, Tamm, Germany) and saved to computer using Axoscope 8 (Axon Instruments). Experiments were carried out in parallel on control and transfected cells from the same batch of cells. Transfected cells were identified by expression of enhanced green fluorescent protein. Previous studies in our laboratory have established that 95% of cells co-express proteins from both plasmids in the transfection (11, 36). Recordings were alternated between untransfected and transfected cells using the same carbon fiber to eliminate any effects of interfiber variability. For all analyses, data were derived from cells from multiple cell preparations.

Analysis—Amperometric data were exported from the Axoscope and subsequently analyzed using Origin (Microcal Software, Northampton, MA). Spikes were selected for analysis, provided that the spike amplitude was greater than 40 pA, to remove any confounding effects of diffusion by selecting fusion events not occurring directly underneath the end of the carbon fiber. Spike feet were defined from foot onset (time at which the amperometric current rose 2.5× above noise level) to spike onset (time at which the amperometric spike current began to rise, determined by differentiation of the amperometric trace). All of the data are shown as mean ± S.E. or as a box plot of median values. Significance was tested using nonparametric Mann-Whitney U test specifically because the data were nonparametrically distributed; thus, this was the appropriate statistical test.

RESULTS

Effect of Cyclin-dependent Kinase 5 on Total Secretion from PC12 Cells—Overexpression of P25, an activator protein of Cdk5, in adrenal chromaffin cells enhances secretion of growth hormone induced by 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) (27). DMPP is a nicotinic receptor agonist, and therefore induced secretion is dependent on levels of receptor activation and calcium influx, as well as late stage mechanisms of exocytosis. It has subsequently been shown that Cdk5 phosphorylates P/Q-type voltage-dependent calcium channels that are known to be present in neurosecretory cells and that this phosphorylation can alter calcium influx (30). Therefore, we tested whether the effect of Cdk5 on total amounts of exocytosis...
was independent of calcium entry by assaying secretion of growth hormone in digitonin-permeabilized PC12 cells (33). Cells were transfected with mammalian expression vectors encoding Cdk5, its activator protein P35, or a dominant-negative mutant, D144N (dnCdk5), along with a plasmid encoding growth hormone to allow assay of secretion from transfected cells. For all three constructs, overexpression of the protein had no effect on total levels of calcium-induced secretion (Fig. 1). To test the hypothesis that a concomitant increase in both Cdk5 and P35 was required to enhance secretion, both plasmids were co-transfected into cells with growth hormone, and the resultant extent of total secretion was assayed. Co-expression of Cdk5 and P35 also failed to elicit any increase in total growth hormone secretion (Fig. 1).

**Effect of Cdk5 on Individual Secretory Events in Adrenal Chromaffin Cells**—To test whether Cdk5 has an effect on the dynamics of individual secretory events, mammalian expression vectors encoding either wild type Cdk5 or dnCdk5 were transfected into cultured bovine adrenal chromaffin cells. Individual exocytotic events were monitored by carbon fiber amperometry following exposure of the cells to a mixture of digitonin and calcium to directly activate exocytosis (Fig. 2). Each transient current spike recorded by the carbon fiber electrode in amperometric mode represents a single secretory event. Previous work has shown that use of digitonin permeabilization does not modify the characteristics of exocytotic events in chromaffin cells (36, 37). The use of a cell-permeabilized system circumvents any subsidiary upstream effect on receptor activation or calcium channel kinetics. Recordings were alternated between untransfected cells and cells overexpressing either Cdk5 (Fig. 2A, i) or dnCdk5 (Fig. 2B, i) within the same dish to remove any potential effects of carbon fiber variability. Consistent with a lack of effect of Cdk5 on total amounts of secretion, the frequency of amperometric spikes was unaffected by either construct (Fig. 2, A, ii, and B, ii). Furthermore, the frequency distribution of individual fusion events did not appear to be altered by expression of Cdk5 or dnCdk5 (Fig. 2, A, iii, and B, iii).

Although the total number of exocytotic events and rate of secretion were unaffected by Cdk5, closer examination of indi-
individual spike parameters revealed that Cdk5 had a critical
effect on the kinetics of single fusion events. Although the total
charge of amperometric spikes was unaffected in cells overex-
pressing Cdk5 (Fig. 3A), the enzyme significantly increased the
timing of amperometric spikes. The half-
width (B), rise time (C), and fall time (D) of amperometric spikes were all signifi-
cantly decreased in cells expressing Cdk5. Spike parameters and statistical compar-
ison were taken from the same batch of cells as presented in Fig. 2A.

FIG. 3. Overexpression of Cdk5 speeds up individual exocytotic
events. A, Cdk5 did not affect the aver-
age amperometric spike charge. Cdk5
overexpression did, however, shorten the
timing of amperometric spikes. The half-
width (B), rise time (C), and fall time (D) of amperometric spikes were all signifi-
cantly decreased in cells expressing Cdk5.

Effect of Cdk5 on Fusion Pore Dynamics
— Transient current
prespikes preceding the amperometric spike proper are termed
"feet" and represent the steady-state flux of neurotransmitter
released through a stable fusion pore (38–40). To assess neu-

FIG. 4. Dominant-negative inhibi-
tion of Cdk5 increases quantal size
and slows individual exocytotic
events. A, dnCdk5 significantly in-
creased amperometric charge. dnCdk5
has the opposite effect to Cdk5 on the
temporal parameters of amperometry. The half-width (B), rise time (C), and full
time (D) of amperometric spikes were all increased. Spike parameters and statisti-
cal comparison were taken from the same batch of cells as presented in Fig. 2B.
rotransmitter flux through the fusion pore prior to full release, the feet of the amperometric spikes were analyzed (for examples of amperometric feet, see Fig. 6A). Amperometric feet are not ubiquitous events, instead occurring in 10–30% of spikes (38, 40). We found that Cdk5 overexpression or inhibition of activity by dnCdk5 did not alter the number of spikes with quantifiable feet (Fig. 6B). Closer examination of individual foot parameters, however, demonstrated that although Cdk5 overexpression had no obvious effect (Fig. 7A), dnCdk5 did modify the dynamics of the fusion pore (Fig. 7B). dnCdk5 significantly increased the total charge (or total number of catecholamine molecules released; Fig. 7B, i) and maximal current (Fig. 7B, iii) of the amperometric foot. There was no significant effect on foot duration in dnCdk5 cells (Fig. 7B, ii) suggesting that the conductance, but not the stability, of the fusion pore was affected.

**Mutation of Munc18 Phosphorylation Site for Cdk5**—The data above suggest a regulatory role for Cdk5 in fusion pore dynamics and during the late stages of exocytosis. Cdk5 is known to phosphorylate one protein implicated in the final steps of exocytosis, Munc18 (27, 41). Phosphorylation of Munc18 occurs at Thr-574 and results in a substantial reduction of the binding affinity of Munc18 for syntaxin. Phosphorylation reduces Munc18-syntaxin binding to 5% of wild type protein (41). We first investigated whether we could use a phosphomimetic mutation (T574E) to test whether Munc18 was the phosphorylation target for the effects of Cdk5 on fusion pore dynamics and the late stages of exocytosis. For example, we previously have used successfully a Munc18 phosphomimetic for protein kinase C phosphorylation, which decreased the binding affinity of Munc18 for syntaxin by 81% (42). However, the T574E mutation was not found to be phosphomimetic (Fig. 8), causing only a minor 2.5% reduction in binding affinity to syntaxin. As Cdk5 is constitutively active, we could alternatively investigate the involvement of Munc18 as a Cdk5 substrate by mutation of the phosphorylation site to a non-phosphorylatable residue such as alanine. This was also not phosphomimetic for syntaxin binding (Fig. 8), although there was a similar minor 2.5× reduction in binding affinity as was seen with the T574E mutation.

As we could not test a phosphomimetic Munc18 to mimic overexpression of Cdk5, we instead investigated the effect of occlusion of the Cdk5 phosphorylation site of Munc18 on individual secretory events and assayed with carbon fiber amperometry (Fig. 9A). This is functionally analogous to the removal of endogenous Cdk5 when overexpressing the dnCdk5 construct. Overexpression of T574A Munc18 had an identical effect on amperometric spike parameters as the dnCdk5 construct, increasing quantal size by 33% (Fig. 9A). Additionally, the kinetics of exocytosis were significantly slowed, resulting in a longer rise time (Fig. 9C), half-width (Fig. 9D), and fall time (Fig. 9E) of the amperometric spike. Consistent with the effects of Cdk5 and dnCdk5 constructs, the amplitude (Fig. 10A),
frequency (Fig. 10B), and the time course (Fig. 10C) of individual amperometric spikes were not affected by T574A expression. Not all functional effects of dnCdk5, however, were mimicked in cells expressing T574A Munc18. Analysis of amperometric feet in T574A-expressing cells revealed that the Munc18 mutation had no effect on neurotransmitter flux through the fusion pore (Fig. 11). There were no significant differences in foot charge (Fig. 11, i), duration (Fig. 11, ii), or maximum current (Fig. 11, iii) of T574A in control spikes. Foot frequency was unaffected by expression of Munc18 T574A (p = 0.92). Taken together, these results lend support to the hypothesis that Munc18 is only one of the potential phosphorylation targets for the regulation of the late stages of single exocytotic events by Cdk5.

**DISCUSSION**

Exocytosis and endocytosis are tightly coupled processes, and the modulation of one requires coordination with regulation of the other (43). Protein phosphorylation is a known modulator of both exo- and endocytosis and is implicated in the selection of fusion mode (8, 9, 13). We have examined the role of phosphorylation of Munc18 by protein kinase C in the regulation of exocytosis in chromaffin cells by using carbon fiber amperometry (11). Changes in the kinetic parameters of the amperometric spikes could be because of multiple factors, but we have argued that these can reflect a switch between modes of exocytosis with kiss-and-run exocytosis giving only partial granule emptying and reduced quantal size (11). Kiss-and-run exocytosis has been well established in neuroendocrine cells (9, 44, 45) and more recently convincingly demonstrated at central synapses (46, 47). Partial release from chromaffin granules has been shown to be a common event in a recent study based on imaging of the release of a fluorescent granule content protein (5). Both protein kinase C activation by phorbol ester treatment (10) and expression of a protein kinase C phosphomimetic Munc18 (11) induce faster exocytotic kinetics and a smaller quantal charge/secretory event consistent with kiss-and-run exocytosis. In agreement with our interpretation, quantal size is also altered in the null mutant of the *Caenorhabditis elegans* orthologue of Munc18 (48). We demonstrate here that Cdk5 regulates the mode of exocytosis in a similar manner to protein kinase C activation. Overexpression of Cdk5 narrowed individ-
ual secretory events consistent with a shift toward kiss-and-run exocytosis, whereas inhibition of Cdk5 increased quantal size and kinetics, indicating an increase in full fusion events. Interestingly, Cdk5 is also essential for the continuation of full clathrin-mediated compensatory endocytosis (25, 26). Cdk5 phosphorylates key slow endocytosis proteins such as dynamin and amphiphysin, and dominant-negative inhibition of Cdk5 blocks slow synaptic vesicle endocytosis. Dynamin itself can regulate the switch to kiss-and-run exocytosis (36, 49). The critical involvement of Cdk5 phosphorylation targets in exocytosis and endocytosis may implicate Cdk5 as a putative participant in a common intracellular pathway regulating both physiological processes.

Cdk5 has been implicated in synaptic transmission and plasticity, both pre- and postsynaptically, although discrepancies exist in the directional effect of Cdk5 on neurotransmitter release. Pharmacological inhibition of Cdk5 activity decreases total secretion (27, 28), and overexpression of Cdk5 increases exocytosis (29). In contrast, Cdk5 inhibition has a positive effect on neurotransmitter release in synaptosomes (30) and in striatal slices (31). In this study we show that contrary to the strong effects of Cdk5 on the late stage mechanisms governing individual fusion events, neither overexpression nor inhibition of Cdk5 had any effect on total amounts of secretion or frequency of secretory events in permeabilized cells. It is known that Cdk5 phosphorylates P/Q-type voltage-dependent calcium channels and that inhibition by roscovitine alters calcium influx (30). We would suggest that the lack of effect on total secretion demonstrated here is because of potentially confounding effects on excitation and calcium influx being circumvented by the utilization of a cell permeabilization strategy. Similar to Cdk5 regulation of synaptic transmission, the effects on short term plasticity are also conflicting. Paired pulse facilitation is increased in hippocampal slices by pharmacological inhibition of Cdk5 (30), whereas paired pulse facilitation is decreased in a mouse knockout of the Cdk5 activator protein P35 (50). The regulation of the extent and timing of individual secretory events by Cdk5 may be involved in the presynaptic mechanisms underlying Cdk5-dependent regulation of synaptic plasticity.

The effects of Cdk5 on amperometric spikes could potentially be the result of an alteration to catecholamine loading of granules. We can rule out this possibility for the following reasons. First, pharmacologically increasing or decreasing catecholamine loading, with L-DOPA or reserpine, respectively, causes an increase or decrease in amperometric spike amplitude (34, 51, 52). However, there was no effect of either Cdk5 or dnCdk5 expression on individual spike amplitude. Moreover, foot frequency is inversely related to catecholamine content; increasing catecholamine content decreases foot frequency (40). Manipulation of Cdk5, however, did not affect foot frequency. We therefore conclude that the effects of Cdk5 demonstrated here are indeed the result of an effect on the late stages of exocytosis.

Our data suggest that Cdk5 may regulate exocytosis in part through its phosphorylation of Munc18, as occlusion of the Cdk5 phosphorylation site of Munc18 has the same effect on amperometric spikes as Cdk5 inhibition. Alterations to the late stage kinetics of exocytosis and quantal size are unlikely to be achieved through the regulation of endocytic proteins (25, 26, 53), which are involved downstream of the exocytotic mechanism and would not account for changes to the amperometric...
spike rise time demonstrated here. A survey of the known key components and regulators of the exocytotic machinery indicates that only Munc18 and Munc13 have recognizable consensus sites for Cdk5 phosphorylation. It is therefore possible that Munc18 is an effector for the regulation of the late stages of exocytosis by Cdk5. In light of a structural inaccessibility of the Cdk5 phosphorylation site of Munc18 (54) and a lack of effect of the T574A mutation on amperometric feet, other presynaptic target effectors for Cdk5 must also be functionally important.

Precisely how phosphorylation of Munc18 by Cdk5 would affect the final steps of exocytosis is not clear. Despite extensive research, the actual function of Munc18 during exocytosis has remained controversial perhaps because of Munc18 having functions in multiple steps in exocytosis. Knock-out studies have implicated an early stage role during docking at the neuromuscular junction in C. elegans (48) and in the mouse chromaffin cell (55), whereas central synapses in the mouse knockout (56) and overexpression studies in the chromaffin cell (11, 34) have pointed to a critical late stage role for Munc18. Phosphorylation of Munc18 by Cdk5 could modulate exocytosis via the reduced affinity of Munc18 for syntaxin (41). A postulated function of Munc18 is to act as a negative clamp on fusion by binding tightly to syntaxin and limiting its participation in

the SNARE complex (57–59), thereby regulating exocytosis. Alternatively, excess Munc18 may have a subsequent late stage effect downstream of its initial syntaxin interaction, either directly or through other proteins, such as Doc2 (60), Mint (61), granuphilin (62), or phospholipase D (63). At present, the ultimate function(s) for Munc18 during the late stages of exocytosis remain unclear.

The amperometric foot has been suggested to represent the release of catecholamine via a stable but briefly lived fusion pore prior to the main exocytotic step (1, 38, 64). The duration of the foot, representative of fusion pore stability, can be modified by long term alterations to the expressed isoforms of specific presynaptic proteins, such as synaptotagmin (39). Cdk5 inhibition, however, increased the size of the amperometric foot without affecting foot duration suggesting that dnCdk5 increases fusion pore conductance. Specific mutations within the transmembrane segment of syntaxin can reduce fusion pore conductance, and thus, syntaxin has been postulated to form in part the fusion pore itself (65). Although it is unlikely that Cdk5 itself is involved in pore formation, the enzyme does indeed bind syntaxin directly (41) and may influence pore conductance through this protein interaction. A lipidic connection between vesicle and plasma membranes is thought to impart great stress on the fusion pore, causing a collapse of the vesicle into the plasma membrane (66). As Cdk5 inhibition also promotes a switch to full fusion events, it is a tempting hypothesis that larger fusion pore flux increases the probability of full

---

**Fig. 10.** Spike amplitude and the number of exocytotic events are not altered in cells expressing T574A mutation of Munc18. A, amplitude of individual amperometric spikes was identical in control and T574A cells. Spikes were analyzed from the same data presented in Fig. 9. B, amperometric spike frequency is unaltered in cells expressing T574A. C, T574A overexpression had no apparent effect on the frequency time course of amperometric spikes.

**Fig. 11.** Occluding Cdk5 phosphorylation of Munc18 does not affect amperometric foot parameters. I, overexpression of Munc18 (T574A) had no effect on (i) total charge, (ii) duration, or (iii) maximal current of amperometric feet in adrenal chromaffin cells.
Cdk5 Regulates the Late Stages of Exocytosis

expansion and thus full fusion. Such a hypothesis would imply that the size of the pore and its duration are both critical determining factors in the switch from kiss-and-run to full fusion.

Inhibition of Cdk5 activity by expression of the dominant-negative mutant produced an increase in the quantal size of the amperometric spikes, slower termination of the release event (inset, half-life), and an increase in the maximum current during the prespike foot. These effects, although statistically significant, were relatively small; so what could be their physiological significance? The most likely relevance of these effects alone or in combination would be from such changes occurring during synaptic vesicle exocytosis under the influence of constitutive Cdk5 activity. It has been speculated that small changes in transmitter flux through a fusion pore or in quantal size could determine whether or not the synapses of the neurotransmitter are "silent" depending on whether the concentration of glutamate rises rapidly enough in the synaptic cleft before its clearance to activate the low affinity α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors (67, 68). Indeed small (~28%) changes in quantal size have been measured as a mechanism underlying paired pulse depression (69).

The kinetics of release from synaptic vesicles cannot be reported as a mechanism underlying paired pulse depression (69). The kinetics of release from synaptic vesicles cannot be determined factors in the switch from kiss-and-run to full exo- and endocytosis. In conclusion, we have shown that Cdk5 can regulate exocytosis and modify the fusion pore itself. Overexpression of a mutant form of Munc18, non-phosphorylatable by Cdk5, only partially mimics the effects of dominant-negative inhibition of Cdk5 on single exocytic events supporting the hypothesis that Cdk5 regulates secretion through multiple presynaptic targets, including Munc18. These results may be significant in indicating a mechanism for short term regulation of the kinetic mode of exocytosis through protein phosphorylation and impair Cdk5 in a common mechanism for coordinated control over exo- and endocytosis.

REFERENCES

1. Alvarez de Toledo, G., Fernandez-Chacon, R., and Fernandez, J. M. (1993) Nature 361, 554 – 559
2. Albillos, A., Dernick, G., Horstmann, H., Almers, W., Alvarez de Toledo, G., and Turner, K. M. (1999) J. Cell Sci. 112, 1653 – 1658
3. Barclay, J. W., Craig, T. J., Fisher, R. J., Ciufo, L. F., Evans, G. J. O., Morgan, A. E., and Burgoyne, R. D. (2000) J. Neurosci. 20, 5354 – 5358
4. Shannon, A., Zhang, L., Fisher, R. J., Washbourne, P., Wilson, M. C., and Burgoyne, R. D. (2001) J. Neurosci. 21, 9377 – 9387
5. Graham, M. E., Washbourne, P., Wilson, M. C., and Burgoyne, R. D. (2001) J. Cell Sci. 114, 4377 – 4387
6. Graham, M. E., O'Callaghan, D. W., McMahon, H. T., and Burgoyne, R. D. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 1214 – 1219
7. Chow, R. H., von Ruden, L., and Neher, E. (1992) Nature 356, 60 – 63
8. Wang, C.-T., Grishanin, R., Earles, C. A., Chang, P. Y., Martin, T. F. J., and Chen, S. (2003) Biochim. Biophys. Acta 1597, 111 – 115
9. Sombers, L. A., Hancher, H. J., Colliver, T. L., Wittenberg, N., Cans, A. O., Arbault, S., Amatore, C., and Ewing, A. G. (2004) J. Neurosci. 24, 303 – 309
10. Lee, S. Y., Wenk, M. R., Kim, Y., Nairn, A. C., and De Camilli, P. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 127 – 132
11. Gellens, F. E., Dossous, M. M., and Zijlstra, D. (1993) Nature 364, 507 – 509
12. Ales, E., Tabares, L., Poyato, J. M., Valero, V., Lindau, M., and Alvarez de Toledo, G. (1999) Nat. Cell Biol. 1, 40 – 44
13. Chen, T. K., Kwon, Y. T., Brunson, R., Dikkes, P., Li, E., and Tsai, L.-H. (1997) Neuron 18, 29 – 42
14. Kwon, Y. T., Tsai, L.-H., and Crandall, J. E. (1999) J. Comp. Neurol. 415, 418 – 424
15. Connell-Crowley, L., de la Monte, S., and Gygi, S. (2000) J. Neurosci. 20, 859 – 869
16. Patrick, G. N., Zukerberg, L., Mikoshiba, K., Takei, K., and Tsai, L.-H. (2000) Science 287, 485 – 488
17. Connell-Crowley, L., de la Monte, S., and Gygi, S. (2000) J. Neurosci. 20, 859 – 869
18. Kwon, Y. T., Tsai, L.-H., and Crandall, J. E. (1999) J. Comp. Neurol. 415, 418 – 424
19. Connell-Crowley, L., de la Monte, S., and Gygi, S. (2000) J. Neurosci. 20, 859 – 869
20. Patrick, G. N., Zukerberg, L., Mikoshiba, K., Takei, K., and Tsai, L.-H. (2000) Science 287, 485 – 488
21. Connell-Crowley, L., de la Monte, S., and Gygi, S. (2000) J. Neurosci. 20, 859 – 869
22. Abuhasan, M., Williams, R. A., Jakowski, A., Kowz, K. P., McCarthy, S., Coskun, T., Carlo, A., Seymour, P. A., and Burkhardt, J. E., Nelson, R. B., and McNeish, J. D. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 2910 – 2915
23. Hanes, J., Karsch, S., and Ryu, S. H. (2004) J. Neurosci. 24, 1897 – 1906
24. Misura, K. M. S., Bock, J. G., Lyons, C., Scheller, R. H., and Wies, W. I. (2000) Nature 404, 355 – 362
25. Voets, T., Toonen, R., Brian, E. C., de Wit, H., Moser, T., Betting, J., Sudhoff, T. C., Neher, E., and Verhave, M. (2001) Nature 411, 581 – 589
26. Verhage, M., Vries, K. J., Roshol, H., Burbach, J. P. H., Gispen, W. H., and Verheijen, R. (2004) J. Neurosci. 24, 1837 – 1846
27. Groblewski, E., Pevsner, J., and Xu, T. (2000) J. Biol. Chem. 275, 43733 – 43738
28. Mikkola, K. H., Fischer, R. J., and Burgoyne, R. D. (2000) Science 287, 485 – 488
29. Zhang, L., Fisher, R. J., Washbourne, P., Wilson, M. C., and Burgoyne, R. D. (2001) J. Cell Sci. 114, 4377 – 4387
30. Tomizawa, K., Ohta, J., Matsushita, M., Moriwaki, A., Li, S.-T., Takei, K., and Tsai, L.-H. (2000) Neuron 22, 259 – 267
31. Cherrigu, K., Svenningson, P., and Greengard, P. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 2191 – 2196
32. Barclay, J. W., Craig, T. J., Fisher, R. J., and Burgoyne, R. D. (1997) J. Neurochem. 69, 559 – 567
Regulation of the Fusion Pore Conductance during Exocytosis by Cyclin-dependent Kinase 5

Jeff W. Barclay, Marcos Aldea, Tim J. Craig, Alan Morgan and Robert D. Burgoyne

J. Biol. Chem. 2004, 279:41495-41503.
doi: 10.1074/jbc.M406670200 originally published online July 23, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M406670200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 69 references, 33 of which can be accessed free at
http://www.jbc.org/content/279/40/41495.full.html#ref-list-1