Brain Myosin-V, a Calmodulin-carrying Myosin, Binds to Calmodulin-dependent Protein Kinase II and Activates Its Kinase Activity*

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Myosin-V, an unconventional myosin, has two notable structural features: (i) a regulatory neck domain having six IQ motifs that bind calmodulin and light chains, and (ii) a structurally distinct tail domain likely responsible for its specific intracellular interactions. Myosin-V copurifies with synaptic vesicles via its tail domain, which also is a substrate for calmodulin-dependent protein kinase II. We demonstrate here that myosin-V coinmunoprecipitates with CaM-kinase II from a Triton X-100-solubilized fraction of isolated nerve terminals. The purified proteins also coinmunoprecipitate from dilute solutions and bind in overlay experiments on Western blots. The binding region on myosin-V was mapped to its proximal and medial tail domains. Autophosphorylated CaM-kinase II binds to the tail domain of myosin-V with an apparent Kd of 7.7 nM. Surprisingly, myosin-V activates CaM-kinase II activity in a Ca2+-dependent manner, without the need for additional CaM. The apparent activation constants for the autophosphorylation of CaM-kinase II were 10 and 26 nM, respectively, for myosin-V versus CaM. The maximum incorporation of 32P into CaM-kinase II activated by myosin-V was twice that for CaM, suggesting that myosin-V binding to CaM-kinase II entails alterations in kinetic and/or phosphorylation site parameters. These data suggest that myosin-V, a calmodulin-carrying myosin, binds to and delivers CaM to CaM-kinase II, a calmodulin-dependent enzyme.

The primary intracellular receptor for Ca2+ in neuronal cells is calmodulin, which mediates the calcium signal by reversible, Ca2+-regulated binding to many target enzymes, which include the calmodulin-dependent protein kinases (reviewed in Ref. 1). Recently, unconventional myosins that carry calmodulins as light chains have been identified in nervous tissue and implicated in neuronal processes such as phototransduction (21). CaMKII is an abundant protein in brain with a broad substrate specificity. It associates with the actin cytoskeleton (18), as well as with synaptic vesicles (19) and postsynaptic densities (20), and is involved in neuronal processes such as neurotransmitter synthesis and release, ion channel regulation, and long-term potentiation (reviewed in Refs. 1 and 21). Its kinase activity is regulated by Ca2+/calmodulin and autophosphorylation in a complex manner (see Ref. 1). In a cellular milieu, how CaMKII is regulated, what it phosphorylates, and when may be determined in part by the subcellular colocalization of the participating components, including calmodulin as well as specific substrates. A general role for the calmodulin-carrying, unconventional myosins in the subcellular localization of calmodulin has been suggested (22).

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In this paper we present biochemical evidence, which suggests that BM-V can bind to and deliver calmodulin to a calmodulin-requiring enzyme, CaMKII. We show that CaMKII co-

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immunoprecipitates with BM-V from a Triton X-100-soluble extract of synaptosomes. Furthermore, the purified proteins bind to each other in both immunoprecipitation and overlay experiments. We use the overlay technique to determine the binding affinity and to map the binding site(s) on the BM-V molecule. Finally, we show that both the autophosphorylating activity of CaMKII and substrate phosphorylation of BM-V are activated in this complex.

**EXPERIMENTAL PROCEDURES**

**Materials**—[γ-32P]ATP (5000 Ci/mmol) was purchased from Amersham Pharmacia Biotech and NEN Life Science Products. Electrophoresis chemicals, molecular mass standards, imidazole, ATP (grade II), EDTA, EGTA, phenol, glycerol, maltose, IPTG, diithiothreitol, ampicillin, tetracycline, chloramphenicol, apritin, benzamidine, and lysozyme were purchased from Sigma. Nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate were obtained from Sigma or Promega. Anti-rabbit and anti-mouse IgGs conjugated to alkaline phosphatase and anti-rabbit and anti-mouse IgGs conjugated to horseradish peroxidase were purchased from Sigma. 5-bromo-4-chloro-3-indolyl phosphate were obtained from Sigma or Promega. Anti-rabbit and anti-mouse IgGs conjugated to alkaline phosphatase and anti-rabbit and anti-mouse IgGs conjugated to horseradish peroxidase were obtained from Sigma.

**Preparation of BM-V and CaMKII**—BM-V was purified from chick brain essentially as described by Cheney (24). Approximately 8 calmodulins copurify per BM-V with this procedure (14), which includes several precipitation steps and two chromatography columns, indicating their tight association with BM-V. Final fractions containing pure BM-V were pooled, dialyzed against 20 mM imidazole-HCl, pH 7.4 (containing 75 mM KCl, 2.5 mM MgCl2, 0.1 mM EDTA, 1 mM DTT), and stored up to 2 weeks at 4 °C or with 30% sucrose at -20 °C. CaMKII was purified from bovine brain either fresh or frozen, by the method of Gopalkrishna and Anderson (25), followed by purification on HPLC to remove low molecular weight contaminants, as follows: 0.5 ml of calmodulin (0.2 ml in 26 ml HEPES, pH 7.2, containing 0.5 mM EGTA) from the Gopalkrishna and Anderson preparation was loaded onto a MonoQ HR 5/5 column (Amersham Pharmacia Biotech) and eluted with a linear gradient of NaCl (50–350 mM in 10 ml) over 40 min at 8 ml/min; 485–497 ml fractions were collected in 2 ml fractions. CaMKII eluted at about 495 ml NaCl and is separated from a closely pre-eluting contaminant by judicial fraction collection and reapplication to the column. The final preparation of calmodulin is highly purified as judged by Coomassie Blue and silver staining on low molecular weight gels.

**Purification of Proteins**—Myosin-V was purified from chick brain essentially as described by Cheney (24). Approximately 8 calmodulins copurify per BM-V with this procedure (14), which includes several precipitation steps and two chromatography columns, indicating their tight association with BM-V. Final fractions containing pure BM-V were pooled, dialyzed against 20 mM imidazole-HCl, pH 7.4 (containing 75 mM KCl, 2.5 mM MgCl2, 0.1 mM EDTA, 1 mM DTT), and stored up to 2 weeks at 4 °C or with 30% sucrose at -20 °C. CaMKII was purified from bovine brain either fresh or frozen, by the method of Gopalkrishna and Anderson (25), followed by purification on HPLC to remove low molecular weight contaminants, as follows: 0.5 ml of calmodulin (0.2 ml in 26 ml HEPES, pH 7.2, containing 0.5 mM EGTA) from the Gopalkrishna and Anderson preparation was loaded onto a MonoQ HR 5/5 column (Amersham Pharmacia Biotech) and eluted with a linear gradient of NaCl (50–350 mM in 10 ml) over 40 min at 3 ml/min; 485–497 ml fractions were collected in 2 ml fractions. CaMKII eluted at about 495 ml NaCl and is separated from a closely pre-eluting contaminant by judicial fraction collection and reapplication to the column. The final preparation of calmodulin is highly purified as judged by Coomassie Blue and silver staining on low molecular weight gels.

**Cloning and Expression of Fusion Proteins**—Molecular cloning techniques used were essentially as described by Sambrook et al. (27). The subcloning of the head, neck, and complete tail domains of chicken BM-V in fusion with maltose-binding protein (MBP; 42 kDa) in the pHi902 vector was described by Espejo et al. (13). The medial tail domain was subcloned in fusion with glutathione S-transferase (GST; 27.5 kDa) in the pGEX vector (Amersham Pharmacia Biotech). The protein visualization, blotting using appropriate antibodies. The sequence encoding the globular tail was also derived from the 32a crystal structure of the virus. The protein was stored at -20 °C in 30% sucrose.
the cycle of events of synaptic transmission (reviewed in Ref. 34). Thus, these results suggest that BM-V may also be an eventual component of these supramolecular structures. **Purified CaMKII Coimmunoprecipitates with BM-V or Its Bacterially Expressed Tail Domain**—To assay for direct binding between BM-V and CaMKII, we performed in vitro coimmunoprecipitation experiments from a dilute mixture of the two purified proteins using the BM-V tail antibodies (Fig. 3A). Coimmunoprecipitation of CaMKII and BM-V was observed in the absence of ATP (reaction 2), no phosphorylation condition as well as under conditions where autophosphorylation was promoted (reaction 3). Both non-phosphorylated (seen in lanes 2p and 3p) and phosphorylated forms of αCaMKII (the slower migrating band recognized by the αCaMKII antibody seen only in lane 3p) were detected. Controls demonstrated that the precipitation of CaMKII in this assay was dependent on the presence of both BM-V and the BM-V tail antibodies. Similarly, the experiment.
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CaMKII coimmunoprecipitated with the bacterially expressed tail domain (Fig. 3B). Under conditions where autophosphorylation of CaMKII was promoted (reaction 4, note the requirement for calmodulin), it appeared that the phosphorylated α-subunit (based on electrophoretic mobility) was the principal component that coimmunoprecipitated with the tail domain (lane 4p).

To permit direct demonstration of the incorporation of $^{32}$P into the phosphorylated species, reaction mixtures of BM-V and CaMKII, diluted 2- and 8-fold in relation to the CaMKII concentration of Fig. 3A, were incubated for 1 min at 37°C upon addition of 10 μM [$\gamma$-$^{32}$P]ATP. The reaction was then diluted to 3 ml containing 1 μCi non-radioactive ATP and immunoprecipitation performed as described above. Autoradiography of the immunoprecipitated proteins separated on a SDS-PAGE gel (Fig. 4) demonstrated that (a) the α- and β-subunits of CaMKII were autophosphorylated and BM-V phosphorylated, and (b) the phosphorylated species were coimmunoprecipitated by tail antibodies. The relative intensity of the bands was not affected by 4-fold dilution of substrate and enzyme in the kinase reaction, suggesting that the phosphorylated species were effectively bound during the reaction and fully immunoprecipitated under these dilute conditions.

CaMKII Binds to the BM-V Heavy Chain and Its Bacterially Expressed Tail Domain; BM-V Binds to Both α and β Subunits of CaMKII—To further characterize the binding between BM-V and CaMKII, we used the “overlay” technique where Western blots of protein samples were incubated with protein ligand, followed by detection of binding by ligand-specific antibodies. When a Western blot of purified BM-V or of the MBP-tail fusion protein expressed in bacteria was incubated with purified CaMKII, its binding to the BM-V heavy chain and to the tail domain was detected by the CaMKII monoclonal antibody (Fig. 5, lanes 2 and 5). Reciprocally, when a blot of CaMKII was incubated with purified BM-V, both α and β subunits of CaMKII were detected by BM-V tail antibodies (Fig. 5, lane 8). These data strongly suggest that there is direct binding between these two proteins.

Autophosphorylated CaMKII Binds to the BM-V Whole Tail Domain with High Affinity, and the Binding Site Is Centered around the Proximal Tail Region—In order to estimate the affinity of the CaMKII binding to BM-V, we developed a method of ligand detection on the immobilized tail domain with the overlay technique using autophosphorylated [$^{32}$P]CaMKII as the ligand probe. As shown in Fig. 6A, analysis of the overlaid blot by phosphor image scanning demonstrated that [$^{32}$P]CaMKII was bound to the immobilized tail at incubation concentrations in the nanomolar range. The data from a typical experiment, plotted in Fig. 6B, showed an apparently hyperbolic increase in [$^{32}$P]CaMKII binding with increased concentration. The reciprocal plot of the data (inset) gave an apparent $K_d$ of 7.7 nM. Four equivalent experiments gave $K_d$ values that ranged from 1.1 to 7.7 nM. In order to compare the binding of non-phosphorylated versus autophosphorylated CaMKII,
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CaMKII and BM-V showed Ca\(^{2+}\) tions (Fig. 8). The surprising result was that a mixture of proteins under several conditions of [32P]CaMKII to recombinant proteins corresponding to several regions of BM-V (Fig. 7A). Strong binding was observed on the whole tail domain (lane 8) and the proximal tail segment (lane 5). Weaker, but clearly detectable binding was observed for the medial tail region (lane 6). No binding was detected over the head (labeled as the globular tail (lane 7) segments, or over the GST or MBP proteins alone or proteins in the non-induced bacterial extract. We also have detected very weak binding to the expressed neck domain in separate experiments (data not shown), but due to difficulties in the bacterial expression of this region, we have not obtained clearly comparative results. These composite data are illustrated on a linear map of BM-V (Fig. 7B) and suggest that the binding site for CaMKII is centered around the proximal tail region with some participation from adjacent regions.

**Fig. 5.** The reciprocal binding of BM-V and CaMKII is shown by an “overlay” assay. Western blots of BM-V, MBP-Tail purified on an amylose column and CaMKII (CKII) were incubated either with CaMKII (lanes 2 and 5), with BM-V (lane 8) or with blocking solution only (lanes 1, 3, 4, 6, 7, and 9) as described under “Experimental Procedures.” Lane 10 is a Coomassie Blue-stained gel of the purified CaMKII used in these studies. The blots were probed with a monoclonal antibody against the α-subunit of CaMKII (lanes 1, 2, 4, 5, and 9) or with BM-Tail antibodies (lanes 3, 6, 7, and 8). The positions of BM-V heavy chain (hcBM-V), undegraded fusion protein (MBP-Tail) and α and β subunits of CaMKII (CKII) are indicated by arrowheads to the right of the figure.

equivalent overlay experiments were done except that they were probed with anti-αCaMKII, followed by enzymatic detection (Fig. 6C). The result demonstrated that autophosphorylated CaMKII bound much more strongly than non-phosphorylated CaMKII.

Based on these experiments, we attempted to map the binding site on the BM-V molecule by examining the binding of [32P]CaMKII to recombinant proteins corresponding to several regions of BM-V (Fig. 7A). Strong binding was observed on the whole tail domain (lane 8) and the proximal tail segment (lane 5). Weaker, but clearly detectable binding was observed for the medial tail region (lane 6). No binding was detected over the head (labeled as the globular tail (lane 7) segments, or over the GST or MBP proteins alone or proteins in the non-induced bacterial extract. We also have detected very weak binding to the expressed neck domain in separate experiments (data not shown), but due to difficulties in the bacterial expression of this domain, we have not obtained clearly comparative results. These composite data are illustrated on a linear map of BM-V (Fig. 7B) and suggest that the binding site for CaMKII is centered around the proximal tail region with some participation from adjacent regions.

**BM-V Activates the Protein Kinase Activity of CaMKII without Requirement for Exogenous Calmodulin**—Previous work from our laboratory showing that BM-V is a substrate for CaMKII (17) was confirmed here in Fig. 4 using purified proteins. In order to better characterize this activity in terms of Ca\(^{2+}\) and calmodulin requirements, we performed phosphorylation reactions with the purified proteins under several conditions (Fig. 8). The surprising result was that a mixture of CaMKII and BM-V showed Ca\(^{2+}\)-dependent autophosphorylation of the kinase subunits and substrate phosphorylation of BM-V without requirement for exogenous calmodulin. Since this activity was abolished by micromolar concentrations of the calmodulin antagonist, trifluoperazine, these data suggest that BM-V, which has calmodulin light chains bound to its neck domain, was contributing one or more of its calmodulins to the regulatory domain of CaMKII. The immunoprecipitation of BM-V from aliquots used in kinase activation experiments resulted in the diminution of kinase activity (data not shown),
thus indicating that the presence of native BM-V was necessary for the full activation effect on CaMKII.

In order to quantitate this activation, the incorporation of $^{32}$P into CaMKII induced by BM-V was determined. The time course of the incorporation was not linear under the conditions studied (Fig. 9) and, in fact, suggested the occurrence of a burst of autophosphorylating activity within 15 s of initiation of the reaction. We therefore used the shortest reaction time attainable in practice (15 s) to determine the effect of increasing BM-V concentration on $^{32}$P incorporation. The activation of CaMKII by BM-V, together with that by exogenous calmodulin on the same preparation of CaMKII and under the same reaction conditions, is illustrated in Fig. 10A. Reciprocal plots of these data, shown in Fig. 10B, gave values of 10 and 26 nM for the apparent activation constants of BM-V and calmodulin, respectively, which is consistent with an exchange of about 2–3 calmodulins from BM-V to CaMKII under the conditions of the experiment. Notably, the maximum incorporation of $^{32}$P into CaMKII activated by BM-V was about twice that by exogenous calmodulin. Although the protocol used here does not permit us to distinguish between the initial steady-state rate and a possible initial burst phase (Fig. 9), the data suggest that BM-V not only supplies calmodulin, but additionally the binding of BM-V to CaMKII entails alterations in kinetic and/or autophosphorylation site parameters.

**DISCUSSION**

An important target of Ca$^{2+}$/calmodulin in neuronal cells is CaMKII, whose kinase activity is stringently regulated by the Ca$^{2+}$–dependent binding of calmodulin. CaMKII is a multifunctional enzyme with a wide amplitude of potential cellular substrates (reviewed in Refs. 1, 21, and 35). Thus, its subcellular localization and specific associations within its immediate molecular vicinity are likely to be important factors in its cellular function and control. In the pre-synaptic region, for example, CaMKII is bound to synapsin I and synaptic vesicles where it regulates, via phosphorylation, the interactions between these components and the actin cytoskeleton (36, 37), an important regulatory process of neurotransmitter release (reviewed in Ref. 38). CaMKII is also associated with postsynaptic densities, submembranous actin-cytoskeleton structures believed to be involved in receptor regulation and synaptic plasticity (reviewed in Refs. 39 and 40). Autophosphorylation of CaMKII affects its reversible association with postsynaptic densities (41) and recent studies identified the polypeptides, p190 and p140, as major CaMKII-binding proteins (42).
proteins have been identified that bind calmodulin in the ab-
cytokinesis contractile ring in yeast (47). Furthermore, several
1
figure. Ca2
and Ca2
(44), centrosomes and the mitotic apparatus (45), the contract-
subcellular compartments, for example, postsynaptic densities
the cell calmodulin is indeed discretely localized to several
ent in relatively high concentrations in brain (43). However, in
access to target proteins, being small, highly soluble, and pres-
1
were initiated by the addition of 2.4
m
Ci of [γ-32P]ATP and stopped after 2 min at 35 °C with SDS-PAGE sample buffer. The experiments were:
1, BM-V alone; 2, CaMII alone; 3, BM-V + CaMII, no Ca2
; 4, BM-V + CaMII + Ca2
; 5, CaMII alone + Ca
; 6, BM-V + CaMII + Ca2
+ TFP. Samples of the reactions were analyzed by SDS-PAGE and autoradiography of the dried gel (Autorad.). A Western blot of an equivalent gel was probed with BM-V tail antibodies and with mono-
clonal antibodies against the α subunit of CaMII (Blot) to illustrate the protein content of the reactions. The heavy chain of BM-V (hcBM-V) and CaMKII (CKII, α- and β-subunits) are indicated to the right of the figure. Ca2
-activated protein kinase activity was not detected in the BM-V fraction (data not shown).

Calmodulin would not be expected a priori to have limited
access to target proteins, being small, highly soluble, and present
in relatively high concentrations in brain (43). However, in the
cell calmodulin is indeed discretely localized to several
subcellular compartments, for example, postsynaptic densities (44), centrosomes and the mitotic apparatus (45), the contract-
ile vacuole of Dictyostelium (46), and the growing bud and cytokinesis contractile ring in yeast (47). Furthermore, several
proteins have been identified that bind calmodulin in the ab-
ence of Ca2
+, such as neuromodulin (48) and the unconven-
tional myosins (reviewed in Ref. 12). The notion that calmod-
lin-carrying myosins may play a role in the determination of the
subcellular localization of calmodulin has been suggested (22, 46, 49). Porter and collaborators (22) even suggested that
calmodulin could translocate from myosin to other proteins as
part of a mechanism of enzyme regulation.

In this paper, we present biochemical evidence that BM-V,
an unconventional myosin which harbors at least 8 calmodu-
ins on its neck domains, binds to CaMII and activates its
kinase activity in the presence of Ca2
+. Using a combination of
immunoprecipitation and Western blot overlay techniques, we
showed binding (i) between the native proteins for both phos-
phorylated and non-phosphorylated species; (ii) between dena-
tured-renatured (Western blotted) polypeptides, corresponding
to the heavy chain of BM-V and the α and β subunits of
CaMII, to the appropriate alternate native protein; and (iii)
between autophosphorylated CaMKII and bacterially
expressed proteins corresponding to the whole tail, medial tail,
and proximal tails of the BM-V molecule, but not to the head
domain nor to the C-terminal globular tail domain. The
binding was resistant to 600 mM NaCl and 1% Triton X-100,
and under the conditions measured (Western blots of the bac-
terially expressed tail domain overlaid with autophosphory-
lated CaMKII) was of relatively high affinity. The binding of
non-phosphorylated CaMII to the tail domain was also
detected, although very much weaker and not quantifiable with
our methods. We also demonstrated that BM-V was able to
activate the kinase activities of CaMKII in a Ca2
+-dependent,
trifluoperazine-inhibited manner without the need for addi-
tional calmodulin. Comparison between the apparent activa-
tion constants for BM-V versus calmodulin suggested that each
BM-V provided 2 or more calmodulins. Interestingly, activation
of the autophosphorylation of CaMKII by BM-V resulted in a
higher maximum level of incorporation of 32P than that by
purified calmodulin. Our data suggested that this activation
included an initial burst of autophosphorylation, but our ex-
perimental protocol did not allow us to distinguish this burst
from the initial steady-state rate of autophosphorylation. How-
ever, this result did suggest that BM-V not only supplies cal-
modulin to CaMII, but that its binding alters the kinetic
properties or autophosphorylation sites of CaMII. These bio-
chemical results obtained in vitro are consistent with the pos-
sibility that these two proteins interact in the intracellular
milieu.

The question is whether an interaction between CaMII and
BM-V is indeed plausible based on what is known about the
subcellular locations and functions of these two proteins. In a
recent report, BM-V was shown to be associated with synaptic
vesicles via the synaptobrevin-synaptophysin complex (7).
These authors suggested that BM-V may be involved in the
recruitment of synaptic vesicles to the pre-synaptic membrane.
We also have detected BM-V in synaptic vesicle preparations,3
although we demonstrated here its coimmunoprecipitation
with syntaxin, an integral membrane protein of the synaptic

3 F. Mani, E. M. Espreafico, and R. E. Larson, unpublished data.
plasma membrane, from detergent extracts of synaptosomes. Integral membrane proteins of synaptic vesicles and the synaptic plasma membrane, together with associated and linking proteins, form multimeric complexes during different stages of the lifecycle of synaptic vesicles (reviewed in Refs. 34 and 38), which resist detergent extractions (50). Specifically, syntaxin and SNAP-25 tightly bind synaptobrevin as a priming event for vesicle fusion to the plasma membrane (51), although this complex seems to require the dissociation of synaptophysin (52). In accordance, we did not detect synaptophysin in the immunoprecipitate with BM-V and syntaxin (Fig. 2). Thus, the present data on BM-V associations are not necessarily in conflict. Clearly, further studies on the subfractionation of synaptosomes will have to be done to establish the precise interactions. In any case, the data do support the basic claim that BM-V is associated with components of the multimeric complexes involved in the synaptic vesicle exocytic cycle.

Bi and collaborators (53) have provided evidence for essential roles of both motor proteins, kinesin and myosin, in the recruitment of vesicles to the release sites of Ca\textsuperscript{2+}-regulated exocytosis in living cells. Without identifying the myosin, these studies indicated that a myosin-mediated step affected Ca\textsuperscript{2+}-regulated exocytosis at a point upstream from the final fusion event, but downstream from the kinesin transport event. Importantly, the inhibition of CaMKII had similar effects at the same stage of exocytosis as did the inhibition of myosin. A crucial function of synaptic vesicle-bound CaMKII at this stage of exocytosis is the phosphorylation of synapsin I, which tethers synaptic vesicles to the actin cytoskeleton in its unphosphorylated form and releases this interaction when phosphorylated (37).

There is much evidence to suggest that BM-V has a role in vesicle transport (reviewed in Refs. 10 and 11), including small brain-derived vesicles containing the synaptic vesicle marker protein, SV2 (54). The IQ motifs in the domain of BM-V have high homology to the calmodulin-binding sequence of neuregulin (GAP-43), which has been suggested to have a role in reversibly sequestering calmodulin at specific locations near calmodulin-requiring enzymes in the cell (48). Similarly, calmodulin binds to BM-V at low Ca\textsuperscript{2+} concentrations and is at least partially released at micromolar Ca\textsuperscript{2+} (15, 16).

A plausible hypothesis that unites the data we have presented here with that of others is that, upon elevation of intracellular free Ca\textsuperscript{2+}, calmodulin could translocate from BM-V to CaMKII, which in turn would autophosphorylate and then phosphorylate in its immediate environment synapsin I and BM-V. In the former case, the tethering effect of synapsin I on synaptic vesicles to the actin cytoskeleton would be released. In the latter case, we do not know what effect, if any, phosphorylation of the BM-V tail domain by CaMKII (17) would have, but we can speculate that new ties to the actin cytoskeleton via BM-V might occur that may participate in the mobilization of the synaptic vesicles from the “reserve pool” to the “releasable pool.”

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