Function of Calmodulin in Postsynaptic Densities

III. Calmodulin-binding Proteins of the Postsynaptic Density

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ABSTRACT A method has been developed for binding calmodulin, radioiodinated by the lactoperoxidase method, to denaturing gels and has been used to attempt to identify the calmodulin-binding proteins of cerebral cortex postsynaptic densities (PSDs). Calmodulin primarily bound to the major 51,000 Mr protein in a saturatable manner; secondarily bound to the 60,000 Mr region, 140,000 Mr region, and 230,000 Mr protein; and bound in lesser amounts to a number of other proteins. The major 51,000 Mr calmodulin-binding protein is one of unknown identity. Binding of iodinated calmodulin to these proteins was blocked by EDTA, EGTA, chlorpromazine, and preincubation with unlabeled calmodulin. Calmodulin iodinated by the chloramine-T method, which inactivates calmodulin, did not bind to the PSD but bound nonspecifically to histone. Calmodulin did not bind to proteins from a variety of sources for which calmodulin interactions have not been found. Except for three proteins, all of the proteins of synaptic membranes that bind calmodulin could be accounted for by proteins of the PSD which are a part of the synaptic membrane fraction. The major 51,000 Mr protein and the corresponding iodinated calmodulin binding were greatly reduced in cerebellar PSDs and this difference between cerebral cortex and cerebellar PSDs is discussed in light of the possible function of calmodulin in synaptic excitation responses.

Calmodulin has been identified biochemically as a major component of postsynaptic densities (PSDs) (20, 21) and its presence has also been confirmed immunohistochemically (35, 52). The relatively large amounts of calmodulin in the postsynaptic density as well as the presence there of both a calmodulin-activated protein kinase (21, 23, 24) and a calmodulin activated cyclic nucleotide phosphodiesterase (4, 21, 22) suggest an important role for this molecule in the regulation of neurotransmission. Because of the many important functions in which calmodulin is involved (cf. reference 49), it was deemed important to try to examine the interactions of PSD calmodulin with other PSD proteins. We know that it must interact with the kinase and the phosphodiesterase (21, 23, 24), and it would be instructive to know if it interacts with or binds other PSD proteins. Because the identity and function of most of the PSD proteins are unknown, a calmodulin-binding study would also aid in the tentative identification of at least some of them. To undertake such a study, it was felt necessary to separate the proteins electrophoretically, and in the case of the PSD proteins, to do so under denaturing conditions, because many of them are insoluble in non-denaturing solutions. In this study, a technique has been developed based upon several methods (1, 6, 8, 43) with which ligands could be bound to proteins on SDS gels. We used this technique to try to identify the calmodulin-binding proteins of the PSD on SDS polyacrylamide gels using radioiodinated calmodulin. This technique was found to be specific and indicated the presence of at least five calmodulin-binding proteins in the PSD. The primary binding components were the major 51,000 Mr protein of the cerebral cortex PSD (15, 29), which is phosphorylated by the calmodulin-activatable protein kinase (21, 23, 24) and which is postulated to be a unique component of asymmetric synapses (10, 11, 21), and a band in the gel which co-migrated with purified brain calcineurin and cyclic nucleotide phosphodiesterase. Independently of our binding studies (9, 12, this paper), Glenney and Weber (19) used a similar technique to find two calmodulin-binding proteins (Mr = 47,000 and 56,000) in chick brain acetone powder.
Binding of Radiiodinated Calmodulin Proteins on SDS Gels

The method of binding ¹²⁵I-calmodulin to proteins on SDS gels was developed from previous binding techniques (1, 6, 43) and has been previously described by us (12). Proteins in 2% SDS were electrophoresed in linear 5–15% polyacrylamide slab gels (2 mm x 1 cm x 18 cm) containing 0.1% SDS. After electrophoresis the gels were fixed with 25% isopropanol-10% acetic acid for 12 h with at least four changes by shaking the gels in plastic trays. All washing and binding was done at 25°C. The gels were washed for 5–10 min in distilled water and then washed with buffer A (50 mM Tris, pH 7.6; 0.2 M NaCl; 1 mM EDTA; 1 mM 2-mercaptoethanol) and then applied to a DEAE-Sephadex A-25 column equilibrated with 300 mM NaCl in buffer A. The elution of calmodulin was monitored with SDS polyacrylamide gel electrophoresis (PAGE). Calmodulin was iodinated by the method of Richman and Klee (41) with the following slight modifications to increase the specific activity (∼17-fold). Iodination was performed (25°C) in a reaction volume of 1 ml that contained 0.05 M sodium phosphate (pH 7.0), 1 mM CaCl₂, 1 mg calmodulin, 12 μg lactoperoxidase, and 0.4 mM CaCl₂. 2 μCi ¹²¹I Na was added to the reaction mixture and the reaction was initiated by the addition of 5 μl of H₂O₂ (diluted 1:500). After 7 min, 5 μl of H₂O₂ (1:500) was again added to the reaction mixture and the reaction was allowed to proceed for an additional 7 min. The iodinated calmodulin was isolated as described by Richman and Klee (41) and then was reduced by ethylation. The iodinated calmodulin was iodinated by the Richman-Klee method, which is biologically active (41), gave the same pattern of binding to SDS gels as did the calmodulin iodinated by our modification of their method.

| Treatment | ¹²⁵I-Calmodulin re-constituted % |
|-----------|----------------------------------|
| Control   | 100                              |
| + Chlorpromazine, 1 mM          | 40                               |
| + EDTA, 1 mM                    | 0                                |
| + EGTA, 1 mM                    | 0                                |
| + Unlabeled CaM, 5 μg           | 0                                |
| + BSA, 5 μg                     | 100                              |
| Chloramine-T calmodulin         | 0                                |

Cerebral cortex PSDs (150 μg) were depleted of calmodulin as described before (20). 1 μg iodinated calmodulin (1 x 10⁶ cpm) was incubated with PSDs at 37°C for 10 min in the presence of 0.5 mM CaCl₂. After incubation the PSDs were spun down in an Eppendorf microcentrifuge (Brinkmann Instruments, Inc., Westbury, NY), washed once with the incubation mixture, and finally, the pellets were counted on a Geiger counter. The 100% value indicates that all the added radioactivity was recovered in the pelleted PSDs. Chloramine-T calmodulin indicates that calmodulin was iodinated using chloramine-T (28) instead of our modification of the lactoperoxidase method (41).
FIGURE 1 Binding of radioiodinated calmodulin to proteins from cerebral cortex PSDs separated on SDS polyacrylamide gels. The method of binding is given in Materials and Methods with 175 μg PSD proteins being used in all slots. The M₆ of the major calmodulin-binding proteins are shown on the left. Slot 7 shows the Coomassie Blue staining of the PSD preparation. Slot 2 shows the autoradiograph of the gel after treatment with radioiodinated calmodulin. Slots 3–8 show autoradiography of the requisite controls. The binding of calmodulin was performed in the presence of: slot 3, 2 μM EGTA; slot 4, 0.1 mM EDTA; slot 5, 1 mM chloropromazine. Slot 6 shows the effect of preincubation with 1 mg unlabeled calmodulin for 2 h, while slot 7 shows the effect of preincubation with 5 mg BSA for 2 h. Slot 8: the gel was washed with 1 mM EGTA for 12 h after binding of radioiodinated calmodulin and then the autoradiograph was made. Autoradiographs were exposed at -90°C for 6 h using a Dupont Cronex Lightning-Plus YH enhancing screen.

The proteins at lesser molecular weights. A more comprehensive map of the binding is shown later in Fig. 6. Various controls indicate that the radioiodinated calmodulin is binding specifically. All binding activity is removed by the addition of either EGTA (slot 3), EDTA (slot 4), or chloropromazine (slot 5), or by preincubation of the gel with unlabeled calmodulin (1 mg in 10 ml) (slot 6). Also if the gel is first labeled, washing the gel subsequently in EGTA removes all binding (slot 8). The addition of a randomly chosen protein, BSA, did not only block binding but actually enhanced the binding of calmodulin to the gel (compare slots 2 and 7). This enhancement of binding by preincubation of the gel in 1 mg/ml BSA before incubation with radioiodinated calmodulin was >10 times the control in various experiments. This increase in binding seems to be specific because it occurs only with proteins on the gel that bind calmodulin in the absence of BSA. We do not know why this occurs, but some possible reasons could include (a) neutralizing static charges which are possibly generated on the surface of the gel during washing, (b) the removal by BSA of residual SDS, acetic acid, or isopropanol, or (c) the blocking by BSA of nonspecific sites allowing a greater relative concentration of calmodulin to stain the specific sites. This effect of BSA has been taken advantage of in all subsequent experiments.

A second set of controls is shown in Fig. 2, which compares the binding of radioiodinated calmodulin to PSD proteins and to histone. While histones were found to be capable of binding calmodulin, we believe this is caused by nonspecific interactions because calmodulin is acidic and histones are basic. To test this hypothesis, gels having either PSD proteins or histone were both incubated with either calmodulin labeled by the lactoperoxidase method or with calmodulin labeled by the chloramine-T method. While both these preparations were capable of binding to histones (slots 4 and 6), only the lactoperoxidase-iodinated calmodulin was found to bind to PSD proteins (compare slots 3 and 5). Because the chloramine-T method inactivates calmodulin (25, 45, 47), we conclude that the interaction of the lactoperoxidase-iodinated calmodulin with PSD proteins is specific and is not caused by simple ionic interactions.

Further added support that this binding is specific is shown in Fig. 3. Proteins from a number of sources do not bind radioiodinated calmodulin labeled by the lactoperoxidase method. Proteins from both E. coli (slot 1) and spinach chloroplast membranes (slot 2) do not bind calmodulin. Both pyruvate kinase (slot 3), an enzyme that is inhibited by calcium, and the inhibitory subunit of troponin (slot 4), which binds calmodulin in some in vitro assays (2) do not bind radioiodinated calmodulin. Because the electroplax of Electrophorus electricus has been reported to contain calmodulin (14), the purified acetylcholine receptor from Torpedo, a related organism, was tested and found not to bind calmodulin (slot 5).

FIGURE 2 Comparison of the binding of calmodulin radioiodinated by lactoperoxidase with the binding of calmodulin radioiodinated by chloramine-T to proteins from cerebral cortex PSDs and to histones. Slot 1, Coomassie Blue staining of PSD proteins (175 μg); slot 2, Coomassie Blue staining pattern of histones (15 μg); slot 3, binding of lactoperoxidase-iodinated calmodulin to PSD proteins; slot 4, binding of lactoperoxidase-iodinated calmodulin to histones; slot 5, binding of chloramine-T-iodinated calmodulin to PSD proteins; slot 6, binding of chloramine-T iodinated-calmodulin to histones. Autoradiographs were exposed at -90°C for 6 h using a Dupont Cronex Lightning-Plus YH enhancing screen.
either that the protein does not bind calmodulin, or that it gels is indeed specific. On the other hand some proteins that binding of radioiodinated calmodulin to proteins on denaturing protein. All of the above control results indicate that the found in these six preparations, only one band binds calmo-

All these experiments show that out of some 150 protein bands denaturing gel containing a crude extract from black widow spider venom glands, a single binding protein is found (slot 6). However, when radioiodinated calmodulin is added to a denaturing gel containing a crude extract from black widow spider, a single binding protein is found (slot 6). All experiments show that out of some 150 protein bands found in these six preparations, only one band binds calmodulin, and this one protein may or may not be a true binding protein. All of the above control results indicate that the binding of radioiodinated calmodulin to proteins on denaturing gels is indeed specific. On the other hand some proteins that have been found to interact specifically with calmodulin in other systems do not bind radioiodinated calmodulin on gels. Thus a positive binding reaction in the gel system would indicate specific binding, but a negative reaction would indicate either that the protein does not bind calmodulin, or that it does, but has not reattached correctly in the assay and has lost its capacity to bind calmodulin.

Fig. 4 shows a saturation curve of the binding of radioiodinated calmodulin to a gel containing 175 μg of PSD proteins. The amount of label incorporated into the major 51,000 M_r protein is compared with the amount of calmodulin added. The amount of calmodulin binding comes close to saturation but not completely. This is caused most likely by calmodulin only binding to the surface of the gel (cf. Fig. 5), and increasing the amount of calmodulin to very high levels will slowly continue to push the equilibrium of the binding towards the interior of the gel. However, the near saturation again demonstrates the specific binding in this system.

The process by which iodinated calmodulin binds to proteins on SDS gels is indicated by the curves in Fig. 5. In panel A is a plot of the amount of radioactivity incorporated into the major 51,000 M_r protein as a function of increasing amounts of PSD. The resulting curve is not linear but logarithmic, indicating the technique is not quantitative except possibly on a logarithmic scale. If the amount of bound radioiodinated calmodulin is instead plotted as a function of the width of the major 51,000 M_r protein band, a linear relationship is observed. These data indicate calmodulin is binding only to the surface of the gel. Thus the thickness of the gel has no strong influence on the binding of calmodulin, except that for a given amount of protein a thinner gel will result in a wider band. Because the binding of calmodulin is dependent on the width of a band and not on the total amount of protein, the technique is very sensitive. Using the preincubation with BSA (cf. above), we routinely are able to detect less than the 100-300 ng of a calmodulin-binding protein which are detectable by Coomassie Blue staining.

A comparison of the binding of radioiodinated calmodulin to synaptic membranes, cerebral cortex PSDs, and cerebellar PSDs is shown in Fig. 6. The pattern of binding to a preparation of cerebral cortex PSDs (slot 5) is very similar to that of a synaptic membrane preparation from which they are derived (slot 4), suggesting that the majority of the calmodulin-binding proteins in synaptic membranes are caused by the presence of PSDs in this fraction. This finding agrees well with the immunohistochemistry results showing the presence of calmodulin at the postsynaptic density and not at other synaptic membrane sites (35, 52). However, there are three binding proteins at 29,000, 125,000, and 130,000 M_r that are found in the synaptic membrane preparation that are not found in the PSD preparation. These proteins might possibly be either adenyl cyclase and/or the calcium-activatable ATPase as both these enzyme activities have been found in synaptic membranes, but have not been found in the PSD (15). A comparison of the patterns of calmodulin binding on gels to proteins from cerebral cortex (slot 5) and cerebellar (slot 6) PSDs shows the cerebellar

![Figure 3](https://example.com/image.png)

**FIGURE 3** Lack of binding of calmodulin to proteins from various sources on SDS gels. All samples were treated identically as described in Materials and Methods and are directly comparable to Figs. 1 and 2. In all cases, slot a shows the Coomassie Blue-staining patterns of proteins on SDS gels, while slot b shows the autoradiograms of such preparations after pretreatment with radioiodinated calmodulin as given in Materials and Methods. The following are the various protein preparations: 1, 75 μg E. coli; 2, 125 μg spinach chloroplast membranes; 3, 40 μg pyruvate kinase; 4, 10 μg of the inhibitory subunit of troponin; 5, 20 μg purified acetylcholine receptor from Torpedo; 6, 125 μg crude extract from the venom glands of the black widow spider. Autoradiographs were exposed as described in Fig. 1.

**FIGURE 4** Saturation curve of calmodulin binding to the major 51,000 M_r protein. Binding to the major 51,000 M_r protein was determined by scanning the autoradiogram after binding to 175 μg PSD proteins run on the gel, cutting out the 51,000 M_r peak from the scan, and weighing it. The highest value of binding was arbitrarily set at 100%, determined from densitometry tracings of the autoradiograms, exposed within the linear range of radioactivity vs. autoradiograph density. The background is a clear autoradiogram.
less quantities in cerebellar than in cerebral cortex PSDs (com-
nucleotide phosphodiesterase has also been described by us in the PSD (22), while others have described the presence of calcineurin there (52), both of these being in the 60,000 M<sub>r</sub> region of the gel which binds calmodulin.

Although we do not know the identity of the major 51,000 M<sub>r</sub> protein, some previous studies have given some clues as to its likely function. In a previous study in which two types of PSDS were isolated (10, 11), those isolated from cerebral cortex were found to be an enriched fraction of PSDS from symmetric or Gray type I synapses while those isolated from cerebellum were found to be an enriched fraction of PSDS from symmetric or Gray type II synapses. At present we have little idea as to why we have an enrichment of these presumably type I PSDS from cerebral cortex and an enrichment of these presumably type II PSDS from cerebellum, because both types of synapses are found in both these brain areas. An extensive review of the literature led Eccles (17) to propose that type I synapses mediate excitation responses and type II synapses mediate inhibitory responses. The major differences in the protein composition between these two preparations is that the cerebellar PSDS have much smaller amounts of the major 51,000 M<sub>r</sub> protein than do the cerebral cortex PSDS, and they contain 50% less calmodulin than do the cerebral cortex PSDS. Minor differences also exist in that the cerebellar PSDS lack the minor calmodulin-binding proteins at 115,000, 76,000, 70,000, 40,000, and 36,000 M<sub>r</sub>, which are found in the cerebral cortex PSDS. This finding suggests that the major 51,000 M<sub>r</sub> protein, calmodulin, and possibly the other listed calmodulin-binding proteins are intimately involved in the excitation process at the synapses. The process by which the 51,000 M<sub>r</sub> protein, which is the major protein of the PSD, may control excitation is by way of both (a) modulation of the action of the protein by direct interaction with calmodulin and (b) modulation of the action of the protein by the calcium-calmodulin-dependent phosphorylation (23). How these two systems interact is unknown, but we do know that phosphorylation of the 51,000 M<sub>r</sub> protein by the calcium-calmodulin-dependent protein kinase did not release bound calmodulin from PSDS as indicated by SDS PAGE, indicating that calmodulin could be bound to the phosphorylated protein.

Although we are now only beginning to obtain identification of a few calmodulin-binding proteins, a question can be raised at this time as to the role, if any, of calmodulin in postsynaptic function. The large number of calmodulin-binding proteins at the postsynaptic site plus numerous recent reports in the literature on calmodulin indicate that calmodulin may be important in a large variety of synaptic functions. Firstly, calmodulin plays a role in calcium flux between the intracellular and extracellular environment. Phospholipid metabolism has been implicated as a controlling factor in calcium entry (26), and a number of the enzymes involved in the metabolism of phospholipids require calcium, and one of the primary enzymes, phospholipase A<sub>2</sub>, has been shown to be activated by calmodulin (51). Also, it may be that it is Ca<sup>2+</sup>-calmodulin (32, 44) and not just calcium that activates the ATPase which catalyzes the removal of calcium from inside the cells. Secondly, calmodulin is intimately involved in the regulation of cAMP metabolism, because calmodulin activates synaptic membrane adenyl cyclase (7, 13) and the PSD phosphodiesterase (4, 22), thus controlling both the synthesis and degradation of cAMP.

In this context, calmodulin may also modulate the role of neurotransmitter receptors in nerve signal conduction, as many neurotransmitters exert their effect through a coupled adenyl cyclase. Receptors that are not coupled to cAMP may also be under the control of calcium-calmodulin, as the binding of glutamic acid to its receptor is enhanced by calcium (5). Thirdly, calmodulin may be involved in excitatory responses by way of mediating the effects of calcium. Chlorpromazine, a drug that binds and inactivates calmodulin (34), caused a reduction in miniature endplate potential amplitudes at the neuromuscular junction (3). Also Mn<sup>2+</sup>, a divalent cation which can partially bind calmodulin (20), partially inactivated the excitatory postsynaptic potential in cat spinal cord (31). Also, calcium has been shown to be required for desensitization or receptor inactivation in the neuromuscular junction (37, 40). If hippocampal CA1 neurons are repetitively fired they develop a long-lasting after-hyperpolarization as a result of a calcium-activated potassium current (27), and the long-term potentiation in the hippocampus, which may involve memory, has an absolute requirement for calcium (16). Finally, tubulin has been identified as part of the PSD (15, 18, 29, 39) and in a few cases microtubules have been seen extending to the PSD (50). As calmodulin has been found to possibly control microtubule polymerization-depolymerization (38), calmodulin may also be controlling the function of tubulin in the PSD.

We thus propose as a working hypothesis that (a) calmodulin and the major 51,000 M<sub>r</sub> protein are involved in the modulation and possible generation of postsynaptic excitatory responses, (b) the interaction of calmodulin with other postsynaptic proteins mediates some of the above-described calcium-requiring events, and (c) these calcium-requiring postsynaptic responses occur in the PSD.

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REFERENCES

1. Adair, W. S., D. Juvinich, and U. W. Goodnow. 1978. Localization of cellular antigens in sodium dodecyl sulfate-polyacrylamide gels. J. Cell Biol. 79:281-285.

2. Argov, Z. and Y. Yaari. 1979. The action of chlorpromazine at an isolated cholinergic synapse. Brain Res. 177:301-309.

3. Baudry, M. and G. Lynch. 1979. Regulation of glutamate receptors by calmodulin. Nature (Lond.). 278:748-749.

4. Bigelis, R., and K. Burridge. 1978. The immunological detection of yeast nonsense termination fragments on sodium dodecylsulfate polyacrylamide gels. Biochim. Biophys. Res. Commun. 82:322-327.

5. Baudry, M., and G. Lynch. 1979. Regulation of glutamate receptors by calmodulin. Brain Res. 177:301-309.

6. Bigelis, R., and K. Burridge. 1978. The immunological detection of yeast nonsense termination fragments on sodium dodecylsulfate polyacrylamide gels. Biochim. Biophys. Res. Commun. 82:322-327.

7. Brosnien, N., T. Y. C. Huang, B. Mcl. Breckenridge, and D. J. Wolf. 1975. Identification of a calcium binding protein as a calcium dependent regulator of brain adenylate cyclase. Proc. Natl. Acad. Sci. U. S. A. 72:546-55.

8. Burridge, K. 1976. Changes in cellular glycoproteins after transformation: identification of specific glycoproteins and antigens in sodium dodecyl sulfate gels. Proc. Natl. Acad. Sci. U. S. A. 73:4657-4661.

9. Carlin, R. K., D. J. Grab, and P. Siekewitz. 1980. The calmodulin binding proteins of the postsynaptic density. Fed. Proc. 39:1658.

10. Carlin, R. K., D. J. Grab, and P. Siekewitz. 1979. Characterization of postsynaptic densities from different brain parts. J. Cell Biol. 82:322-327.

11. Carlin, R. K., D. J. Grab, and P. Siekewitz. 1980. Isolation and characterization of postsynaptic densities from various brain regions: enrichment of different types of postsynaptic densities. J. Cell Biol. 86:831-843.

12. Carlin, R. K., D. J. Grab, and P. Siekewitz. 1980. The binding of radio-iodinated calmodulin to proteins on desaturing gels. Ann. N. Y. Acad. Sci. 356:73-74.
22. Grab, D. J., R. K. Carlin, and P. Siekevitz. 1977. The structure of postsynaptic densities isolated from dog cerebral cortex. I. Overall morphology and protein composition. J. Cell Biol. 74:181-203.

23. Grab, D. J., R. K. Carlin, and P. Siekevitz. 1981. Function of calmodulin in postsynaptic densities. J. Cell Biol. 89:433-439.

24. Grab, D. J., and P. Siekevitz. 1979. Calmodulin-dependent protein kinase activity in postsynaptic densities. J. Cell Biol. 83(3 Pt. 2):723a (Abstr.).

25. Graf, E., A. G. Filoteo, and J. T. Penniston. 1980. Preparation of 125I-calmodulin with retention of full biological activity: its binding to human erythrocyte ghosts. Biochim. Biophys. Acta. 605:99-108.

26. Hawthorne, J. N., and M. R. Pickard. 1979. Phospholipids in synaptic function. Prog. Brain Res. 51:3-14.

27. Kelly, P. T., and C. W. Cotman. 1978. Synaptic proteins: characterization of tubulin and actin. J. Neurosci. 8:49-56.

28. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of iodine-131 labeled human IgG. Arch. Biochem. Biophys. 103:719-726.

29. Kuo, C., S. Ichida, T. Matsuoka, S. Kakiuchi, and H. Yoshida. 1979. Regulation of ATP-dependent Ca\(^{2+}\)-dependent protein kinase by Ca\(^{2+}\)-dependent modulator protein. J. Biol. Chem. 254:670-676.

30. LaPorte, D. K., and D. R. Storm. 1978. Detection of calcium-dependent regulatory protein binding components using \(^{125}\)I-labeled calcium-dependent regulatory protein. J. Biol. Chem. 253:3374-3377.

31. Leven, R. M., and B. Weiss. 1978. Specificity of the binding of trifluoperazine to the calcium-dependent activator of phosphodiesterase and to a series of other calcium-binding proteins. Biochim. Biophys. Acta. 405:99-108.

32. Lin, C. J., R. D. Dedman, B. R. Britskik, and A. R. Means. 1980. Localization of calmodulin in rat cerebellum by immunoselection microscopy. J. Cell Biol. 85:473-480.

33. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

34. Maxfield, F. A. 1966. The effect of calcium on the desensitization of membrane receptors at the neuromuscular junction. J. Gen. Physiol. 49:863-876.

35. Matus, A. L., R. B. Walters, and S. Moghal. 1975. Immunohistochemical demonstration of tubulin associated with microtubules and synaptic junctions in mammalian brain. J. Neurocytol. 4:333-344.

36. Manthey, A. A. 1966. The effect of calcium on the desensitization of membrane receptors at the neuromuscular junction. J. Gen. Physiol. 49:863-876.

37. Naito, W. L., and R. L. Parsons. 1979. Factors in the inactivation of postsynaptic membrane receptors of frog skeletal muscle. J. Gen. Physiol. 56:218-249.

38. Richman, P. G., and C. B. Klee. 1978. Interaction of \(^{125}\)I-labeled Ca\(^{2+}\) dependent regulator protein with cyclic nucleotide phosphodiesterase and its inhibitory protein. J. Biol. Chem. 253:6323-6326.

39. Richman, P. G., and C. B. Klee. 1978. Interaction of \(^{125}\)I-labeled Ca\(^{2+}\) dependent regulator protein with cyclic nucleotide phosphodiesterase and its inhibitory protein. J. Biol. Chem. 253:6323-6326.

40. Rosas, J. A., P. T. Kelly, R. H. Pesin, and C. W. Cotman. 1979. Protein and glycoprotein composition of synaptic junctions from discrete synaptic regions and different species. Brain Res. 168:151-167.

41. Siskovitz, P. 1973. The differentiation of rat liver endoplasmic reticulum membranes: apocytochrome P450 as a membrane protein. J. Supramol. Struct. 1:471-489.

42. Sobott, K., S. Ichida, H. Yoshida, R. Yamazaki, and S. Kakiuchi. 1977. Occurrence of a Ca\(^{2+}\)- and modulator protein-activatable ATPase in the synaptic plasma membranes of brain. FEBS (Fed. Eur. Biochem. Soc.) Lett. 91:199-202.

43. Thiry, P., A. Vandenhoore, M. Vandenhoore-Poel, J. Rathé, and J. Christophe. 1980. The activation of brain adenyl cyclase and brain cyclic-nucleotide phosphodiesterase by seven calmodulin derivatives. Eur. J. Biochem. 103:409-414.

44. Wallace, R. W., E. A. Tallant, and W. Y. Cheung. 1980. High levels of a heat-labile calmodulin-binding protein (CaM-BP) in bovine neostriatum. Biochemistry 19:1831-1837.

45. Walsh, M., and F. C. Stevens. 1977. Chemical modification studies on the Ca\(^{2+}\)-dependent protein modulator of cyclic nucleotide phosphodiesterase. Biochemistry. 16:2742-2749.

46. Watsonson, D. M., W. G. Harrellson, P. M. Keller, F. Sharief, and T. C. Yamazan. 1976. Structural similarities between the Ca\(^{2+}\)-dependent regulatory proteins of 3',5'-cyclic nucleotide phosphodiesterase and actomyosin ATPase. J. Biol. Chem. 251:4501-4513.

47. Watsonson, D. M., and F. F. Vincenzi, editors. 1980. Calmodulin and cell functions. Ann. N. Y. Acad. Sci. 356.

48. Westrum, L. E., and E. G. Gray. 1977. Microtubules associated with postsynaptic thickenings. J. Neurocytol. 6:505-518.

49. Wonig, P. Y. K., and W. Y. Cheung. 1979. Calmodulin stimulates human platelet phosphodiesterase. Biochem. Biophys. Res. Commun. 90:473-480.

50. Wood, J. E., R. W. Wallace, J. N. Whitaker, and W. Y. Cheung. 1980. Immunohistochemical localization of calmodulin and a heat-labile calmodulin-binding protein (CaM-BP) in basal ganglia of mouse brain. J. Cell Biol. 84:66-76.