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Ca^{2+}/calmodulin-binding peptides block phototransduction in *Limulus* ventral photoreceptors: Evidence for direct inhibition of phospholipase C

EDWIN A. RICHARD*, SMITA GHOSH†, JOHN M. LOWENSTEIN†, AND JOHN E. LISMAN*‡

*Biology and †Biochemistry Departments, Brandeis University, Waltham, MA 02254-9110

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ABSTRACT **Phototransduction in *Limulus* photoreceptors involves a G protein-mediated activation of phospholipase C (PLC) and subsequent steps involving $InsP_3$-mediated release of intracellular Ca^{2+}. While exploring the role of calmodulin in this cascade, we found that intracellular injection of Ca^{2+}/calmodulin-binding peptides (CCBPs) strongly inhibited the light response. By chemically exciting the cascade at various stages, we found the primary target of this effect was not in late stages of the cascade but rather at the level of G protein and PLC. That PLCδ_1 contains a calmodulin-like structure raised the possibility that PLC might be directly affected by CCBPs. To test this possibility, *in vitro* experiments were conducted on purified PLC. The activity of this enzyme was strongly inhibited by CCBPs and also inhibited by calmodulin itself. Our results suggest that the calmodulin-like region of PLC has an important role in regulating this enzyme.**

Phototransduction in *Limulus* photoreceptors is a complex excitation cascade that has sufficient amplification to produce large electrical events to single photons (1). The initial stage of this cascade resembles the rhodopsin/G protein interaction found in vertebrate photoreceptors, but the enzyme activated by G protein is phosphoinositide-specific phospholipase C (PLC) rather than cGMP-phosphodiesterase. PLC, in turn, generates $InsP_3$ (2), and the resulting $InsP_3$-mediated Ca^{2+} release (3–5) leads to activation of nonspecific cation channels (2, 6), perhaps through an intermediate step involving cGMP (7, 8).

Invertebrate photoreceptors contain a high concentration of calmodulin (9). In the region of the photoreceptor specialized for phototransduction, the microvillar region, the concentration may be as high as 0.5 mM (10). Because the light-induced elevation of Ca^{2+} plays an obligatory role in the excitation process in *Limulus* (11), we suspected that a calmodulin-dependent process might play an important role in a late stage of the excitation process, perhaps coupling the $InsP_3$-mediated elevation of Ca^{2+} to the opening of ion channels. If this were the case, calmodulin peptide antagonists should reduce the response to light. The experiments reported here show Ca^{2+}/calmodulin peptide antagonists do indeed have this effect. However our results show that the primary site of this effect is not at a late stage of transduction but rather an early stage involving PLC.

PLC isoforms are found in all eukaryotic cells and are involved in signal transduction, including sensory, learning-related synaptic plasticity, and oncogenesis (for reviews see refs. 12 and 13). Recently, the crystal structure of PLCδ_1 has been obtained (14). This structure revealed EF-hand domains that resemble the structure of calmodulin with Ca^{2+} bound (the "calmodulin-like" domain). Sequence alignment and α-helix prediction suggest the existence of similar structures in all PLC isozymes. The regulatory role of this region is unclear, but it seemed possible from our physiological results that it might be the target of calmodulin antagonists. In the second part of this study we show that purified PLC is directly inhibited by Ca^{2+}/calmodulin-binding peptides (CCBPs).

MATERIALS AND METHODS

Electrophysiology. Preparation of the ventral nerve and perfusion with artificial sea water were carried out as reported previously (15). Cells were impaled with an electrode containing Ca^{2+}/calmodulin-binding or control peptide. Peptides were introduced into cells by 60–120 small pressure pulses, and the injections were monitored by an infrared video camera. Total injected volumes were estimated (16) as no more than 5% of total cell volume and usually less, i.e., the maximal intracellular peptide concentration was less than 300 μM. A second intracellular electrode was used for pressure injection of other test compounds and voltage clamp. Beam intensity was approximately 1.0 mW/cm^2 and reduced by using neutral density filters.

Electrode Solutions. The first electrode contained 6 mM Ca^{2+}/calmodulin-binding or control peptide, 1 mM EDTA, 100 mM KCl, neutralized with Mops or Hepes, pH 7–7.5. Control peptides (LADVAEQRHLAKK and KKALHRQEAVDAL) were a gift from Leslie Griffith. Ca^{2+}/calmodulin-dependent protein kinase II peptide 290–309 (CMKII 290–309) and vasoactive intestinal peptide (VIP) were obtained from commercial sources. Peptides were dissolved in distilled water and lyophilized to reduce organic acid content before use. For experiments using voltage clamp, the second electrode contained 3 M KCl. For excitation by Ca^{2+}, the second electrode contained a solution of 2 mM *N*-(2-hydroxyethyl)ethylenedinitrilo-*N*,*N*′,*N*′-triacetic acid (HEDTA), 1.8 mM $CaCl_2$, and 170 mM KCl, pH 7.6. For excitation by $InsP_3$, the second electrode contained a solution of 100 μM $InsP_3$, 170 KCl, and 10 mM Hepes, pH 7.2 (4).

Activation of G Proteins. A solution of 25 μM GTP[γS], 170 KCl, and 10 mM Hepes, pH 7.2 was introduced intracellularly by using multiple small injections of GTP[γS]. Nucleotide exchange was activated by light exposure, and then the cell was dark-adapted (17).

Biochemistry. Phospholipase C was purified (18) and assays were performed with 4 μM free Ca^{2+} (19) as described previously, except that calmodulin, Ca^{2+}/calmodulin-binding

Abbreviations: CCBP, Ca^{2+}/calmodulin-binding peptide; CMKII 290–309, Ca^{2+}/calmodulin-dependent protein kinase II peptides 290–309; HEDTA, *N*-(2-hydroxyethyl)ethylenedinitrilo-*N*,*N*′,*N*′-triacetic acid; $InsP_3$, inositol 1,4,-trisphosphate; PLC, phospholipase C; VIP, vasoactive intestinal peptide.

‡To whom reprint requests should be addressed. e-mail: lisman@binah.cc.brandeis.edu.

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peptides, and control peptide were preincubated with phospholipase C for 30 min before starting the reaction.

RESULTS

CCBPs Block Phototransduction. We investigated the role of calmodulin by injecting the Ca^{2+}/calmodulin-binding peptide derived from the pseudosubstrate region of calmodulin-dependent kinase II (20, 21). The peptide was injected into the cytoplasm of the photoreceptor through an intracellular microelectrode. The injection was done under visual control; the final cytoplasmic concentration of peptide was estimated to be no more than several hundred micromolar. Fig. 1*A* shows that the peptide rapidly reduced the photoresponse and was capable of decreasing the response to a flash by more than two orders of magnitude (Figs. 2 and 3). This inhibition slowly reversed, presumably as a result of degradation of the peptide by endogenous proteases (22). Injection of comparable and

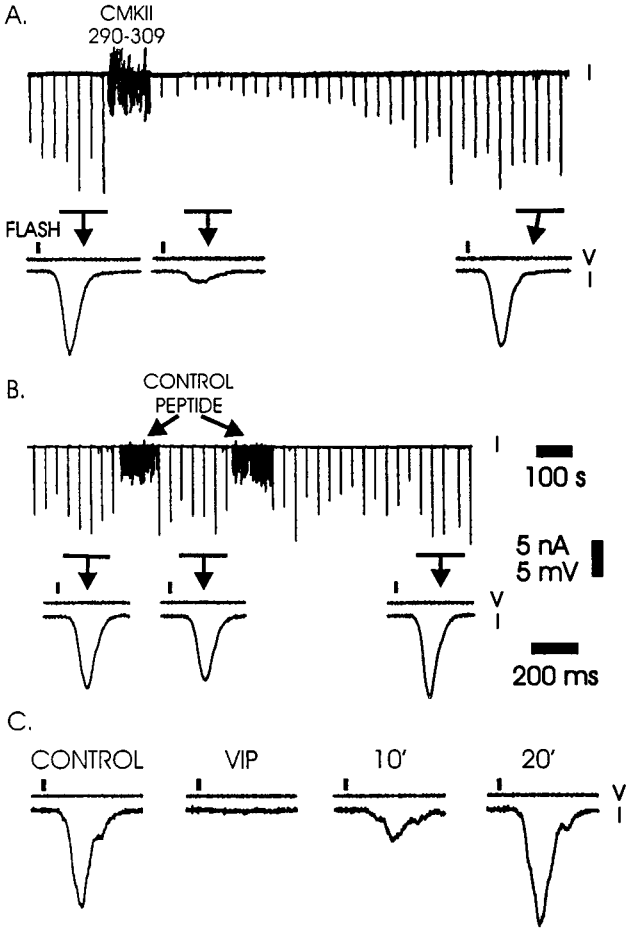

FIG. 1. Effect of injection of Ca^{2+}/calmodulin-binding peptides CMKII 290–309 and VIP into *Limulus* ventral photoreceptors. (*A*) Effects of intracellular pressure injection of CMKII 290–309 on the light-induced current. At the top is a continuous current trace (100-s scale bar). A dim light flash every 30 s induced inward current (downward deflection). During the period marked "CMKII 290–309," the peptide was injected with multiple, small pressure pulses. This produced a large, but reversible decrease in the light response. Directly underneath are response averages (n = 5, 200-ms scale bar) during periods indicated by the bars and arrows. Solid rectangles represent the flash. Voltage (V) and current traces (I) are as indicated. (*B*) Effect of control peptide on the light-induced current. Approximately twice the number of injections of similar volume were made than in *A*. (*C*) Effect of VIP injection on the light-induced current. Single responses are shown before injection, immediately following, 10 s later, and 20 s later.

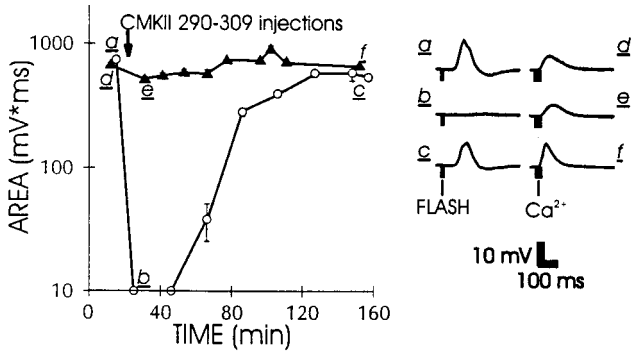

FIG. 2. Comparison of the inhibitory effects of CMKII 290–309 on the excitation (depolarization) produced by light (open circles) or Ca^{2+} injection (solid triangles). a–f match averaged (n = 4–5) areas (*Left*) to time course (*Right*) before a d, after b e, and following recovery from c f peptide injection. The two light response data points immediately after peptide injection represent upper limits on the area.

greater amounts of control peptide had no effect (Fig. 1*B*). To see whether the inhibition of the light response was a general feature of CCBPs we tested the peptide hormone VIP, which also binds to Ca^{2+}/calmodulin with high affinity (23). Injection of VIP desensitized cells in a similar manner to CMKII 290–309 (Fig. 1*C*). We conclude that one or more steps in the transduction process are strongly inhibited by injection of CCBPs.

CCBPs Block Either G Protein or PLC. We initially suspected that CCBPs might act at a late stage of the cascade and involve the processes by which $InsP_3$-mediated Ca^{2+} release affects the opening of ion channels. If this was the case, the peptide should strongly reduce the excitation caused by intracellular Ca^{2+} injection, just as peptide reduces the response to light. Previous work showed that brief injections of Ca^{2+} into the transducing lobe of the photoreceptor excite this photoreceptor. The conductance opened in this way appears to be the same as that opened by light (11, 24).

Ca^{2+} injection from electrodes containing high concentrations of Ca^{2+} are notoriously difficult because the electrodes usually become blocked within minutes, presumably as a result of Ca^{2+} precipitates forming in the electrode tip. We developed a new method for injecting Ca^{2+} that involves the Ca^{2+} buffer HEDTA (25). Injection of a solution containing 2 mM HEDTA (in the microelectrode) with no added Ca^{2+} failed to excite cells (Fig. 4*A*). However, if 1.8 mM Ca^{2+} was added to 2 mM HEDTA, pH 7.6, the free Ca^{2+} in the solution would be $\approx$8.5 μM. Injection of this solution gave highly reproducible excitation to consecutive injections (Fig. 4*A*). An initial "use effect" often occurred with responses increasing to a stable level during the first set of injections (Fig. 4*B*). The response amplitude and duration depended on the duration of

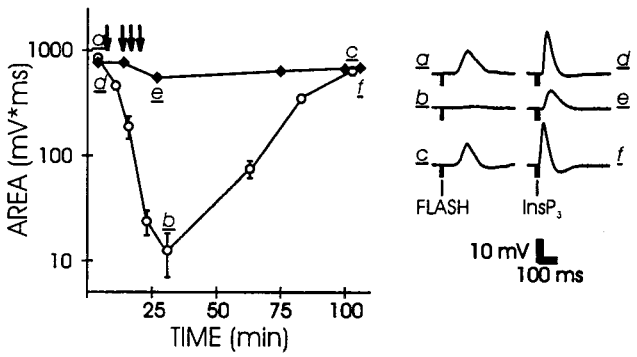

FIG. 3. Comparison of the effects of CMKII 290–309 on the excitation produced by light (open circles) and $InsP_3$ (solid diamonds). For details see Fig. 2.

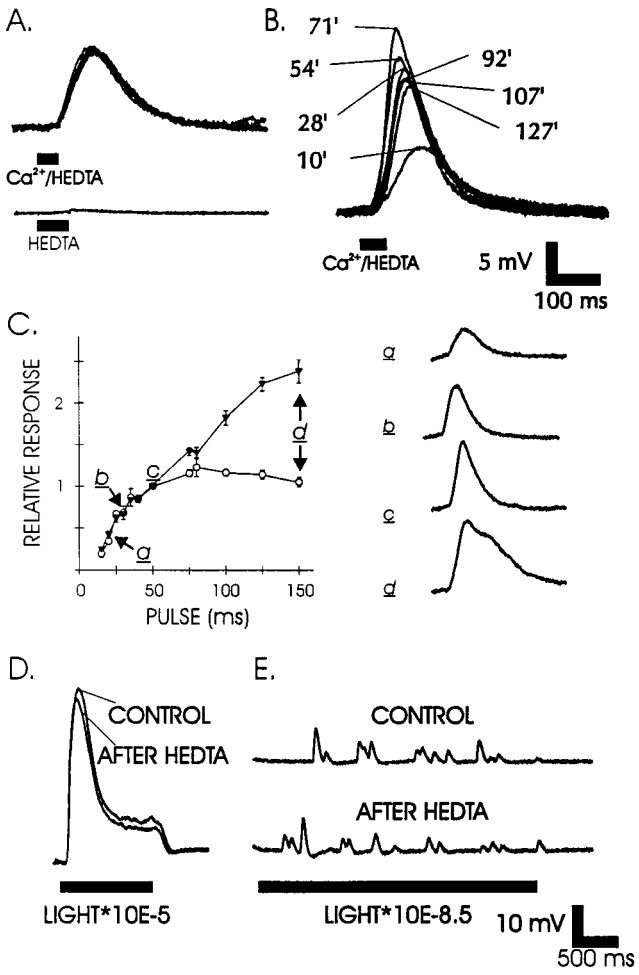

FIG. 4. Characterization of the excitation produced by intracellular pressure injection of 1.8 mM Ca^{2+}/2 mM HEDTA, pH 7.6 solution. (*A*) Superposed depolarizations produced by five consecutive injections of Ca^{2+}/HEDTA solution (40-ms pressure pulses spaced 1 min apart). Below is the effect of 50 ms pressure pulse injection of 2 mM HEDTA only in a control experiment. (*B*) Superposed responses to Ca^{2+}/HEDTA injections by 50-ms pressure pulses at times indicated over more than 2 h. (*C*) Relationship between pressure pulse length and response peak amplitude (open circles) and areas (solid triangles). a–d match amplitude and pulse duration (*Left*) to voltage trace (*Right*). Bars represent the standard error of 3–10 measurements over the 2-h experiment. Responses were normalized by dividing response amplitude and area by those of concurrent 50-ms pressure pulse responses. (*D*) Lack of effect of HEDTA buffer on the light response after >100 injections in the same control experiment. (*E*) Lack of effect of HEDTA buffer on the response to single photons after >100 injections in the same control experiment. The stimulating light beams were attenuated as indicated.

the pressure pulse (Fig. 4*C*). As duration increased, the response amplitude saturated, but the response area continued to increase. An important rationale for use of HEDTA is that it does *not* buffer intracellular Ca^{2+} effectively. Ca^{2+} buffers have been shown to greatly reduce the response to light (11, 26, 27). HEDTA fails as an intracellular Ca^{2+} buffer because of poor selectivity for Ca^{2+} relative to Mg^{2+} (only about 16-fold) (25). Once injected into cells HEDTA should equilibrate as a Mg^{2+} salt (estimated at 97%), because the intracellular Mg^{2+} concentration (mM) is orders of magnitude higher than resting Ca^{2+} concentration (sub-μM) (28, 29). HEDTA fails to deplete cell Mg^{2+} levels as each injection is rapidly diluted >100-fold into the total cell volume. Intracellular Mg^{2+} levels presumably are replenished from the bath solution (48 mM Mg^{2+}). Indeed, normal responses to light (Fig. 4*D*), including unchanged responses to single photons (Fig. 4*E*), were observed after >100 injections of either 2 mM HEDTA, as in Fig. 4, or the Ca^{2+}/HEDTA.

By using the reproducible responses to Ca^{2+} injection, we tested whether these responses were reduced by CCBPs. Fig. 2 shows that the response to Ca^{2+} injection was not strongly inhibited by CMKII 290–309, whereas the excitation by light in the same cell was strongly inhibited. To exclude the possibility that the lack of effect was because of saturation of the response to Ca^{2+} at the site of injection, we used a pressure pulse producing only 50–70% of maximal response amplitude (as in Fig. 4*C*). The minor effects of CCBP on the response to Ca^{2+} injection produced under these conditions indicate that the primary effect of the Ca^{2+}/calmodulin-binding peptide on excitation is upstream from the excitatory role of Ca^{2+} in the cascade.

If CCBPs affected the $InsP_3$ receptor, thereby blocking $InsP_3$-mediated Ca^{2+} release, the peptide should have strong effects on the excitation produced by $InsP_3$ injection. This form of excitation also was relatively insensitive to CMKII 290–309 (Fig. 3). This indicates that the primary action of Ca^{2+}/calmodulin-binding peptide is upstream from the production of $InsP_3$ in the cascade.

We next examined the excitation produced at an early step of the transduction by using the G protein activator GTP[γS] (30). After injection of this activator into the cell and stimulation of guanine nucleotide exchange by light there was a sustained increase in the frequency of discrete events in the dark, as previously reported for this and other G protein activators (17, 31, 32). Fig. 5 shows that the frequency of these events was greatly reduced by CCBP, in parallel with the reduction in the response to light. The reduction in event rate because of CMKII 290–309 was observed in three additional experiments. We lack sufficient data to characterize the rare GTP[γS]-induced events observed during maximal desensitization to light after peptide injection, but they appeared normal in shape and normal, or slightly reduced, in size. We interpret the pattern of change as follows: GTP[γS] directly activates G proteins, bypassing the need for activation by rhodopsin. Active G protein subunits bind and activate PLCβ (33–35). Although the stoichiometry has not been measured, the discrete events likely reflect the activation of individual PLCβ molecules. If Ca^{2+}/calmodulin-binding peptide eliminated the activity of some fraction of G protein or PLCβ, the result would be a reduction in the frequency of discrete events, as observed. However, those activated PLCβ molecules that are not inhibited by CCBP would generate normal-size discrete waves, as observed. We conclude that the CCBP primarily acts downstream from rhodopsin and upstream from $InsP_3$; the results can be explained by an effect of peptide on either G protein or PLCβ.

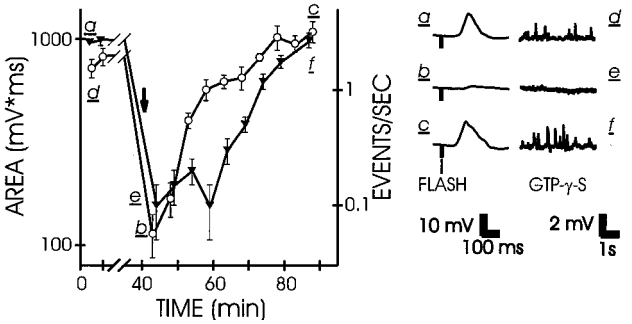

FIG. 5. Comparison of the effects of CMKII 290–309 on the excitation produced by light (open circles, left axis) and by GTP[γS] (solid inverted triangles, right axis). The GTP[γS]-induced activity is quantified by the rate of discrete events. For details see Fig. 2.

CCBPs Inhibit PLC *in Vitro*. The occurrence of a calmodulin-like domain in PLCδ_1 (14) and our results (Figs. 1–3 and 5) suggested the possibility that CCBPs might interact directly with PLC. Therefore, we tested whether the activity of pure PLC isoforms are affected by the CCBPs. Both PLCδ and γ isozymes were strongly inhibited by the CCBPs, VIP and CMKII 290–309 (Fig. 6) that inhibited the light response in our physiological experiments (Figs. 1–3 and 5). The inhibition occurred in the range of 1–100 μM peptide. Control peptide (100 μM) had no effect on PLC activities. PLCβ isozyme, including the norpA PLC involved in invertebrate phototransduction (36, 37), was not available in pure form.

Essen *et al.* (14) noted that sequence similarity occurs between representatives of known PLC isozymes in the calmodulin-like domain and showed that the program PHD predicts α-helical structure consistent with EF-hands in β and γ isoforms. This suggests that the calmodulin-like structure has been conserved in all PLC isoforms.

One interesting possibility is that the calmodulin-like structure in PLC binds to some other region of the enzyme that resembles a calmodulin-binding peptide. If this linkage exists, it may be necessary for enzyme activity, because deletion of the calmodulin-like domain abolishes activity (38–40). This type of an interaction might be broken by calmodulin as well as by Ca^{2+}/calmodulin-binding peptides. Consistent with this prediction, addition of 10 μM calmodulin to *in vitro* assays decreased PLCδ_1, δ_3, and γ_1 isoform activities greater than 30%.

DISCUSSION

Our central finding is that the light response of *Limulus* ventral photoreceptors can be desensitized more than two orders of magnitude by CCBPs. At the low light intensities used in this study, there was no evidence of a component of the response that was insensitive to this inhibition. We used two different Ca^{2+}/calmodulin-binding peptides, and both were potent inhibitors of the light response. A third CCBP, M5 (41), has been reported to decrease the light response in *Drosophila* (42, 43).

Our physiological results indicate that the primary site of inhibition by CCBPs is at the G protein/PLC stage of the transduction cascade. Our *in vitro* results by using three pure isoforms of PLC show that CCBPs can directly inhibit PLC activity. Therefore, the *in vivo* effects of CCBPs as calmodulin antagonists appear less specific than previously thought. The use of CCBPs in living cells as probes for calmodulin-dependent processes must be carefully examined.

Given the abundant calmodulin found in invertebrate photoreceptors (9, 10), it is somewhat surprising that CCBPs are effective at inhibiting the light response through their interaction with PLC. However, in the cases of *Drosophila* photoreceptors, brush border epithelia cells, and the brain, the bulk of calmodulin appears to be bound to Ca^{2+}-independent high affinity sites (10, 44, 45). In addition, CCBPs have much lower affinity for calmodulin (μM) compared with Ca^{2+}/calmodulin (nM) (21, 46). Thus, at low levels of Ca^{2+} in dark-adapted cells, the free concentration of any form of calmodulin may be too low to prevent inhibition of PLC by CCBPs. Consistent with this interpretation are the findings by Arnon *et al.* (42, 43) that the CCBP M5 did not inhibit the light response in wild-type *Drosophila* photoreceptors exposed to bright lights in the presence of bath Ca^{2+} (when Ca^{2+} would be high). However, when bath Ca^{2+} or calmodulin was reduced (presumably lowering Ca^{2+}/calmodulin), an inhibition of the light response by M5 was observed.

Although the primary site of action of CCBPs appears to be at PLC, our results suggest that there may be a second site of action late in the transduction cascade. The evidence for a second site is the observation that CCBP produced a small (2-fold) but consistent reduction in the response to $InsP_3$ or Ca^{2+} injection. This result may reflect the depression of free calmodulin levels by CCBPs, consistent with a role for calmodulin in the late stages of transduction. Further work will be needed to understand the mechanism of this effect.

Because the amino-terminal domain of PLC contains a region that strongly resembles the three-dimensional structure of Ca^{2+}/calmodulin, it seems likely that CCBPs are binding to this domain and that this domain has a role in regulating enzyme activity. We have also found that pure PLC is inhibited by calmodulin, indicating that there is a region of the enzyme with calmodulin-binding properties. Together these results suggest a possible model of PLC function in which the endogenous calmodulin-like domain binds to the endogenous calmodulin-binding domain; disruption of this linkage by either calmodulin or CCBPs leads to inhibition of enzyme activity.

Our finding that PLC activity can be inhibited by Ca^{2+}/calmodulin suggests a new site for the control of PLC transduction cascades. Negative feedback by Ca^{2+} is important for reducing transduction gain when photoreceptors become light-adapted (15, 26, 46). Roles for calmodulin in this process have been inferred from the effects on the *Limulus* photoresponse

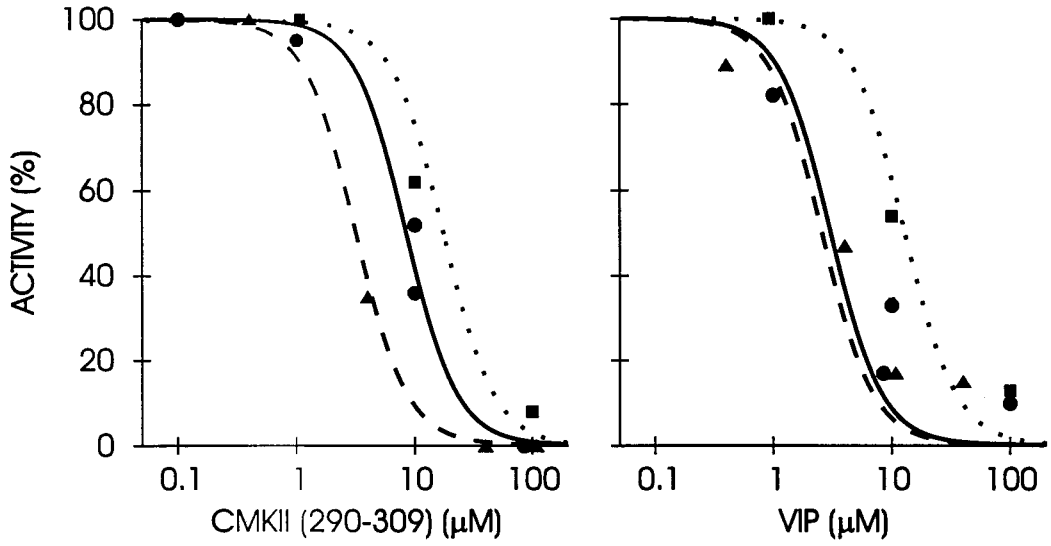

FIG. 6. Inhibitory effects of Ca^{2+}/calmodulin-binding peptides CMKII 290–309 and VIP on phospholipases C δ_1 (circles), δ_3 (triangles), and γ_1 (squares). Data points represent the mean of duplicate assays normalized to controls lacking peptide.

of inhibiting Ca^{2+}/calmodulin-dependent protein kinase II and calcineurin (47) and electrophysiology of *Drosophila ninaC* mutants (48, 49). Work on head membrane extracts from *Drosophila* has found that high Ca^{2+} exerts an inhibitory effect on PLC activity (50, 51). Our results suggest that this may be a direct effect of Ca^{2+}/calmodulin on PLC. Because PLC activity can lead to rapid Ca^{2+} release in photoreceptors and other cell types (for reviews see refs. 12 and 13), the inhibition of PLC by Ca^{2+}/calmodulin could provide negative feedback needed to limit further Ca^{2+} release.

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1. Borsellino, A. & Fuortes, M. G. F. (1968) *J. Physiol.* **196,** 507–539.
2. Brown, J. E., Rubin, L. J., Ghalayini, A. J., Tarver, A. P., Irvine, R. F., Berridge, M. J. & Anderson, R. E. (1984) *Nature (London)* **311,** 160–163.
3. Brown, J. E. & Rubin, L. J. (1984) *Biochem. Biophys. Res. Commun.* **125,** 1137–1142.
4. Payne, R., Corson, D. W., Fein, A. & Berridge, M. J. (1986) *J. Gen. Physiol.* **88,** 127–142.
5. Payne, R. & Fein, A. (1987) *J. Cell Biol.* **104,** 933–937.
6. Fein, A., Payne, R., Corson, D. W., Berridge, M. J. & Irvine, R. F. (1984) *Nature (London)* **311,** 157–160.
7. Bacigalupo, J., Johnson, E. C., Vergara, C. & Lisman, J. E. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7938–7942.
8. Johnson, E. C., Robinson, P. R. & Lisman, J. E. (1986) *Nature (London)* **324,** 468–470.
9. de Couet, H. G., Jablonski, P. P. & Perkin, J. L. (1989) *Cell Tissue Res.* **244,** 315–319.
10. Porter, J. A., Yu, M., Doberstein, S. K., Pollard, T. D. & Montell, C. (1993) *Science* **262,** 1038–1042.
11. Shin, J., Richard, E. A. & Lisman, J. E. (1993) *Neuron* **11,** 845–855.
12. Minke, B. & Selinger, Z. (1992) in *Sensory Transduction*, eds. Corey, D. P. & Roper, S.D., (Rockefeller University Press, New York), pp. 201–217.
13. Noh, D. Y., Shin, S. H. & Rhee, S. G. (1995) *Biochim. Biophys. Acta* **1242,** 99–113.
14. Essen, L. O., Perisic, O., Cheung, R., Katan, M. & Williams, R. L. (1996) *Nature (London)* **380,** 595–602.
15. Lisman, J. E. & Brown, J. E. (1972) *J. Gen. Physiol.* **59,** 701–719.
16. Corson, D. W. & Fein, A. (1983) *Biophys. J.* **44,** 299–309.
17. Bolsover, S. R. & Brown, J. E. (1982) *J. Physiol.* **332,** 325–342.
18. Ghosh, S. & Lowenstein, J. M. (1996) *Gene* **176,** 249–255.
19. Ghosh, S., Pawelczyk, T. & Lowenstein, J. M. (1997) *Protein Expression Purif.* **9,** 262–278.
20. Payne, M. E., Fong, Y. L., Ono, T., Colbran, R. J., Kemp, B. E., Soderling, T. R. & Means, A. R. (1988) *J. Biol. Chem.* **263,** 7190–7195.
21. Hanley, R. M., Means, A. R., Ono, T., Kemp, B. E., Burgin, K. E., Waxham, N. & Kelly, P. T. (1987) *Science* **237,** 293–297.
22. Fernandez, A., Mery, J., Vandromme, M., Basset, M., Cavadore, J. C. & Lamb, N. J. (1991) *Exp. Cell Res.* **195,** 468–477.
23. Stallwood, D., Brugger, C. H., Baggenstoss, B. A., Stemmer, P. M., Shiraga, H., Landers, D. F. & Paul, S. (1992) *J. Biol. Chem.* **267,** 19617–19621.
24. Payne, R., Corson, D. W. & Fein, A. (1986) *J. Gen. Physiol.* **88,** 107–126.
25. Martell, A. E. & Smith, R. M. (1974) *Critical Stability Constants: Amino Acids* (Plenum, New York), Vol. 1.
26. Lisman, J. E. & Brown, J. E. (1975) *J. Gen. Physiol.* **66,** 489–506.
27. Frank, T. M. & Fein, A. (1991) *J. Gen. Physiol.* **97,** 697–723.
28. Ukhanov, K. Y., Flores, T. M., Hsiao, H. S., Mohapatra, P., Pitts, C. H. & Payne, R. (1995) *J. Gen. Physiol.* **105,** 95–116.
29. O'Day, P. M. & Gray-Keller, M. P. (1989) *J. Gen. Physiol.* **93,** 473–494.
30. Pfeuffer, T. & Helmreich, E. J. M. (1975) *J. Biol. Chem.* **250,** 867–876.
31. Fein, A. & Corson, D. W. (1981) *Science* **212,** 555–557.
32. Erickson, M. A., Robinson, P. & Lisman, J. (1992) *Science* **257,** 1255–1258.
33. Devary, O., Heichal, O., Blumenfeld, A., Cassel, D., Suss, E., Barash, S., Rubinstein, C. T., Minke, B. & Selinger, Z. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6939–6943.
34. Hepler, J. R., Kozasa, T., Smrcka, A. V., Simon, M. I., Rhee, S. G., Sternweis, P. C. & Gilman, A. G. (1993) *J. Biol. Chem.* **268,** 14367–14375.
35. Smrcka, A. V. & Sternweis, P. C. (1993) *J. Biol. Chem.* **268,** 9667–9674.
36. Bloomquist, B. T., Shortridge, R. D., Schneuwly, S., Perdew, M., Montell, C., Steller, H., Rubin, G. & Pak, W. L. (1988) *Cell* **54,** 723–733.
37. Pearn, M. T., Randall, L. L., Shortridge, R. D., Burg, M. G. & Pak, W. L. (1996) *J. Biol. Chem.* **271,** 4937–4945.
38. Bristol, A., Hall, S. M., Kriz, R. W., Stahl, M. L., Fan, Y. S., Byers, M. G., Eddy, R. L., Shows, T. B. & Knopf, J. L. (1988) *Cold Spring Harbor Symp. Quant. Biol.* **53,** 915–920.
39. Ellis, M. V., Carne, A. & Katan, M. (1993) *Eur. J. Biochem.* **213,** 339–347.
40. Ghosh, S. (1996) Ph.D. thesis (Brandeis University, Waltham, MA).
41. Kennelly, P. J., Edelman, A. M., Blumenthal, D. K. & Krebs, E. G. (1987) *J. Biol. Chem.* **262,** 11958–11963.
42. Arnon, A., Cook, B., Montell, C., Selinger, Z. & Minke, B. (1997) *Science* **275,** 1119–1121.
43. Arnon, A., Cook, B., Gillo, B., Montell, C., Selinger, Z. & Minke, B. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 5894–5899.
44. Alexander, K. A., Cimler, B. M., Meier, K. E. & Storm, D. R. (1987) *J. Biol. Chem.* **262,** 6108–6113.
45. Glenney, J. R. J., Bretscher, A. & Weber, K. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 6458–6462.
46. Payne, R., Walz, B., Levy, S. & Fein, A. (1988) *Philos. Trans. R. Soc. London. B* **320,** 359–379.
47. Kass, L. & Bray, W. O. (1996) *J. Photochem. Photobiol. B* **35,** 105–113.
48. Hofstee, C. A., Henderson, S., Hardie, R. C. & Stavenga, D. G. (1996) *Visual Neurosci.* **13,** 897–906.
49. Porter, J. A., Minke, B. & Montell, C. (1995) *EMBO J.* **14,** 4450–4459.
50. Inoue, H., Yoshioka, T. & Hotta, Y. (1988) *J. Biochem. (Tokyo)* **103,** 91–94.
51. Running Deer, J. L., Hurley, J. B. & Yarfitz, S. L. (1995) *J. Biol. Chem.* **270,** 12623–12628.