Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 2943–2947, April 1989
Neurobiology

Protein kinase C is not necessary for peptide-induced suppression of M current or for desensitization of the peptide receptors

(phorbol esters/sympathetic neurons/substance P/whole-cell voltage clamp/staurosporine)

MARTHA M. BOSMA AND BERTIL HILLE

Department of Physiology and Biophysics, SJ-40, University of Washington Medical School, Seattle, WA 98195

Contributed by Bertil Hille, January 23, 1989

ABSTRACT **Frog sympathetic ganglion cells were studied under whole-cell voltage clamp to determine whether protein kinase C (PKC) mediates peptide-induced suppression of M current (I_M) or desensitization of peptide receptors. Low concentrations (10 nM) of chicken II luteinizing hormone-releasing hormone (LHRH) or substance P (SP) suppressed I_M; in addition, higher concentrations (1 μM) desensitized receptors. Desensitization is homologous (specific to the peptide) and lasts at least 25 min. Two stimulators of PKC, phorbol 12-myristate 13-acetate and dioctanoylglycerol, partially depressed I_M and occluded the response to SP but not to LHRH. The two actions of PKC stimulators were blocked by PKC inhibitors (staurosporine, a pseudosubstrate peptide, and H-7), but SP- and LHRH-mediated suppression of I_M and receptor desensitization were not affected. Thus, we conclude that PKC is not necessary for normal I_M suppression or receptor desensitization.**

Agonist-dependent modulation of synaptic efficacy is a common theme in the integration of information within the nervous system. In sympathetic and hippocampal neurons, smooth muscle cells, and the neuroblastoma cell line NG108-15, excitability is affected by a specific agonist-modulated voltage-dependent K current (for review, see ref. 1). This outward current is called M current (I_M) because it was first shown to be suppressed by acetylcholine acting on muscarinic receptors (2). I_M normally stabilizes the membrane potential of the cell so that suppression of I_M enhances excitatory synaptic inputs. In frog sympathetic neurons, I_M is suppressed by some peptide neurotransmitters, including luteinizing hormone-releasing hormone (LHRH) and substance P (SP), giving a slow and long-lasting response termed the late slow excitatory postsynaptic potential (3–8). The SP response in frog sympathetic neurons undergoes desensitization (8), and we describe here desensitization of the LHRH response as well.

Attempts have been made to find specific second messengers underlying receptor–I_M channel coupling. In frog sympathetic ganglion cells, coupling requires a pertussis toxin-insensitive GTP binding protein (G protein) (9), and agonist action is accompanied by an increased turnover of inositol phospholipids and a transient increase in intracellular free calcium $[Ca]_i$ (10). However, neither buffering $[Ca]_i$ to high or low levels nor including inositol trisphosphate ($InsP_3$) in the pipette changes the agonist responses (10, 11). In frog sympathetic ganglion cells, smooth muscle, and NG108-15 cells, phorbol esters or diacylglycerols depress I_M partially (10, 12–15), leading one group to propose that protein kinase C (PKC) is the mediator of agonist-induced suppression of I_M (12–14). In hippocampal neurons, however, phorbol esters do not depress I_M, whereas $InsP_3$ added to the pipette does (16). Our study is aimed at clarifying the role of PKC both in the response of I_M to agonist and in desensitization of the peptide receptors that mediate suppression of I_M. We demonstrate that PKC is not the major effector of either action.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

METHODS

Cells and Electrical Recording. Neurons were dissociated from lumbar sympathetic ganglia of *Rana pipiens* (9). Fresh cells were taken out of the culture medium for each new recording. Recordings were made only from the large cells, presumed to be B cells. Recording pipettes had resistances of 1–1.5 MΩ. All potentials were corrected by subtracting a 10-mV junction potential. Experiments were done at room temperature (≈22°C). Whole-cell, voltage clamp currents were recorded with a List EPC-7 patch clamp (List Electronics, Darmstadt, F.R.G.), low-pass filtered at 400 Hz, and recorded digitally using the BASIC-FASTLAB System (Indec Systems, Sunnyvale, CA). I_M was defined by Brown and Adams (2) as a noninactivating voltage-gated K current that is suppresssed by muscarinic agonists. We record I_M by their classical protocol. The cell is held at −35 mV, where other outward K currents inactivate, so that the standing outward current is primarily I_M (see Fig. 1*A*). When the suppression of I_M is quantified in the text, we first correct for leak currents as described (9).

Solutions. Standard Ringer's solution contained 115 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, and 10 mM Hepes buffer (pH 7.4). The pipette (internal) solution contained 100 mM potassium aspartate, 20 mM KCl, 5 mM NaCl, 5 mM Hepes buffer, 1 mM potassium EGTA, 2 mM $MgCl_2$, 1.5 mM potassium ATP, 0.1 mM GTP, and 0.1 mM leupeptin (pH 7.4). Phorbol 12-myristate 13-acetate (PMA) (Sigma), dioctanoylglycerol (diC_8) (Molecular Probes), and staurosporine (Boehringer Mannheim) were dissolved in dimethyl sulfoxide before use, to a final carrier concentration of 0.5–1%. H-7 (Calbiochem) was dissolved in Ringer's solution. Peptides were obtained from Peninsula Laboratories and stored dessicated at −13°C. External solutions were changed by flowing 1 ml of new solution through the 100-μl bath. This took ≈45 s. A peptide inhibitor of PKC [PKC(19–36)] consisting of residues 19–36 of PKC (17) was kindly supplied by Bruce E. Kemp (Melbourne).

RESULTS

Agonist-Induced Suppression of I_M and Receptor Desensitization. The action of SP was dose dependent, with concentrations of 2–30 nM suppressing I_M almost completely. In Fig. 1*A*, perfusion of 2.5 nM SP into the bath suppressed I_M, as

Abbreviations: diC_8, dioctanoylglycerol; LHRH, luteinizing hormone-releasing hormone; t-LHRH, teleost LHRH; cII-LHRH, chicken II LHRH; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; SP, substance P; I_M, M current; G protein, GTP binding protein.

indicated by the decline of the standing current at −35 mV and by the reduced current relaxations in response to the 600-ms hyperpolarizations shown (*Insets*). I_M remained suppressed for as long as the peptide was present and returned when the peptide was washed out. A second application of 2.5 nM SP elicited a second similar response (data not shown). I_M often returned to levels greater than that before agonist application, a transient phenomenon called overrecovery (9).

At higher concentrations of SP, the time course of suppression was different. Fig. 1*B* shows current during bath application of 1 μM SP. During the first minute of SP, the current was suppressed but then returned at a rate of ≈40%/min and overrecovered even while the agonist was still in the bath. This pattern of response was seen in 42/45 cells tested at 1 μM SP. Hence, as others have reported (8, 10), the SP response desensitizes. A slow partial desensitization was sometimes seen with 20 nM SP (2/5 cells), and a rapid desensitization was seen with 300 nM (2/2 cells). Once exposed to a desensitizing dose of SP, cells no longer responded to SP. Fig. 2*A* shows two applications of 1 μM SP. The experiment is similar to those described in Fig. 1, but for clarity we show only the mean current before each hyperpolarizing pulse instead of the detailed time course of the current. In the first SP application, suppression, desensitization, and overrecovery occurred. The agonist was then washed off. When the same concentration of SP was applied 24 min later, it did not suppress I_M. In three other cells, there was no second SP response when tested 11, 23, or 26 min after the first response.

LHRH also suppresses I_M. Teleost LHRH (t-LHRH; Glu-His-Trp-Ser-Tyr-Gly-**Trp-Leu**-Pro-Gly) suppressed I_M at concentrations of 0.5–1.0 μM (Fig. 1*C*) but caused very little desensitization (only ≈7% return of current per min). Washout of t-LHRH allowed return of I_M and overrecovery. However, application of chicken II LHRH (cII-LHRH; Glu-His-Trp-Ser-**His**-Gly-**Trp**-Tyr-Pro-Gly) at the same concentration suppressed I_M, usually to a greater extent than t-LHRH (18), and then desensitized the LHRH response (Fig. 1*D*). Of 32 cells tested, only 1 did not respond to 1 μM cII-LHRH. At 5–20 nM cII-LHRH, there was 70% suppression but little desensitization (n = 4). At 50 and 100 nM, some desensitization always occurred. Chicken I LHRH (Glu-His-Trp-Ser-Tyr-Gly-**Leu-Gln**-Pro-Gly) at concentrations of 1–10 μM had little effect on I_M in three cells, although when two of the cells were tested subsequently, they responded normally to cII-LHRH. The two active LHRH analogs may act at the same receptor(s), since desensitization of cII-LHRH made the response refractory to t-LHRH as well. Fig. 2*B* shows a series of peptide applications. At first, t-LHRH gave a reversible suppression of I_M. cII-LHRH was then applied, I_M was suppressed, and the response was desensitized. Subsequent application of t-LHRH had no effect on I_M.

Desensitization is homologous for each peptide. In Fig. 2*B*, application of cII-LHRH completely suppressed I_M and desensitized the receptors for t-LHRH, but SP was still able both to suppress I_M and to desensitize its receptor (4/4 cells). In other experiments, the order of peptide application was reversed. SP was applied first, which made the cell unresponsive to a second SP application but did not alter the LHRH-induced suppression or desensitization (10/10 cells; see also Fig. 1 *B* and *C*). Finally, the suppression of I_M by muscarine was not affected by previous SP or LHRH receptor desensitization (7/7 cells). Thus, when homologous desensitization of SP or LHRH receptors occurs, there is no desensitization of other receptors.

Stimulation of PKC. The inositol phospholipid pathway is activated by SP and LHRH in these neurons (10), and phorbol esters and diacylglycerols do affect I_M (10, 12–15). Since phosphorylation by PKC has been shown to desensitize other receptors (see *Discussion*) and has also been suggested to mediate agonist-induced suppression of I_M (12–14), we further investigated the role of PKC.

As has been reported previously, agents that stimulate PKC, such as PMA, depressed I_M partially (Fig. 3*A* and *B*). At 1 μM PMA, there was a 63% ± 8% depression (mean ± SEM; n = 7), which is less than the nearly full suppression obtainable with SP or LHRH. As reported before (10, 12), increasing the phorbol ester concentration did not suppress I_M further. Removing PMA from the bath did not lead to any recovery. PMA also had differential effects on the responses to LHRH and SP. In Fig. 3*A*, application of t-LHRH and cII-LHRH after PMA elicited normal suppression of the remaining I_M, with recovery of current either by removal of agonist (t-LHRH) or by receptor desensitization (cII-LHRH). However, as shown in Fig. 3*B*, SP applied after PMA no longer acted on I_M, whereas cII-LHRH caused suppression. Continued exposure to cII-LHRH did desensitize the LHRH response after the long-term recording had been stopped (data not shown). Thus, PMA specifically occluded the SP response (6/6 cells) but did not alter

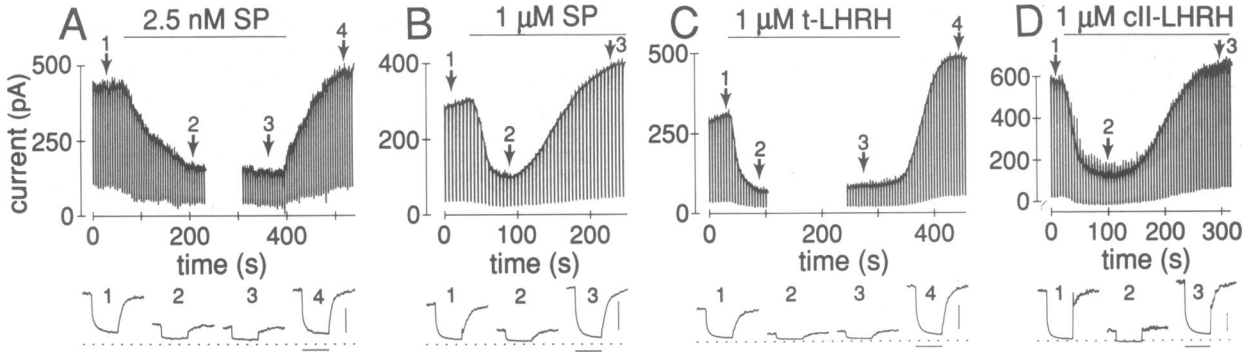

FIG. 1. The actions of SP and LHRH on I_M. Cells were held at −35 mV. Solutions were added to the bath as indicated. When peptides were applied, the holding current was reduced. To confirm that this is actually a suppression of I_M, 600-ms hyperpolarizing pulses to −60 mV were applied every 5 s (downward strokes on slow time scale records). (*Insets*) Current traces recorded during these pulses taken at the numbered arrows are shown on an expanded time scale. I_M can be recognized from its time-dependent relaxations representing a slow closing of channels at −60 mV and a slow reopening at −35 mV. Dashed line indicates zero current. In the blank intervals between sets of pulses, complete current–voltage relations were recorded. (Bars = 200 pA, 600 ms.) (*A*) An application of 2.5 nM SP suppresses I_M reversibly without desensitization. (*B*) An application of 1 μM SP suppresses I_M, rapidly desensitizes the response, and causes overrecovery. The slight upward trend of the recording before agonist addition is caused by the beginning of solution flow around the cells and was seen in many cells even with no addition of agonist. (*C*) Teleost LHRH (t-LHRH) (1 μM) suppresses I_M and desensitizes only very slowly (≈7% return of current per min). Same cell as in *B*, 4 min later. (*D*) Chicken II LHRH (cII-LHRH) (1 μM) suppresses I_M and the response desensitizes rapidly.

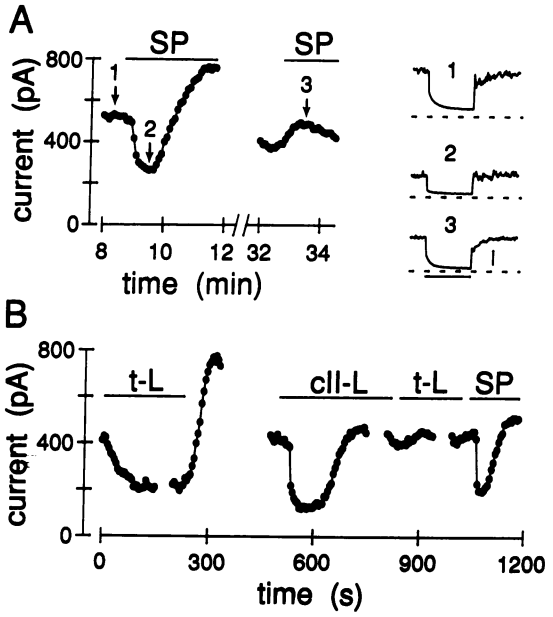

FIG. 2. Desensitization of peptide responses is long lasting and homologous. Each point is an average of the standing current at −35 mV. (*A*) Lasting nature of SP receptor desensitization shown by two applications of 1 μM SP spaced 24 min apart. Time axis shows minutes since breakthrough to whole-cell configuration. (Bars = 200 pA, 600 ms). (*B*) Homologous nature of receptor desensitization shown by successive applications of t-LHRH (t-L), cII-LHRH (cII-L), t-LHRH (t-L), and SP, all at 1 μM. Where the bath solutions were exchanging, we left short gaps in the bars above the currents.

suppression of I_M by either t-LHRH (2/2 cells) or cII-LHRH (5/5 cells).

A stable analogue of diacylglycerol, diC_8, gave similar results but was nearly 100 times less potent. A maximal 61% ± 10% depression of I_M was seen at 90 μM diC_8 (n = 6), and

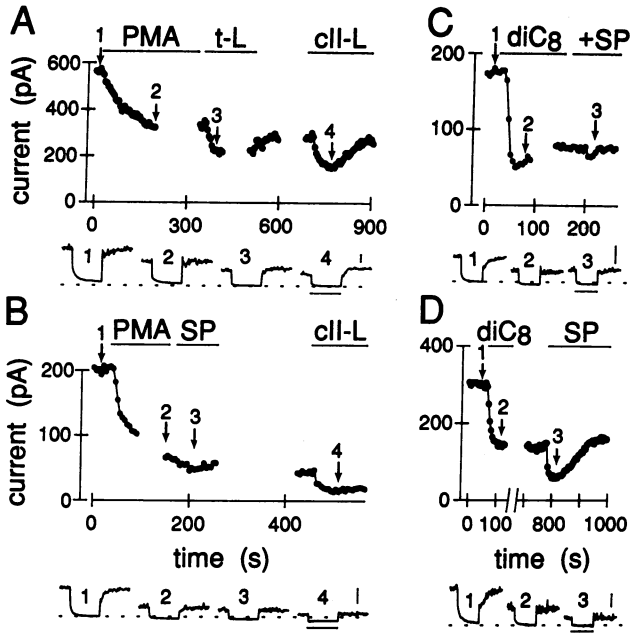

FIG. 3. Stimulators of PKC depress I_M and occlude SP responses. The peptides were applied at 1 μM. (Bars = 100 pA, 600 ms.) (*A* and *B*) Treatment with 1 μM PMA for 3–5 min depressed I_M slowly and blocked responses to SP but not to LHRH. (cII-LHRH is denoted cII-L; t-LHRH is denoted t-L.) (*C* and *D*) Exposure to 90 μM diC_8 depressed I_M rapidly and blocked SP responses reversibly. (*C*) diC_8 was applied first, and SP was subsequently applied with diC_8. (*D*) diC_8 was washed out for 10 min before SP was applied.

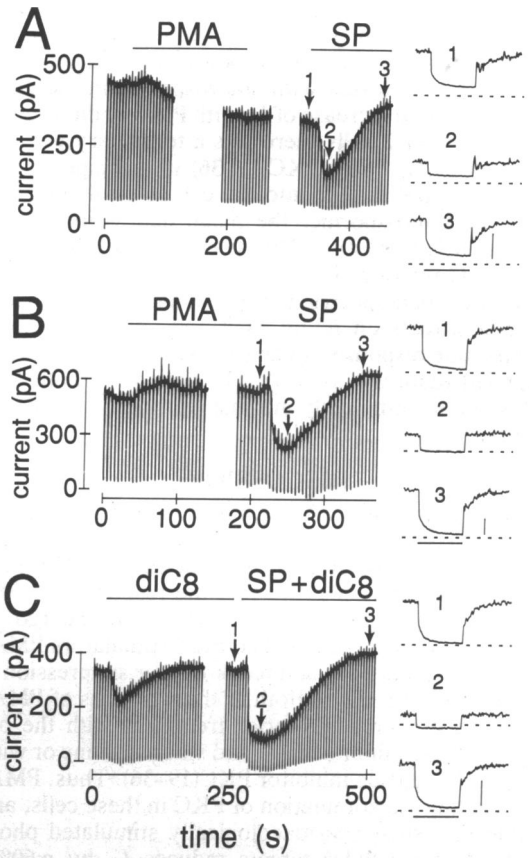

FIG. 4. PKC inhibitors block the depression of I_M by PKC stimulators and allow normal SP responses. Agents applied in the bath were used at the following concentrations: SP, 1 μM; PMA, 1 μM; diC_8, 75 μM. Inhibitors were applied intracellularly via the whole-cell pipette. (Bars = 200 pA, 600 ms.) (*A*) A recording started 20 min after breakthrough to whole-cell mode with a pipette containing 1.5 μM PKC(19–36). (*B*) Twenty minutes after breakthrough with a pipette containing 100 nM staurosporine. (*C*) Sixteen minutes after breakthrough with a pipette containing 150 nM staurosporine.

higher concentrations did not depress I_M more. DiC_8 also occluded the SP response (Fig. 3*C*) (5/5 cells), while LHRH was still able to suppress I_M (data not shown; 2/2 cells). However, in contrast to PMA, the effects of diC_8 were partially reversible. When diC_8 was washed off, in 2/3 cells I_M returned to ≈75% of the levels before diC_8 was added (data not shown). Fig. 3*D* shows one cell in which diC_8 was applied and I_M was depressed. The cell was then extensively washed. Although I_M did not return to control levels in this cell, a normal response of suppression of the remaining I_M and desensitization was seen after SP application.

Inhibition of PKC. To further clarify the role of PKC in receptor–I_M channel coupling and in the desensitization of SP responses, we used three inhibitors of protein kinases. PKC(19–36), a specific pseudosubstrate of PKC with an IC_{50} of 0.2 μM in a cell-free system (17), was used in the pipette at concentrations of 1.5–3 μM. Staurosporine, an inhibitor of PKC with an IC_{50} of 3 nM, and of other kinases at slightly higher concentrations (19), was used in the pipette at 100–200 nM. The third inhibitor, H-7 (20), was applied in the bath at 300 μM.

Fig. 4 shows that including PKC(19–36) or staurosporine in the pipette blocks the effects of activators of PKC. Cells were held for 12–20 min after breakthrough to the whole-cell recording mode to allow the inhibitor to diffuse into the cytoplasm, and the bath was then exchanged for one containing 1 μM PMA (Fig. 4 *A* and *B*) or 75 μM diC_8 (Fig. 4*C*). When the pipette contained either inhibitor, bath application of a PKC stimulator produced only a small depression or even

an increase in I_M. Furthermore, subsequent application of 1 μM SP caused a completely normal sequence of I_M suppression, desensitization of the SP response, and overrecovery of I_M. In 7 of 9 cells tested with staurosporine in the pipette, there was an actual increase of I_M with PMA or diC_8 addition, while in the other 2 cells there was a temporary very small suppression of I_M. With PKC(19–36) in the pipette, which probably does not diffuse into the cell as easily and is less potent than staurosporine, the mean depression of I_M by PMA was 26% ± 9% (n = 10), as compared to almost 65% with no PKC inhibitor in the pipette. Similarly, bath application of the broad-spectrum protein kinase inhibitor H-7 had no adverse effects on I_M by itself and allowed completely normal peptide responses, even with SP (3/3 cells) after PMA was applied (data not shown). The actions of both LHRH peptides were completely normal with any of the PKC inhibitors.

DISCUSSION

The major conclusion of this paper is that PKC is not a necessary mediator of agonist-induced suppression of I_M, of overrecovery, or of homologous desensitization in frog sympathetic ganglia. We find that the responses to SP and LHRH in frog sympathetic neurons can undergo rapid homologous desensitization. The PKC stimulators PMA and diC_8 depress I_M partially and block further suppression of I_M by SP but not by LHRH. Both of these actions of PMA and diC_8 can be prevented by prior treatment with the broad-spectrum kinase inhibitors H-7 and staurosporine or with the PKC-specific peptide inhibitor PKC(19–36). Thus, PMA and diC_8 are acting via stimulation of PKC in these cells, and we conclude that such pharmacologically stimulated phosphorylation of intracellular targets reduces I_M by ≈60% and eliminates responses to SP but not to LHRH. Because we can block the effects of PMA and diC_8 with H-7, staurosporine, and PKC(19–36) (Fig. 4), we are confident that these compounds have actually inhibited PKC in our experiments. Blocking PKC does not prevent any of the effects of agonists, and strongly stimulating PKC does not facilitate or fully mimic effects of agonists. Thus, some pathway(s) other than PKC mediates the suppression of I_M and the desensitization normally caused by SP and LHRH.

Many studies show that transmitter responses can be desensitized when PKC is stimulated by phorbol esters or diacylglycerols. For peptides, examples include responses to angiotensin II, vasopressin, and SP in smooth muscle, to leukotriene D_4 in RBL cells, and to immunoglobulin in lymphocytes (21–26). Extracellular staurosporine blocks the action of diC_8 on immunoglobulin responses at 20 nM and the action of PMA on leukotriene D_4 responses with an IC_{50} of 900 nM (25, 26). Nevertheless, at least in the case of the SP and immunoglobulin responses, these studies concluded that the PKC pathway was not physiologically the most important one for desensitization. For adrenergic receptors an extensive literature has shown that there is phosphorylation of receptors in parallel with the development of desensitization (27–29).

How does PKC interfere selectively with responses to SP? Pfaffinger (9) has shown that suppression of I_M by muscarine and LHRH is mediated by pertussis toxin-insensitive G protein(s). We have found, using various GTP analogs in the pipette, that the SP response also requires G protein(s) (M.M.B., unpublished data). The similarity of the actions of SP, LHRH, and muscarine suggest that they suppress I_M by some final common pathway. Therefore, the selective action of PKC on the response to SP would have to be at an early step before convergence on the common pathway. One possibility is that PKC acts directly on the SP receptor, selectively desensitizing it. If the LHRH and muscarinic acetylcholine receptors are also phosphorylated by PKC, this modification does not desensitize them. An alternative hypothesis is a selective phosphorlyation of G proteins (30). It is possible that several different G proteins can activate the final pathway and that the SP receptor activates only a subset of G proteins that can be modified or uncoupled by PKC. The other receptors may use this same subset and others that are not PKC modified. This hypothesis does not postulate a change at the receptor level.

There is another mechanism for desensitization that does not involve kinases sensitive to the inhibitors that we have used. Rhodopsin kinase and β-adrenergic receptor kinase are enzymes that recognize light-activated rhodopsin and agonist-occupied adrenergic receptor, respectively, as substrates and initiate homologous receptor desensitization without the involvement of G proteins or other second messengers (31, 32). This class of mechanism is consistent with our results and could be used to rationalize the high agonist concentrations needed to desensitize receptors. A few nanomolar SP or cII-LHRH suffices to suppress I_M, but almost 100 times as much is needed for rapid homologous desensitization. Micromolar levels of t-LHRH or muscarine are needed to suppress I_M, and those concentrations induce only slow desensitization (9). Our hypothesis supposes that as little as 1% occupancy of SP or LHRH receptors may suffice to activate the G protein(s) necessary for suppressing I_M. If the action of a receptor kinase is proportional to receptor occupancy and independent of G protein activation, then the rate of desensitization could be increased 100-fold by raising the agonist concentration without appreciably increasing the speed or extent of I_M suppression.

If PKC is activated during an agonist response, its actions on I_M are short-lived and not essential. As muscarine, SP, and LHRH activate phospholipase C in these cells (10), it is probable that PKC would be activated by these agonists. Perhaps this would lead to a depression of I_M and a heterologous loss of SP responsiveness superimposed on the effects of the other non-PKC pathway(s). These phosphorylations, if they exist, must normally be reversed within less than a minute of removing agonist, since I_M returns quickly when agonist is washed off and the SP response is not occluded after an exposure to LHRH (Fig. 2*B*). If PKC is not the normal mechanism for suppressing I_M, what is? Both H-7 and staurosporine inhibit several kinases in a concentration range similar to their effects on PKC. We do not have an internal control to prove that these other enzymes are inhibited in our experiments; nevertheless, our results would not favor mechanisms requiring these kinases. One class of mechanism for suppression of I_M that remains plausible and untested is a direct interaction between activated G protein(s) and the channel.

Whatever the mechanism of homologous peptide receptor desensitization, it is long lasting. It is possible that cellular mechanisms exist that could augment or reverse the desensitization of a response. If such mechanisms were regulated in an associative manner by other neurotransmitters, desensitization could be an important substrate for activity-dependent neural plasticity.

We thank Lea Miller and Don Anderson for valuable technical help and Drs. S. Barnes, L. Bernheim, S. W. Jones, M. D. Leibowitz, W. J. Moody, N. M. Nathanson, and T. Scheuer for very helpful discussions and for reading the manuscript. This work was supported by National Institutes of Health Grant NS08174 and a Neuroscience Research Award from the McKnight Endowment Fund for Neuroscience. M.M.B. was supported by a fellowship from the Muscular Dystrophy Association.

1. Brown, D. A. (1988) *Trends Neurosci.* **11**, 294–299.
2. Brown, D. A. & Adams, P. R. (1980) *Nature (London)* **283**, 673–676.
3. Jan, Y. N., Jan, L. Y. & Kuffler, S. W. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1501–1505.

4. Jan, Y. N., Jan. L. Y. & Kuffler, S. W. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5008–5012.
5. Jan, L. Y. & Jan, Y. N. (1982) *J. Physiol. (London)* **327,** 219–246.
6. Adams, P. R. & Brown, D. A. (1980) *Br. J. Pharmacol.* **68,** 353–355.
7. Adams, P. R., Brown, D. A. & Jones, S. W. (1983) *Br. J. Pharmacol.* **79,** 330–333.
8. Jones, S. W. (1985) *J. Physiol. (London)* **366,** 63–87.
9. Pfaffinger, P. J. (1988) *J. Neurosci.* **8,** 3343–3353.
10. Pfaffinger, P. J., Leibowitz, M. D., Subers, E. M., Nathanson, N. M., Almers, W. & Hille, B. (1988) *Neuron* **1,** 477–484.
11. Adams, P. R., Brown, D. A. & Constanti, A. (1982) *J. Physiol. (London)* **330,** 537–572.
12. Brown, D. A. & Adams, P. R. (1987) *Cell. Mol. Neurobiol.* **7,** 255–269.
13. Higashida, H. & Brown, D. A. (1986) *Nature (London)* **323,** 333–335.
14. Brown, D. A. & Higashida, H. (1988) *J. Physiol. (London)* **397,** 185–207.
15. Clapp, L. H., Sims, S. M., Singer, J. J. & Walsh, J. V. (1988) *Soc. Neurosci. Abstr.* **14,** 1088.
16. Dutar, P. & Nicoll, R. A. (1988) *Neurosci. Lett.* **85,** 89–94.
17. House, C. & Kemp, B. (1987) *Science* **238,** 1726–1728.
18. Jones, S. W. (1987) *Neurosci. Lett.* **80,** 180–184.
19. Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. & Tomita, F. (1986) *Biochem. Biophys. Res. Commun.* **135,** 397–402.
20. Kawamoto, S. & Hidaka, H. (1984) *Biochem. Biophys. Res. Commun.* **125,** 258–264.
21. Satoh, S., Itoh, T. & Kuriyama, H. (1987) *Pflugers Arch.* **410,** 132–138.
22. Aiyar, N., Nambi, P., Whitman, M., Stassen, F. L. & Crooke, S. T. (1987) *Mol. Pharmacol.* **31,** 180–184.
23. Pollock, W. K. & MacIntyre, D. E. (1986) *Biochem. J.* **234,** 67–73.
24. Moskowitz, M. A., Kuo, C., Leeman, S. E., Jessen, M. E. & Derian, C. K. (1987) *J. Neurosci.* **7,** 2344–2351.
25. Vegesna, R. V. K., Wu, H.-L., Mong, S. & Crooke, S. T. (1988) *Mol. Pharmacol.* **33,** 537–542.
26. Cambier, J., Chan, Z.-Z., Pasternak, J., Ransom, J., Sandoval, V. & Pickles, H. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6493–6497.
27. Bouvier, M., Leeb-Lundberg, L. M. F., Benovic, J. L., Carson, M. G. & Lefkowitz, R. J. (1987) *J. Biol. Chem.* **262,** 3106–3113.
28. Bouvier, M., Hausdorff, W. P., DeBlasi, A., O'Dowd, B. F., Kobilka, B. K., Caron, M. G. & Lefkowitz, R. J. (1988) *Nature (London)* **333,** 370–373.
29. Leeb-Lundberg, L. M. F., Cotecchia, S., DeBlasi, A., Carson, M. G. & Lefkowitz, R. J. (1987) *J. Biol. Chem.* **262,** 3098–3105.
30. Katada, T., Gilman, A. G., Watanabe, Y., Bauer, S. & Jakobs, K. H. (1985) *Eur. J. Biochem.* **151,** 431–437.
31. Kuhn, H. & Dreyer, W. J. (1972) *FEBS Lett.* **20,** 1–6.
32. Benovic, J. L., Strasser, R. H., Caron, M. G. & Lefkowitz, R. J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2797–2801.