Activation of Calcium/Calmodulin-dependent Protein Kinase IV in Long Term Potentiation in the Rat Hippocampal CA1 Region*

Received for publication, January 11, 2001, and in revised form, April 10, 2001
Published, JBC Papers in Press, April 16, 2001, DOI 10.1074/jbc.M100247200

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The importance of well characterized calcium/calmodulin-dependent protein kinase (CaMK) II in hippocampal long term potentiation (LTP) is widely well established; however, several CaMKs other than CaMKII are not yet clearly characterized and understood. Here we report the activation of CaMKIV, which is phosphorylated by CaMK kinase and localized predominantly in neuronal nuclei, and its functional role as a cyclic AMP-responsive element-binding protein (CREB) kinase in high frequency stimulation (HFS)-induced LTP in the rat hippocampal CA1 region. CaMKIV was transiently activated in neuronal nuclei after HFS, and the activation returned to the basal level within 30 min. Phosphorylation of CREB, which is a CaMKIV substrate, and expression of c-Fos protein, which is regulated by CREB, increased during LTP. This increase was inhibited mainly by CaMK inhibitors and also by an inhibitor for mitogen-activated protein kinase cascade, although to a lesser extent. Our results suggest that CaMKIV functions as a CREB kinase and controls CREB-regulated gene expression during HFS-induced LTP in the rat hippocampal CA1 region.

Long term potentiation (LTP) in hippocampus (1, 2) is thought to be a model for the molecular mechanism of learning and memory in the mammalian central nervous system. Among several kinds of molecules reported to be involved in LTP (3), protein kinases and phosphatases are particularly important, because the protein phosphorylation and dephosphorylation are essential for the regulation of neuronal functions. We have been studying activation of calcium/calmodulin-dependent protein kinase II (CaMKII; Refs. 4–7), mitogen-activated protein kinase (MAPK; Ref. 7), and protein phosphatase 2A (8) during LTP in the hippocampal CA1 region. CaMKIV (1–7) is particularly implicated in LTP induction, because its activation is accompanied by autophosphorylation (5–7) and the activity is essential for the induction of LTP (9). Although the role of CaMKII in synaptic plasticity has been closely examined by many researchers (1–4), the roles of other CaMK subtypes expressed in neurons are still unknown.

Neurons express at least five known CaMKs: CaMKI (10), CaMKII, CaMKIII (11), CaMKIV (12, 13), and CaMK kinase (14). The activation mechanisms of CaMKI and CaMKIV (14, 15) differ from that of CaMKII; specific threonine residues of CaMKI (Thr177; Ref. 16) and IV (Thr196; Ref. 17) are phosphorylated by CaMK kinase, and phosphorylation is essential for the activation of CaMKI and CaMKIV.

CaMKIV, also called CaMK Gr, is expressed in the nuclei of neurons (18, 19), although its expression is not only limited to the nuclei (19). We previously reported regulation of CaMKIV in cultured rat hippocampal neurons (20, 21) and showed that CaMKIV was activated by N-methyl-D-aspartate glutamate receptor stimulation or high K⁺-induced depolarization (20). Activation of CaMKIV was regulated primarily by protein phosphatases 2A (21–23) and calcineurin (21). Because a major substrate for CaMKIV in the nucleus is cyclic AMP-responsive element-binding protein (CREB; Ref. 24), CaMKIV is thought to regulate gene expression in a neuronal activity-dependent manner. In support of this hypothesis, suppression of CaMKIV expression by antisense oligonucleotides abolished high K⁺- or electrical stimulation-induced CREB phosphorylation in cultured rat hippocampal neurons (25), and CaMKIV-deficient mice showed the decrease in CREB phosphorylation (26, 27). Introduction of a dominant negative CaMKIV or dominant negative CREB into cultured cerebellar Purkinje neurons resulted in defects in the late phase of long term depression in response to glutamate (28). CaMKIV-deficient mice also showed decreased potentiation at a later stage of rat hippocampal CA1 LTP (26). These reports suggest that CaMKIV functions as a CREB kinase and regulates CREB-dependent gene expression in neurons, which may in turn be linked to a protein synthesis-dependent long lasting synaptic plasticity.

To understand the molecular mechanisms of synaptic plasticity and function of CaMKIV, it is essential to know how CaMKIV activity changes following application of conditioning stimuli such as high frequency stimulation (HFS) to induce LTP and how CREB phosphorylation is regulated by CaMKIV and other protein kinases under these conditions. In the present study, we examined CaMKIV activation with simultaneous CREB phosphorylation and stimulation of gene expression during HFS-induced LTP in the rat hippocampal CA1 region.

EXPERIMENTAL PROCEDURES

Materials—The following chemicals and materials were obtained from the indicated sources: [γ-32P]ATP and 125I-protein A from PerkinElmer Life Sciences; calmidazolium (R24517) and H89 from Sigma; KT5720 from Alexis Biochemicals (San Diego, CA); KN93 and KN92 from Seikagaku (Tokyo, Japan); U0126 and U0124 from Calbio-

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* This work was supported by grants-in-aid for scientific research and for scientific research on priority areas from the Ministry of Education, Science, Sports and Culture of Japan and by the Human Frontier Science Program. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: LTP, long term potentiation; CaMK, calcium/calmodulin-dependent protein kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated protein kinase; MEK, MAPK/ERK kinase; CREB, cyclic AMP-responsive element-binding protein; HFS, high frequency stimulation; FITC, fluorescein isothiocyanate; EPSP, excitatory postsynaptic potential; fEPSP, field EPSP; PKA, cyclic AMP-dependent protein kinase; ACSF, artificial cerebrospinal fluid.

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Chem (La Jolla, CA); protein A-Sepharose CL-4B from Amersham Pharmacia Biotech; anti-active MAPK antibody from Promega (Madison, WI); anti-CREB and anti-pCREB antibodies from New England Biolabs (Beverly, MA); anti-ERK2 antibody from Transduction Laboratories (San Diego, CA); anti-e-Fos (Ab-2) antibody from Oncogene (Cambridge, MA); and FTIC-GAPDH anti-rabbit IgG (Ab-1) from Amersham Pharamacia. The thin slices were incubated in Tris-azide buffer containing 1% bovine serum albumin and 0.2% Triton X-100 for 2 h at room temperature and then blocked in PBS containing 10% normal goat serum for 1 h. Bound antibodies were visualized using FITC-conjugated anti-rabbit IgG and analyzed by laser confocal microscopy (Olympus, Tokyo, Japan). Quantification of nuclear CaMKIV phosphorylation was performed as follows. The intensities of the nuclear and cytosolic fluorescence of neurons with FITC were compared in 35–40 neurons, using the Image program, that are located in a 100-μm area between the stimulation and recording electrodes in the CA1 pyramidal cell layer. The number of the neurons in which the intensities of the nuclei are brighter than those of the cytosol was counted. Neurons, which have higher intensities in the nuclei, were defined as positive. The independent experiments of different hippocampal slices were performed four times, and statistical analysis was made.

**RESULTS**

**Activation of CaMKIV, MAPK, and CREB during LTP Inhibition**—We first examined the changes of CaMKIV activity during LTP. Application of HFS to Schaffer-collateral pathways reliably induced the potentiation of fEPSP slope, which lasts over 60 min in the CA1 region (166 ± 17% of baseline at 60 min after HFS; Fig. 1A). CaMKIV activity was determined 3, 10, 30, and 60 min after HFS application and was significantly increased within 3 min (201 ± 12% of control) and 10 min (127 ± 13% of control), returning to basal levels within 30 min after HFS (Fig. 1B). This result was confirmed by immunoblot analysis using the anti-pT196 antibody (21) to assess levels of CaMKIV phosphorylation at Thr196 (Fig. 1C), an essential site for activation of CaMKIV by CaMK kinase. This result is summarized by statistical analysis in Fig. 1D. Phosphorylation of Thr196 was significantly increased 3 min after HFS (226 ± 44% of control) and then returned to the basal level. CaMKIV protein levels were unchanged 3, 10, and 30 min after HFS, although a slight but significant increase (118 ± 7.6% of control; n = 6, p < 0.05) was observed 60 min after HFS and is consistent with the previously reported result (29). We also examined the phosphorylation of p42 mitogen-activated protein kinase (MAPK/extracellular signal-regulated protein kinase 2 (ERK2) and CREB, and phosphorylation of both was detected with phosphospecific antibodies (Fig. 1C, summarized in Fig. 1D). Phosphorylation of p42 MAPK was significantly increased 3 min after HFS (180 ± 15% of control) and then returned to basal levels. Because HFS selectively activated p42 MAPK but not p44 MAPK, changes in phosphorylation of p42MAPK were examined in the present study. This result is consistent with the previous reports (7, 30) that activity of p42 MAPK is transiently increased by HFS in the rat hippocampal CA1 region. In contrast, CREB phosphorylation detected with an anti-phospho-Ser134 antibody increased 3 min (151 ± 14% of control), 10 min (132 ± 10% of control), 30 min (151 ± 24% of control), and 60 min (169 ± 29% of control) after HFS, and the increased phosphorylation was sustained during LTP (Fig. 1D). Although the total amounts of p42 MAPK were unchanged 3 and 10 min after HFS, a slight but significant increase was observed 30 min (116 ± 6.2% of control; n = 6) and 60 min (121 ± 5.9% of control; n = 6) after HFS (p < 0.05). The protein levels of CREB were not significantly changed at any time point after HFS (data not shown).

To determine the localization of activated CaMKIV in neurons, we undertook an immunohistochemical study using the anti-pT196 antibody. In control slices, moderate immunore-
CaMKIV Activation during LTP

**Fig. 1.** Activation of CaMKIV and MAPK with concomitant phosphorylation of CREB during LTP. A, electrophysiological recordings illustrate the average changes in synaptic efficacy in the CA1 region of hippocampal slices (n = 15) produced by HFS (100 Hz, 1-s duration, two times with a 20-s interval between times), which was applied at time 0 (arrow). The averaged fEPSP slope at each time point was normalized to the average base-line response for 20 min before HFS. Inset, representative traces before (left) and 1 h after HFS (right). The calibration bar indicates 1 nV (vertical) and 5 ms (horizontal). B, CaMKIV activity during LTP was determined at the time points indicated in the horizontal label and expressed in comparison with the control. C, representative autoradiograms for phosphorylated CaMKIV (pCaMKIV), phosphorylated p42 MAPK (pMAPK), and phosphorylated CREB (pCREB). After the electrophysiological recordings for the indicated periods with (HFS) or without (Control) HFS, slices were homogenized, and 30 μg of the sample was applied in each lane of SDS-polyacrylamide gel electrophoresis. D, quantitation of the phosphorylation of CaMKIV (black column), p42 MAPK (hatched column), and CREB (gray column). n = 8 for each column. *, p < 0.05; **, p < 0.01.

**Fig. 2.** Immunohistochemical study of HFS-induced CaMKIV activation in CA1 neurons. The slices fixed in 4% paraformaldehyde were treated with the anti-pT196 antibody as described under "Experimental Procedures." A, control slice; B, HFS-applied slice. Both nuclear (arrows) and dendrite (arrowheads) signals were observed. C, HFS-applied slice treated with the anti-pT196 antibody absorbed with the peptide antigen. Note that the cytosolic signal remains but that nuclear and dendritic signals are absent compared with B. The scale bar presented at the bottom of each panel indicates 50 μm. D, comparison of FITC-positive nuclei in 0.01 mm² of CA1. The data from four independent experiments are shown. *, p < 0.01.

Activity was observed in the cytosol (Fig. 2A) but was not competed by absorbance with the peptide antigen (21) (Fig. 2C), indicating that it was nonspecific. The strong nonspecific immunoreactivity in the cytosol would probably mask any small amount of CaMKIV activation in the cytosol. Neuronal nuclei were not stained (Fig. 2A). By contrast, strong immunoreactivity was observed in the nuclei of neurons in HFS-applied slices (Fig. 2B), and that immunoreactivity was absent when the antibody was absorbed with the peptide antigen (Fig. 2C), indicating that it was specific. Although the antibody used in the present study was specific, it seems sometimes that the antibody lacks specificity against non-denatured protein immunocytochemistry. Therefore, the detected immunoreactivity may be called CaMKIV-like immunoreactivity. These results suggest that CaMKIV is activated in the nuclei of CA1 pyramidal neurons by HFS application. Specific immunoreactivity was also observed in the dendrites of neurons (Fig. 2B), because its expression was not limited to nuclei (19). We observed slight expression of CaMKIV in the cytosols and dendrites of cultured neurons (data not shown).

Effect of Calmodulin Antagonist and CaMK Inhibitor on LTP and CREB Phosphorylation—Because it was reported that the major CREB kinase was CaMKIV in response to neuronal stimulation in cultured rat hippocampal neurons (25), we addressed whether CaMKIV functioned as a CREB kinase during LTP. To inhibit CaMKIV activity, we used calmidazolium, a calmodulin antagonist, and KN93, a CaMK inhibitor. Both inhibitors have been previously shown to inhibit CaMKIV activity in cultured rat hippocampal neurons (20). The application of calmidazolium (Fig. 3A) or KN93 (Fig. 3D) inhibited induction of LTP, possibly by inhibiting CaMKII (6, 7). Calmidazolium (n = 7) and KN93 (n = 8) did significantly inhibit CaMKIV phosphorylation without any effect on p42 MAPK phosphorylation (Fig. 3, B and E). HFS-induced CREB phosphorylation was, however, significantly inhibited by both compounds 3 and 30 min after HFS (Fig. 3, B, C, E, and F). By contrast, KN92, an inactive compound similar to KN93, affected neither LTP induction nor CREB phosphorylation (Fig. 3, D and F), indicating specific inhibition by KN93. These results demonstrate a positive correlation between CaMKIV activation and CREB phosphorylation and are consistent with the idea that CaMKIV functions as a CREB kinase during HFS-induced LTP.

Effect of MEK Inhibitor on LTP and CREB Phosphorylation—We next examined the effect of MAPK on HFS-induced CREB phosphorylation. The MAPK cascade is thought to be involved in CREB phosphorylation through the activation of p90 ribosomal S6 kinase 2 (RSK2) (31). We previously reported...
that a high concentration of PD098059, an MEK inhibitor, inhibited LTP induction (7). However, the effect of this inhibitor was positively correlated with inactivation of CaMKII, and therefore we concluded that PD098059 inhibited LTP through inactivation of CaMKII rather than MAPK (7). In this study, we examined the effect of U0126 (32), which specifically inhibits MEK at a lower concentration than PD098059. Under the conditions used, U0126 did not affect the induction of HFS-induced LTP (Fig. 4A), a finding consistent with other reports (33, 34). 10 μM U0126 dramatically inhibited p42 MAPK phosphorylation without any effect on CaMKIV phosphorylation (Fig. 4B). To further inhibit p42 MAPK, 20 and 30 μM U0126 was used, and inhibition of CaMKIV phosphorylation was not observed. On the other hand, CREB phosphorylation was not inhibited 3 min after HFS but was significantly inhibited 30 min after HFS (Fig. 4C). These results suggest that not only CaMKIV but also MAPK pathways function as CREB kinases in HFS-induced LTP. It should be noted that CaMKIV functions as a CREB kinase at a relatively early stage of HFS-induced LTP, whereas MAPK functions at a later point during the time course.

**Effect of PKA Inhibitor on LTP and CREB Phosphorylation**—We examined the effects of H89 and KT5720, inhibitors for PKA, on HFS-induced CREB phosphorylation, because PKA can directly phosphorylate and activate CREB. In our experimental conditions, H89 and KT5720 did not inhibit HFS-induced LTP in the CA1 region (Fig. 5A). In contrast, the mossy fiber CA3 LTP was reduced by PKA inhibitors (Fig. 5B), as previously reported (35), indicating that these inhibitors were active under our experimental conditions. Because H89 has been shown to have nonspecific effects on other kinases than PKA, compared with KT5720, we checked its effect on CaMKIV and MAPK phosphorylation. As shown in Fig. 5C, H89 had no effect on CaMKIV and MAPK phosphorylation. CREB phosphorylation was inhibited neither by H89 nor by KT5720 during LTP in the CA1 region (Fig. 5D). The data from eight (3 min) and seven experiments (30 min) in C and six experiments (3 and 30 min) in F are shown. *, p < 0.05.

**Stimulation of c-Fos Expression during LTP**—To address whether CREB phosphorylation induced by HFS stimulates gene expression, we examined the expression of the c-Fos protein, one of the markers for cyclic AMP-responsive element promoter-mediated gene expression (37). The expression of the c-Fos protein was unchanged 3 and 10 min after LTP induction and then significantly increased 30 (115 ± 10% of control) and 60 (153 ± 13% of control) min after LTP induction (Fig. 6A),
suggesting that the phosphorylated CREB stimulated c-Fos expression. We further tested whether c-Fos expression was regulated by CaMKIV and/or MAPK pathways during LTP. The increase in c-Fos expression at 60 min after HFS was inhibited by the addition of calmidazolium or U0126 (Fig. 6B). Treatment of hippocampal slices with calmidazolium strongly inhibited the HFS-induced c-Fos expression, whereas treatment with U0126 moderately but significantly inhibited c-Fos expression (Fig. 6B). The extent of inhibition by calmidazolium and U0126 was positively correlated with levels of CREB phosphorylation shown in Figs. 3 and 4. These results suggest that both CaMKIV and MAPK function as CREB kinases, which in turn regulate CREB-mediated gene expression in HFS-induced LTP.

**FIG. 4. Effect of a MEK inhibitor on LTP and CREB phosphorylation.** A, changes in fEPSP slopes before and after the application of HFS (arrow) in the CA1 region. The slices were treated with no drug (○, n = 23) or 10 μM U0126 (●, n = 10) for 1 h before and throughout the recording periods. B, representative autoradiograms for phosphorylated CaMKIV (pCaMKIV), phosphorylated p42 MAPK (pMAPK), and phosphorylated CREB (pCREB). The upper and lower panels show samples 3 and 30 min after HFS, respectively. C, quantitation of CREB phosphorylation. The data from eight (3 min) and seven (30 min) experiments are shown. *, p < 0.05.

**FIG. 5. Effect of PKA inhibitors on LTP and CREB phosphorylation.** A and B, changes in fEPSP slopes before and after the application of HFS (arrow) in the CA1 (A) and the CA3 (B) regions. The slices were treated with no drug (○, n = 26 in A and n = 8 in B), 5 μM H89 (▲, n = 9 in A and n = 10 in B), or 10 μM KT5720 (▲, n = 4 in A and n = 5 in B) for 1 h before and throughout the recording periods. C, representative autoradiograms for phosphorylated CaMKIV (pCaMKIV), phosphorylated p42 MAPK (pMAPK), and phosphorylated CREB (pCREB) in CA1 LTP. The upper and lower panels show the samples at 3 and 30 min after HFS, respectively. D, quantitation of CREB phosphorylation during LTP in the CA1 region (n = 13 in control and HFS, n = 9 in HFS + H89, and n = 4 in HFS + KT5720)

**Discussion**

Here we show that CaMKIV is activated accompanied with phosphorylation of Thr^{196} with CREB phosphorylation and stimulation of c-Fos expression during LTP. To our knowledge, this is the first report to show that CaMKIV is activated and involved in stimulation of gene expression during LTP in the hippocampal CA1 region.

The activation of CaMKIV shown in Fig. 1 was transient and returned to the original level in a short time. This activation pattern differed from that of CaMKII, which showed a long lasting activation during LTP (5, 6). In our previous studies, the difference in activation patterns of CaMKII (21, 38) and IV (21) was also observed in cultured hippocampal neurons in
response to glutamate receptor stimulation. Because transient activation of CaMKIV was also reported in Jurkat T cells (22) in response to T cell receptor stimulation, it may be common in most cell types. One explanation for the difference in activation of CaMKII and CaMKIV may be that the latter is dephosphorylated by Ca\(^{2+}\)/calmodulin-dependent protein phosphatase 2B (calcineurin; Ref. 21), whereas CaMKII cannot be dephosphorylated directly by calcineurin (39). Elevation of intracellular Ca\(^{2+}\) is required to activate CaMKIV. Such elevation activates calcineurin, which may rapidly dephosphorylate and inactivate phosphorylated CaMKIV, which differs from the persistent activation of CaMKII.

Although activation of CaMKIV was transient, the present study suggests that CaMKIV acts as a CREB kinase. Experiments with a calmodulin antagonist and protein kinase inhibitors demonstrated that CaMKs are mainly involved in phosphorylation of CREB, with a slight phosphorylation by MAPK, and that PKA has no effect on phosphorylation of CREB. Among CaMKs, the contribution of CaMKII to CREB phosphorylation may be excluded, because c-Fos expression was stimulated during LTP in the present study, and phosphorylation of CREB by CaMKII reportedly had inhibitory effects on gene expression (40). These results were consistent with a recent report (26) that showed reduced CREB phosphorylation and no significant potentiation of fEPSP at 45 min after LTP in hippocampal slices of CaMKIV-deficient mice with normally expressed CaMKII. On the other hand, the c-fos promoter the contains cyclic AMP-responsive element and other motifs such as the serum response element. Because the serum response element was reported to be involved in activation of c-fos promoter by CaMKII (41), stimulation of c-Fos protein expression may also occur during LTP. Because we have no selective inhibitors for CaMK subtypes, it was not possible to identify which subtypes of CaMK are involved in HFS-induced stimulation of c-Fos protein expression.

Involvement of MAPK cascade in hippocampal LTP (7, 30, 33, 34) and learning and memory (42) was recently reported. Consistent with these reports, we confirmed that MAPK was transiently activated in CA1 LTP. It should be noted that MAPK seems to function as a CREB kinase at a later stage of LTP, although MAPK activation was transient. Although CaMKIV and MAPK are transiently activated, such activation in the context of a signaling cascade would be sufficient to trigger changes in gene expression. CaMKIV can directly phosphorylate CREB, which may enable CaMKIV to function as a CREB kinase at a relatively early stage in LTP. MAPK phosphorylates RSK2, which in turn phosphorylates CREB, and the time lag required for the signal transduction to occur may be too great to allow the MAPK cascade to function as a CREB kinase at an early rather than a late stage of LTP. Because c-Fos expression observed 60 min after HFS was inhibited by both inhibitors for CaMKIV and MAPK, activation of both enzymes observed during LTP is enough to be involved in gene expression.

To form long term memory, newly synthesized synaptic components and the formation of a new circuit such as morphologically changed spines may be required (43). This indicates that the stimulation of gene expression and consequent protein synthesis are needed for the formation of memory. In view of the observation on CREB phosphorylation and stimulation of c-Fos expression by CaMKIV and MAPK in the present study, activation of both enzymes may be associated with LTP maintenance and memory formation.

Recent reports showed that PKA is activated during LTP in the CA1 region (44) and is involved in the late phase of LTP (45) and CREB-stimulated gene expression (46). These reports differed from results presented here, because the PKA inhibitors had no effect on LTP induction and CREB phosphorylation. These discrepancies could be due to several reasons. First, there were differences in conditions used to induce LTP in ours and previous studies. In the previous studies, more than twice repetitive 100 Hz trains with 5–10-min intervals (44–46) or low frequency stimulation of 5 Hz (33, 34) were used to induce LTP, which was blocked by inhibitors for PKA (33, 44–46) and MAPK (33, 34), whereas here, LTP induced by two trains of 100 Hz stimulation with a 20-s interval was not inhibited by these inhibitors (33). There is no doubt that conditional changes in the experiments would bring about different results. Second, other reports showed that PKA is involved in LTP maintenance 3 h after HFS but not involved in LTP induction (36, 45). Because the effect of a PKA inhibitor was examined at the early stage of LTP in the present study, the results were consistent with these reports. Third, it has been reported that protein phosphatases, especially protein phosphatase 1, are involved in LTP induction in the PKA pathway (47). In our experimental conditions, however, it seems that PKA may not be activated,

**Fig. 6. Expression of c-Fos during LTP.** A, c-Fos expression was examined during the time course of LTP. The samples were collected at the indicated time points after HFS, and c-Fos expression was determined with a bio-imaging analyzer for autoradiograms. *, p < 0.05; **, p < 0.01. The data from six experiments are shown. B, effects of protein kinase inhibitors on c-Fos expression. All of the samples were collected 60 min after HFS. The slices were treated with no drug (control) and 50 μM calmidazolium (CMZ) for 2–3 h and with 10 μM U0126 for 1–2 h before and throughout the recording periods. The data from five (calmidazolium) and eight (U0126) experiments are shown.
because the PKA inhibitors had no effect on HFS-induced LTP and CREB phosphorylation. In other words, phosphorylation of inhibitor 1 by PKA may not have occurred under the conditions of the present study.

In conclusion, the present study showed that CaMKIV activation was associated with LTP induction and had a positive correlation to CREB phosphorylation and up-regulation of c-Fos expression. Further study will be required to clarify how the actual targets of CaMKIV and MAPK function to alter gene expression required to form long lasting memory.

Acknowledgments—We thank Dr. J. Liu (University of Texas Medical Branch, Galveston, TX) for comments and Dr. I. Itoh and R. Kawakami (Kyushu University, Fukuoka, Japan) for technical advice.

REFERENCES
1. Bliss, T. V. P., and Collingridge, G. L. (1993) Nature 361, 31–39
2. Malenka, R. C., and Nicoll, R. A. (1999) Science 285, 1870–1874
3. Sanes, J. R., and Lichtman, J. W. (1999) Nat. Neurosci. 2, 597–604
4. Fukunaga, K., and Miyamoto, E. (2000) Neurosci. Res. 38, 3–17
5. Fukunaga, K., Stoppari, N., Miyamoto, E., and Muller, D. (1993) J. Biol. Chem. 268, 7683–7687
6. Fukunaga, K., Muller, D., and Miyamoto, E. (1995) J. Biol. Chem. 270, 6119–6124
7. Liu, J., Fukunaga, K., Yamamoto, H., Nishi, K., and Miyamoto, E. (1999) J. Neurosci. 19, 8292–8299
8. Fukunaga, K., Muller, D., Ohimitsu, M., Bakó, E., DePaoli-Roach, A. A., and Miyamoto, E. (2000) J. Neurochem. 74, 807–817
9. Silva, A. J., Stevens, C. F., Tonegawa, S., and Wang, Y. (1992) Science 257, 206–211
10. Nairn, A. C., and Greengard, P. (1987) J. Biol. Chem. 262, 7273–7281
11. Nairn, A. C., Bhagat, R., and Palfrey, H. C. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 7939–7943
12. Sikela, J. M., and Hahn, W. E. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 3038–3042
13. Ohmstedt, C. A., Jensen, K. F., and Sahyoun, N. E. (1989) J. Biol. Chem. 264, 5866–5877
14. Tokumitsu, H., Brickey, D. A., Gidw, J., Hidaka, H., Sikela, J., and Soderling, T. R. (1994) J. Biol. Chem. 269, 28640–28647
15. Soderling, T. R. (1999) Trends Biochem. Sci. 24, 232–236
16. Haribabu, B., Hook, S. S., Selbert, M. A., Goldstein, E. G., Tomhave, E. D., Edelman, A. M., Snyderman, R., and Means, A. R. (1995) EMBO J. 14, 3679–3686
17. Serbert, M. A., Anderson, K. A., Huang, Q.-H., Goldstein, E. G., Means, A. R., and Sweeney, A. M. (1993) J. Biol. Chem. 278, 17016–17021
18. Miyano, O., Kameshita, I., and Fujisawa, H. (1992) J. Biol. Chem. 267, 1198–1203
19. Jensen, K. F., Ohmstedt, C. A., Fisher, R. S., and Sahyoun, N. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2850–2853
20. Kasahara, J., Fukunaga, K., and Miyamoto, E. (2000) J. Neurosci. Res. 59, 594–600
21. Kasahara, J., Fukunaga, K., and Miyamoto, E. (1999) J. Biol. Chem. 274, 9061–9067
22. Park, I.-K., and Soderling, T. R. (1995) J. Biol. Chem. 270, 30464–30469
23. Westpharl, R. S., Anderson, K. A., Means, A. R., and Wadzinski, B. E. (1998) Science 280, 1288–1261
24. Enslen, H., Sun, P., Brickey, D., Soderling, S. H., Klamo, E., and Soderling, T. R. (1994) J. Biol. Chem. 269, 15520–15527
25. Bito, H., Deisseroth, K., and Tsien, R. W. (1996) Cell 87, 1203–1214
26. Ho, N., Liaw, J., Blaese, F., Wei, F., Hanisian, S., Muglia, L. M., Wozniak, D. F., Nardi, A., Arvin, K. L., Holtzman, D. M., Linden, D. J., Zhuo, M., Muglia, L. J., and Chatila, T. A. (2000) J. Neurosci. 20, 6449–6472
27. Ribe, T. J., Rodriguiz, R. M., Khiroug, L., Wetsel, W. C., Augustine, G. J., and Means, A. R. (2000) J. Neurosci. 20, RC107, 1–5
28. Ahn, S., Ginty, D. D., and Linden, D. J. (1999) Neuron 23, 559–568
29. Tokuda, M., Ahmed, B. Y., Lu, Y.-F., Matsui, M., Miyamoto, O., Yamaguchi, F., Konishi, R., and Hatase, O. (1997) Brain Res. 755, 162–166
30. English, J. D., and Sweatt, J. D. (1997) J. Biol. Chem. 272, 19103–19106
31. Xing, J., Ginty, D. D., and Greenberg, M. E. (1996) Science 273, 959–963
32. Favata, M., Horiusch, K. Y., Manos, E. J., Daulerio, A. J., Stradley, D. A., Freaser, W. S., Van Dyk, D. E., Pfitz, W. J., Earl, R. A., Hobbs, F., Copeland, R. A., Magolda, R. L., Scherle, P. A., and Traasjek, J. M. (1998) J. Biol. Chem. 273, 18623–18632
33. Winder, D. G., Martin, R. C., Muzzio, I. A., Rohrer, D., Chrusciainski, A., Kobili, B., and Kandel, E. R. (1999) Neuron 24, 715–726
34. Watabe, A. M., Zaki, P. A., and O’Dell, T. J. (2000) J. Neurosci. 20, 5924–5931
35. Huang, Y.-Y., Li, X.-C., and Kandel, E. R. (1994) Cell 79, 69–79
36. Qi, M., Zhuo, M., Skålhegg, B. S., Brandon, E. P., Kandel, E. R., McKnight, G. S., and Iderza, R. L. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1571–1576
37. Bading, H., Ginty, D. D., and Greenberg, M. E. (1993) Science 260, 181–186
38. Fukunaga, K., Soderling, T. R., and Miyamoto, E. (1992) J. Biol. Chem. 267, 22527–22533
39. Goto, S., Yamamoto, H., Fukunaga, K., Iwasa, T., Matsukado, Y., and Miyamoto, E. (1985) J. Neurochem. 45, 276–285
40. Matthews, R. P., Guthrie, C. H., Wai, L. M., Zhao, X., Means, A. R., and McKnight, S. (1994) Mol. Cell. Biol. 14, 6107–6116
41. Wang, Y., and Simonson, M. S. (1996) Mol. Cell. Biol. 16, 5915–5923
42. Atkins, C. M., Selcher, J. C., Petraitus, J., Carmichael, J. M., and Sweatt, J. D. (1998) Nat. Neurosci. 1, 692–699
43. Lüscher, C., Nolles, A., Malenka, R. C., and Muller, D. (2000) Nat. Neurosci. 3, 545–550
44. Roberson, E. D., and Sweatt, J. D. (1996) J. Biol. Chem. 271, 30436–30441
45. Frey, U., Huang, Y.-Y., and Kandel, E. R. (1993) Science 260, 1661–1664
46. Impar, S., Mark, M., Villares, E. C., Pauer, S., Chavkin, C., and Storm, D. R. (1996) Neuron 16, 973–982
47. Blitzer, R. D., Connor, J. H., Brown, G. P., Wang, T., Shenolikar, S., Iyengar, R., and Landau, E. M. (1998) Science 280, 1940–1943