Research

Depotentiation depends on IP_3 receptor activation sustained by synaptic inputs after LTP induction

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In CA1 neurons of guinea pig hippocampal slices, long-term potentiation (LTP) was induced in field excitatory postsynaptic potentials (EPSPs) or population spikes (PSs) by the delivery of high-frequency stimulation (HFS, 100 pulses at 100 Hz) to CA1 synapses, and was reversed by the delivery of a train of low-frequency stimulation (LFS, 1000 pulses at 2 Hz) at 30 min after HFS (depotentiation), and this effect was inhibited when test synaptic stimulation was halted for a 19-min period after HFS or for a 20-min period after LFS or applied over the same time period in the presence of an antagonist of N-methyl-D-aspartate receptors (NMDARs), group I metabotropic glutamate receptors (mGluRs), or inositol 1, 4, 5-trisphosphate receptors (IP_3Rs). Depotentiation was also blocked by the application of a Ca^{2+} / calmodulin-dependent protein kinase II (CaMKII) inhibitor or a calcineurin inhibitor applied in the presence of test synaptic input for a 10-min period after HFS or for a 20-min period after LFS. These results suggest that, in postsynaptic neurons, the coactivation of NMDARs and group I mGluRs due to sustained synaptic activity following LTP induction results in the activation of IP_3Rs and CaMKII, which leads to the activation of calcineurin after LFS and depotentiation of CA1 synaptic responses.

The stimulation of group I metabotropic glutamate receptors (mGluRs) on hippocampal neurons activates phospholipase C, which hydrolyzes the inositol lipid precursor in the postsynaptic plasma membrane to form inositol 1, 4, 5-trisphosphate (IP_3) and diacylglycerol; the former opens IP_3 receptor (IP_3R) channels and the latter activates protein kinase C (Ben-Ari et al. 1992; Nakanishi 1992). IP_3Rs act as IP_3-gated Ca^{2+}-release channels on the endoplasmic reticulum (ER) of a variety of cells (Miyazaki et al. 1992; Berridge 1993; Mikoshiba 1993). Type 1 IP_3Rs are the major members of the IP_3R family in the central nervous system and are predominantly enriched in hippocampal neurons (Furuichi et al. 1989; Nakanishi et al. 1991).

Prior synaptic activity can influence the subsequent induction of synaptic plasticity in the hippocampus. A type of synaptic plasticity, named depotentiation, in which low-frequency afferent stimulation at 1–2 Hz reverses preestablished long-term potentiation (LTP), has been observed at mossy fiber-CA3 pyramidal neuron synapses (Chen et al. 2001; Yamazaki et al. 2011) and at Schaffer collateral/commissural pathway CA1 synapses (Fujii et al. 1991). Using type 1 IP_3R-deficient mice (Matsumoto et al. 1996), we found that the LTP or depotentiation induced at CA1 synapses was increased or attenuated, respectively, in which the mean magnitude of the responses after the delivery of either a short period of high-frequency stimulation (HFS, 10 pulses at 100 Hz) or low-frequency stimulation (LFS, 1000 pulses at 1 Hz) was significantly greater than that observed in wild-type mice (Fujii et al. 2000).

Previously (Sugita et al. 2016), we found that bath application of 2-aminoethoxydiphenyl borate (2-APB), an antagonist of IP_3Rs and/or store-operated calcium channels (Maruyama et al. 1997; Iwasaki et al. 2001; Bootman et al. 2002; Peppiatt et al. 2003), during priming HFS significantly decreased the magnitude of depotentiation in hippocampal CA1 neurons, and suggested that IP_3Rs remain activated after HFS and that the modulation of IP_3R activity induced during and/or after the subsequent LFS dephosphorylates postsynaptic proteins, leading to a decrease in LTP amplitude. We also found that the induction of depotentiation at CA1 synapses was inhibited by the application of FK506, a calcineurin inhibitor (Liu et al. 1991), to CA1 neurons for 20 min from the end of LFS, and suggested that the dephosphorylation of postsynaptic proteins as a result of calcineurin activation, which occurs during LFS, is maintained by test synaptic stimulation after LFS (Sugita et al. 2016).

Studies on the hippocampal CA1 region have shown that N-methyl-D-aspartate receptor (NMDAR) activation occurs in postsynaptic cells when test synaptic stimulation is given at a frequency as low as 0.05 Hz (Yang 2000; Yamazaki et al. 2012), and the coactivation of NMDARs and mGluRs by test synaptic stimulation of postsynaptic cells determines whether LTP or long-term depression (LTD) is induced at CA1 synapses (Fujii et al. 2003, 2004). In our previous studies (Fujii et al. 1991, 1996, 2000), we reported a type of synaptic plasticity in hippocampal CA1 neurons that we referred to as "LTP suppression," in which a train of LFS given prior to the delivery of HFS suppresses LTP induction. We recently demonstrated that, in this LFS-induced LTP suppression, stopping test synaptic stimulation after priming LFS negates the effects of LFS on subsequent LTP induction and can be replicated by perfusion with an antagonist for NMDARs, group I mGluRs, or IP_3Rs or with a calcineurin inhibitor (Fujii et al. 2016).

Previously (Yamazaki et al. 2012), we reported a type of depotentiation in CA1 neurons in which LTP induced in field excitatory postsynaptic potentials (EPSPs) by the delivery of LFS (80 pulses at 1 Hz) was reversed by the reapplication of the same LFS at 20 min later, and suggested that the induction of depotentiation depends

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on the coactivation of NMDARs and IP_3Rs in postsynaptic neurons. However, when preestablished LTP induced by HFS (100 Hz at 100 pulses) is depotentiated in CA1 neurons by the subsequent delivery of LFS (1000 pulses at 2 Hz), it is not known whether the delivery of test synaptic stimulation after priming HFS determines if depotentiation is induced in CA1 neurons. In the present study, we used a pharmacological approach to study the effects of test synaptic stimulation on the depotentiation induced at CA1 synapses in hippocampal slices from mature guinea pigs.

Results

LTP induction does not require test synaptic stimulation after HFS

LTP of the synaptic responses was induced in hippocampal CA1 neurons by the delivery of HFS (a tetanus of 100 pulses at 100 Hz) in standard perfusate when test synaptic stimulation at 0.05 Hz was continued throughout the experiment or withheld for a 19-min period immediately after HFS. Figure 1A and B show an example time course and summarized time course, respectively, for the change in the slope of the field EPSP (S-EPSP) or amplitude of the population spike (A-PS) in response to HFS when test synaptic stimulation at 0.05 Hz was either continued throughout the experiment (control, $n = 7$) or was stopped for the 19-min period from 1 to 20 min after HFS (stimulation off, $n = 6$).

When test synaptic stimulation at 0.05 Hz was continued throughout the experiment, mean LTP in the S-EPSP measured at 25–30 or 95–100 min after HFS was 168.3 ± 5.6% or 156.4 ± 6.0%, respectively, of the pre-HFS levels (Fig. 1B, left panel), while mean LTP in the A-PS (Fig. 1B, right panel) measured at 25–30 or 95–100 min after HFS was 166.1 ± 3.7% or 168.8 ± 4.7%, respectively, of the pre-HFS levels.

When test synaptic stimulation at 0.05 Hz was stopped for the 19-min period from 1 to 20 min after HFS, but was applied before and after this period (stimulation off), mean LTP in the S-EPSP measured at 25–30 or 95–100 min after HFS was 165.9 ± 9.0% or 164.9 ± 7.1%, respectively, of the pre-HFS levels (Fig. 1B, left panel), neither result being significantly different from the corresponding value for control LTP. Mean LTP in the A-PS measured at 25–30 or 95–100 min after HFS was 158.1 ± 13.3% or 174.3 ± 16.9%, respectively, of the pre-HFS levels (Fig. 1B, right panel), again not significantly different from the corresponding value for control LTP.

Thus, stable LTP was induced in both the S-EPSP and A-PS when test synaptic stimulation at 0.05 Hz was stopped for the 19-min period from 1 to 20 min after HFS, showing that the induction of LTP triggered by HFS does not require test synaptic stimulation immediately after HFS.

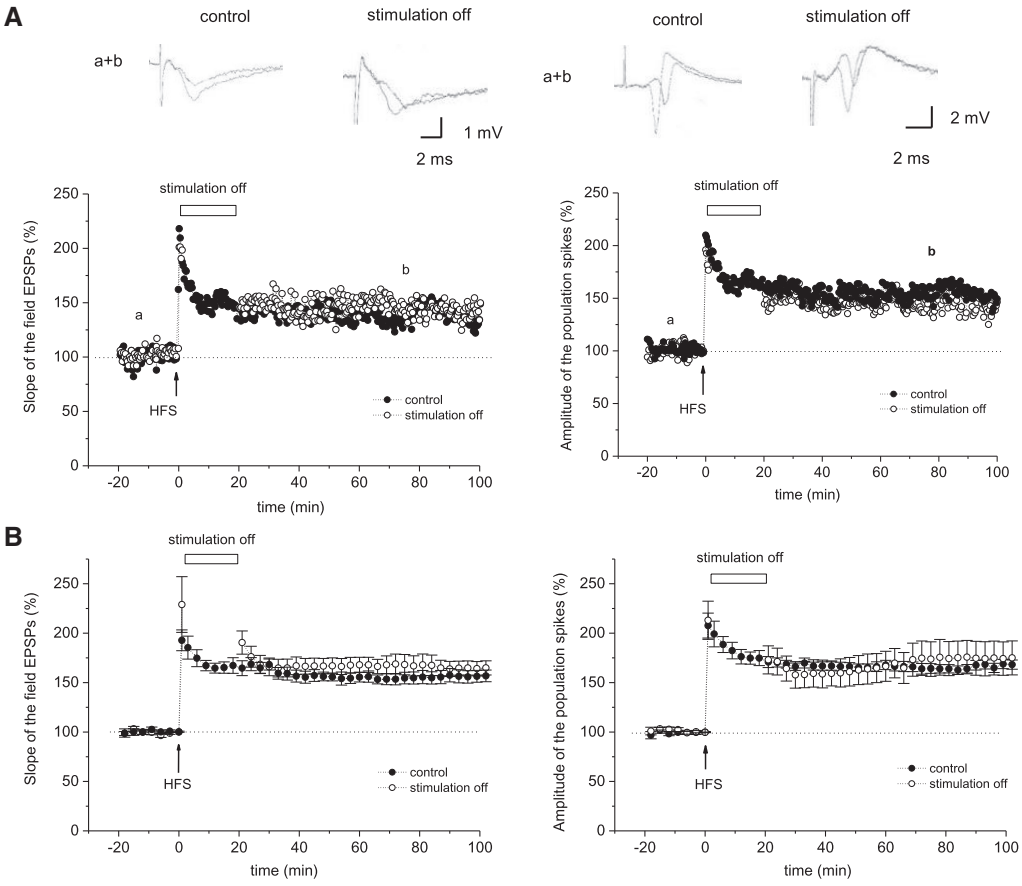

Figure 1. Effect of stopping test synaptic stimulation immediately after HFS on LTP induction. (*A*) Sample waveforms and a typical time course of LTP in the S-EPSP (*left* panel) or A-PS (*right* panel) induced by HFS (100 pulses at 100 Hz) when test synaptic stimulation at 0.05 Hz was either continued throughout the experiment (control) or was stopped for the 19-min period of 1 to 20 min after HFS (stimulation off). The sample waveforms were taken at the times indicated as **a** and **b** in the time course figure. (*B*) Summarized time course for the change in the S-EPSP (*left* panel) or A-PS (*right* panel) induced by HFS (100 pulses at 100 Hz) when test synaptic stimulation was either continued throughout the experiment (control, $n = 7$) or was stopped for the 19-min period of 1 to 20 min after HFS (stimulation off, $n = 6$). The horizontal bar marks the stimulation-off period and the arrow represents the application of HFS. In these, and all subsequent time course figures, the ordinate shows the S-EPSP or A-PS expressed as a percentage of the averaged value measured during the 10-min period before HFS. The symbols and bars represent the mean ± S.E.M.

LTP induction does not depend on CaMKII activated by test synaptic stimulation after HFS

In hippocampal CA1 neurons, HFS (100 pulses at 100 Hz) activates Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which phosphorylates either CaMKII itself or the GluA1 subunit of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) to induce LTP (Bliss and Collingridge 1993; Griffith 2004). When test synaptic stimulation at 0.05 Hz was continued throughout the experiment and the slices were perfused with 10 μM KN-62, a specific inhibitor of CaMKII, for the 10-min period from 9 min before HFS to 1 min after HFS ($n = 6$), as shown in Figure 2, LTP induction was inhibited in the S-EPSP (left panel (1)) and A-PS (right panel (1)). Mean LTP in the S-EPSP measured at 25–30 or 95–100 min after HFS was 112.0 ± 6.6% or 112.0 ± 7.9%, respectively, of the pre-HFS levels (empty circles in left panel). Mean LTP in the A-PS measured at 25–30 or 95–100 min after HFS was 118.7 ± 8.0% or 112.5 ± 6.5%, respectively, of the pre-HFS levels (empty circles in right panel). These results indicate that LTP induction in hippocampal CA1 neurons requires the activity of CaMKII phosphorylated by HFS.

We studied the effects of CaMKII activated by test synaptic stimulation after HFS on LTP induction in CA1 neurons by applying HFS (100 pulses at 100 Hz) to the slices in standard solution, then perfusing the slices with 10 μM KN-62 for the 10-min period from 1 to 11 min after HFS in the presence of test electrical stimulation at 0.05 Hz. When the slices were perfused with 10 μM KN-62 during this period ($n = 6$), as shown in Figure 2, LTP was induced in the S-EPSP (left panel (2)) and A-PS (right panel (2)). Mean LTP in the S-EPSP measured at 25–30 or 95–100 min after HFS was 150.3 ± 8.1% or 163.5 ± 14.6%, respectively, of the pre-HFS levels (filled circles in left panel). Mean LTP in the A-PS measured at 25–30 or 95–100 min after HFS was 158.3 ± 13.3% or 159.6 ± 7.4%, respectively, of the pre-HFS levels (filled circles in right panel). Since KN-62 does not affect the activity of autophosphorylated CaMKII (Tokumitsu et al. 1990) and since LTP induction in hippocampal CA1 neurons requires the activity of CaMKII autophosphorylated during and/or after HFS (Bliss and Collingridge 1993), this result suggests that LTP induction in hippocampal CA1 neurons does not depend on the subsequent activation of CaMKII by test synaptic stimulation during the 10-min period immediately after HFS.

Effects of test synaptic stimulation delivered immediately after 2-Hz LFS on synaptic responses

We examined the effects of test synaptic stimulation delivered immediately after LFS (1000 pulses at 2 Hz) on the synaptic responses of CA1 neurons when LFS was delivered to naïve Schaffer collateral/commissural pathway-CA1 neuron synapses. Figure 3A shows sample wave forms (top traces) and a typical example of the time course of the changes in the S-EPSP (left bottom panel) and A-PS (right one) in response to LFS when test synaptic stimulation was continued (filled circles) or stopped (empty circles) for the 20-min period from 0 to 20 min after the end of LFS (stimulation off). When test synaptic stimulation was continued throughout the experiment (filled circles), we observed that the responses were depressed; these slowly recovered toward pre-LFS control levels, reaching a plateau within 20 min. However, when test synaptic stimulation was stopped for 20 min after LFS (empty circles), its resumption induced responses at the pre-LFS control levels.

The filled circles in Figure 3B show the summarized results for six experiments in which test synaptic stimulation at 0.05 Hz was continued throughout the experiment. In these experiments, the mean value of the S-EPSP (left panel) or A-PS (right panel) measured at 55–60 min after the end of LFS was 100.4 ± 4.5% or 100.1 ± 6.0% of the pre-LFS levels, respectively, showing that LFS delivery induced no significant change in field EPSPs or PSs for up to 60 min. The empty circles in Figure 3B show the summarized results for six experiments in which test synaptic stimulation was stopped for 20 min after LFS (stimulation off). In these experiments, the mean value of the S-EPSP or A-PS (right panel) measured at 55–60 min after LFS was 104.7 ± 3.1% or 106.2 ± 4.2% of the pre-LFS levels, respectively, neither result being significantly different from the corresponding control value. Thus, halting test synaptic stimulation for 20 min immediately after LFS did not affect the level of responses measured at 55–60 min after LFS (1000 pulses at 2 Hz) in naïve CA1 synaptic pathways.

Effects of NMDARs, mGluRs, or IP_3Rs activated immediately after 2-Hz LFS on synaptic responses

When LFS (1000 pulses at 2 Hz) was delivered to naïve CA1 synaptic pathways, it was possible that test synaptic stimulation delivered immediately after LFS could have activated NMDARs, mGluRs, or IP_3Rs at CA1 synapses, while they did not affect the responses measured at 55–60 min after LFS (Fig. 3). Therefore, in the following experiments shown in Figure 4A, we measured the LFS-induced changes in the S-EPSP (left panel) or A-PS (right panel) for up to 60 min after LFS when test synaptic stimulation at 0.05 Hz was continued throughout the experiment, but an antagonist for each of these receptors was added to the perfusate for the 20-min period from 0 to 20 min after the end of LFS.

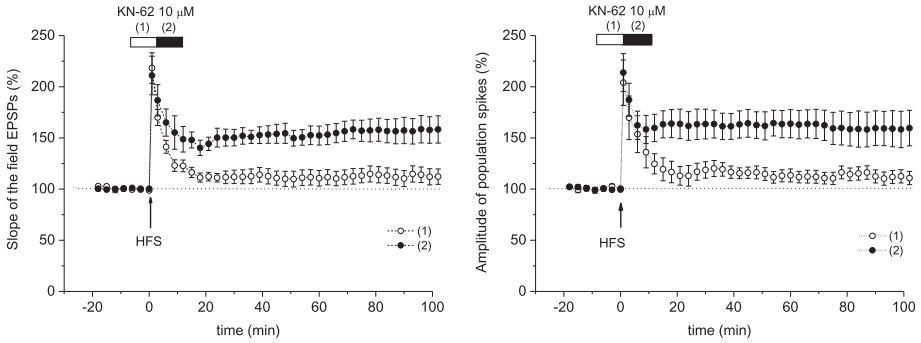

Figure 2. Effect of a CaMKII inhibitor applied for a 10-min period on LTP induction. Summarized time course for the change in the S-EPSP (*left* panel) or A-PS (*right* panel) induced by HFS (100 pulses at 100 Hz) when test synaptic stimulation was continued throughout the experiment and the slices were perfused with 10 μM KN-62 for the 10-min period either from 9 min before HFS to 1 min after HFS ($n = 6$, empty bar and circles (1)) or from 1 to 11 min after HFS ($n = 6$, filled bar and circles (2)). The horizontal bar marks the period of KN-62 application and the arrow represents the delivery of HFS. The symbols and bars represent the mean ± S.E.M.

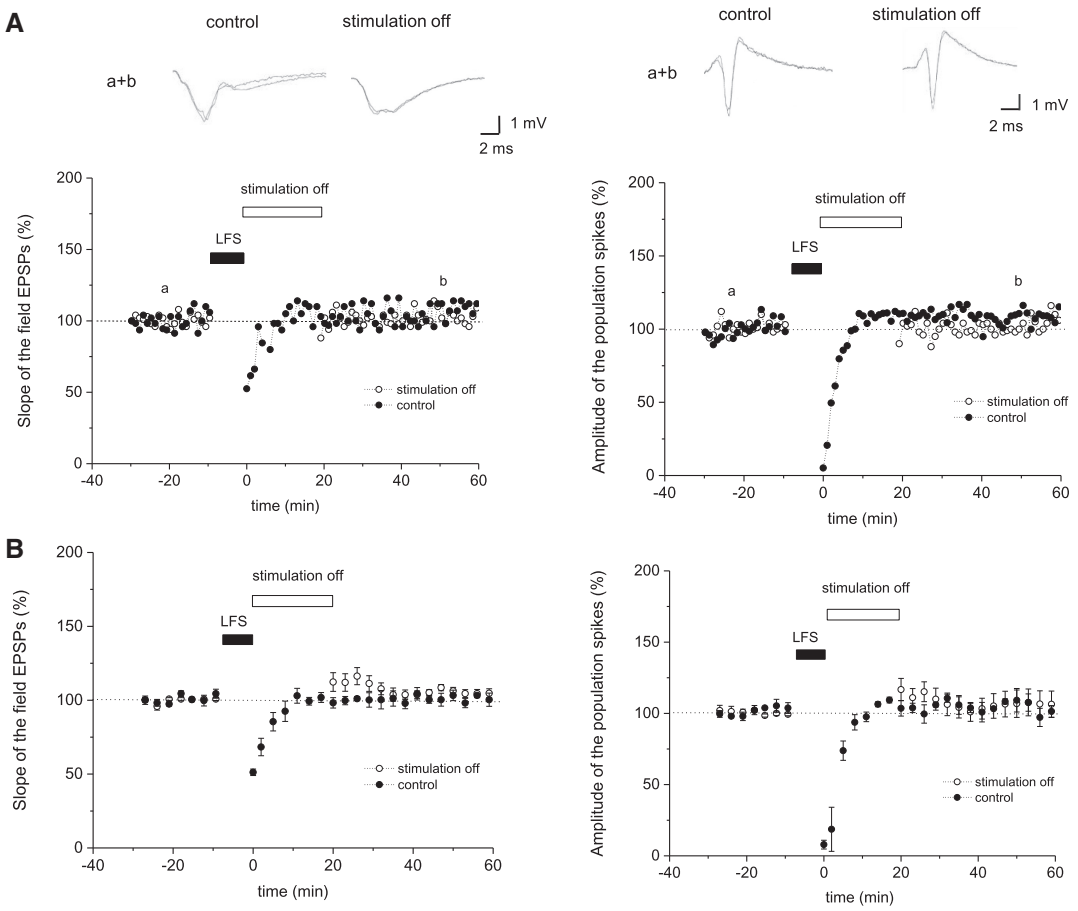

Figure 3. Effect of stopping test synaptic stimulation immediately after LFS on synaptic responses. (*A*) Sample waveforms and a typical time course for the synaptic responses in the S-EPSP (*left* panel) or A-PS (*right* panel) before and after LFS (1000 pulses at 2 Hz) when test synaptic stimulation was either delivered at 0.05 Hz throughout the experiment (filled circles) or stopped for the 20-min period from 0 to 20 min after the end of LFS (unfilled circles). The sample waveforms were taken at the times indicated as **a** and **b** in the time course figure. (*B*) Summarized time course for the change in the S-EPSP (*left* panel) or A-PS (*right* panel) induced by LFS (1000 pulses at 2 Hz) when test synaptic stimulation was either delivered throughout the experiment (filled circles, $n = 6$) or stopped for the 20-min period from 0 to 20 min after the end of LFS (unfilled circles, $n = 6$). The unfilled horizontal bar marks the stimulation-off period and the filled horizontal bar represents the delivery of LFS. In these time course figures, the ordinate shows the S-EPSP or A-PS expressed as a percentage of the averaged value measured during the 10-min period before the delivery of LFS. The symbols and bars represent the mean ± S.E.M.

When test synaptic stimulation was delivered at 0.05 Hz in the presence of 50 µM AP5, an NMDAR inhibitor, for 20 min after LFS (empty circles in Fig. 4A, $n = 7$), test synaptic stimulation delivered just after LFS depressed the responses; these slowly recovered toward pre-LFS control levels, reaching a plateau at 40–50 min after the end of LFS. The mean magnitude of the S-EPSP or A-PS of these responses measured at 55–60 min after LFS was 104.7 ± 4.7% or 100.1 ± 9.8%, respectively, of the pre-LFS levels, neither result being significantly different from the corresponding control value (filled circles in Fig. 3B).

When test synaptic stimulation was delivered in the presence of 100 µM S-4CPG, a specific group I mGluR antagonist, for 20 min after LFS (filled circles in Fig. 4A, $n = 6$), test synaptic stimulation delivered just after LFS depressed the responses; these responses recovered toward pre-LFS control levels, reaching a plateau within 40 min after the end of LFS. The mean magnitude of the S-EPSP or A-PS of these responses measured at 55–60 min after LFS was 102.7 ± 5.9% or 102.2 ± 5.9%, respectively, of the pre-LFS levels, neither result being significantly different from the corresponding control value (filled circles in Fig. 3B).

When test synaptic stimulation was delivered in the presence of 10 µM 2-APB, an IP_3R antagonist, for 20 min after LFS (filled triangles in Fig. 4A, $n = 6$), the responses that were depressed just after LFS recovered toward pre-LFS control levels, reaching a plateau within 40 min after the end of LFS. The mean magnitude of the S-EPSP or A-PS of the responses measured at 55–60 min after LFS was 100.0 ± 7.0% or 105.1 ± 8.4%, respectively, of the pre-LFS levels, neither result being significantly different from the corresponding control value (filled circles in Fig. 3B).

From all of the results shown in Figure 4A, we conclude that the activity of NMDARs, group I mGluRs, and/or IP_3Rs in CA1 neurons for the 20-min period after LFS is not included in the responses measured at 55–60 min after LFS (1000 pulses at 2 Hz) of naïve CA1 synaptic input pathways.

Effects of CaMKII or calcineurin activated immediately after 2-Hz LFS on synaptic responses

We studied whether an inhibitor of CaMKII or calcineurin applied over the same period immediately after LFS (1000 pulses at 2 Hz) affected the synaptic responses when LFS was delivered to naïve CA1 synaptic pathways. In the following experiments, shown in Figure 4B, we observed the LFS-induced changes in the S-EPSP (left panel) or A-PS (right panel) when test synaptic stimulation

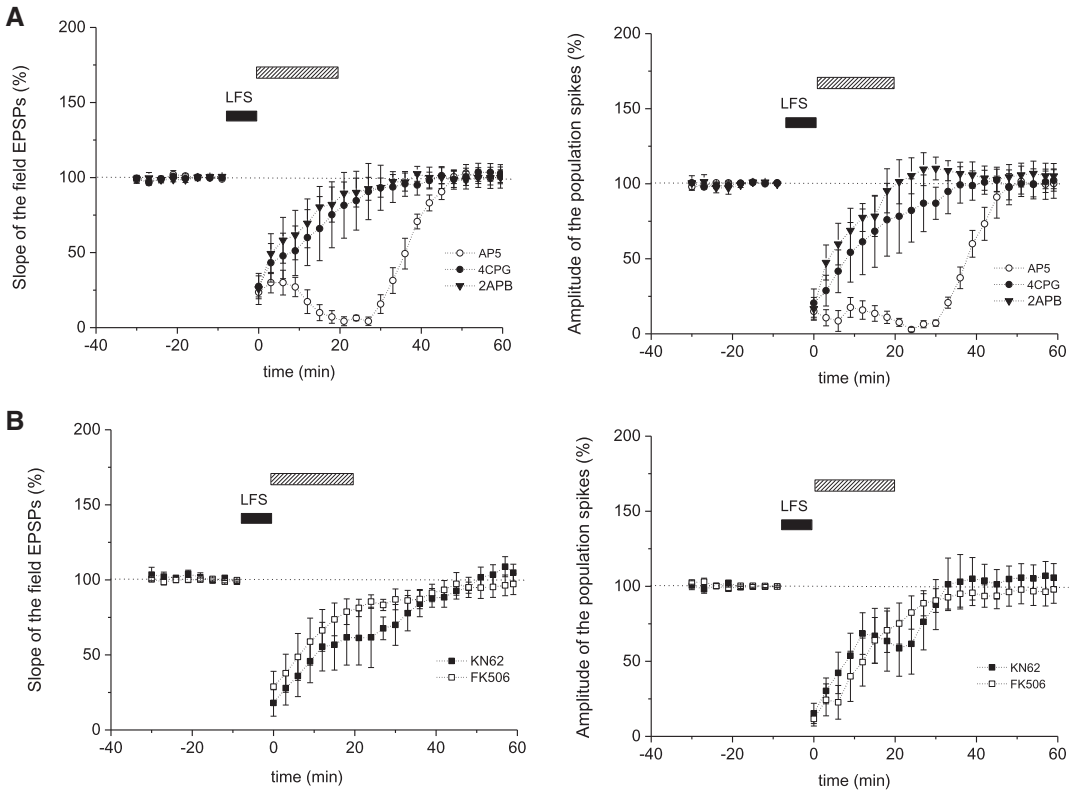

Figure 4. Effects of NMDARs, mGluRs, group I mGluRs, IP_3Rs, CaMKII, or calcineurin activated immediately after LFS on synaptic responses. (*A*) Summarized results for the time course of changes in the S-EPSP (*left* panel) or A-PS (*right* panel) when LFS (1000 pulses at 2 Hz) was delivered to naïve CA1 synaptic inputs and 50 μM AP5 ($n=7$, unfilled circles), 100 μM S-4CPG ($n=6$, filled circles), or 10 μM 2-APB ($n=6$, filled triangles) was applied to the perfusate (gray bar) in the presence of test synaptic inputs during the 20-min period from 0 to 20 min after the end of LFS (black bar). (*B*) Summarized results for the time course of change in the S-EPSP (*left* panel) or A-PS (*right* panel) when LFS (1000 pulses at 2 Hz) was delivered to naïve CA1 synaptic inputs and 10 μM KN-62 ($n=6$) or 1 μM FK506 ($n=6$) was applied (hatched bar) to the perfusate in the presence of test synaptic input during the 20-min period from 0 to 20 min after the end of LFS (gray bar). In these time course figures, the ordinate shows the S-EPSP or A-PS expressed as a percentage of the averaged value measured during the 10-min period before the delivery of LFS. The symbols and bars represent the mean ± S.E.M.

at 0.05 Hz was continued throughout the experiment, but an inhibitor of CaMKII or calcineurin was applied to the perfusate for 20 min from 0 to 20 min after the end of LFS.

When test synaptic stimulation was delivered in the presence of 10 μM KN-62 for 20 min after LFS, the responses that were depressed just after LFS recovered toward pre-LFS control levels, reaching a plateau at 40–50 min after the end of LFS (filled squares in Fig. 4B, $n=6$). The mean magnitude of the S-EPSP or A-PS of the responses measured from 55 to 60 min after LFS was 105.0 ± 5.7% or 105.7 ± 9.4%, respectively, of the pre-LFS levels, neither result being significantly different from the corresponding control value (filled circles in Fig. 3B). In addition, when test synaptic stimulation was delivered in the presence of 1 μM FK506, a specific inhibitor of calcineurin, for 20 min after LFS (empty squares in Fig. 4B, $n=6$), the responses that were depressed just after LFS recovered toward pre-LFS control levels, reaching a plateau within 40 min after the end of LFS. The mean magnitude of the S-EPSP or A-PS of the responses measured from 55 to 60 min after HFS was 97.0 ± 7.0% or 98.0 ± 9.3%, respectively, of the pre-LFS levels, neither result being significantly different from the corresponding control value (filled circles in Fig. 3B). Therefore, we conclude that the activity of CaMKII or calcineurin in CA1 neurons for 20 min immediately after LFS is not included in the level of responses measured at 55–60 min after LFS (1000 pulses at 2 Hz) of naïve CA1 synaptic input pathways.

The activation of CaMKII or calcineurin occurs downstream from NMDAR activation and Ca^{2+}/calmodulin complex formation in the signaling cascade of LTP or LTD in hippocampal CA1 neurons (Bliss and Collingridge 1993; Bear and Abraham 1996). Therefore, from the results shown in Figure 4A and B, we suggest that activation of the signaling pathway, NMDARs activation—Ca^{2+}/calmodulin complex formation—CaMKII activation and/or—calcineurin activation does not occur in CA1 neurons during the 20-min period immediately after LFS, and does not affect the level to which the responses of CA1 neurons recover to at 55–60 min after the end of LFS (1000 pulses at 2 Hz) of naïve CA1 synaptic pathways.

Depotentiation depends on test synaptic stimulation after HFS or LFS

We studied the induction of depotentiation in hippocampal CA1 neurons by LFS (1000 pulses at 2 Hz) applied at 30 min after the delivery of HFS (100 pulses at 100 Hz). In subsequent experiments (Figs. 5–7), we measured the S-EPSP and A-PS during the 5-min period from 55 to 60 min after the end of LFS or the reduction in LTP in the S-EPSP and A-PS (Tables 1–4), and compared these values with the corresponding values for the depotentiation induced in a standard solution by LFS given at 30 min after HFS (first row in Table 1).

Figure 5A shows sample wave forms (top traces) and a typical example of the time course of depotentiation in the S-EPSP (left panel) or A-PS (right panel), while Figure 5B shows the summarized results ($n=7$). When test synaptic stimulation at 0.05 Hz was

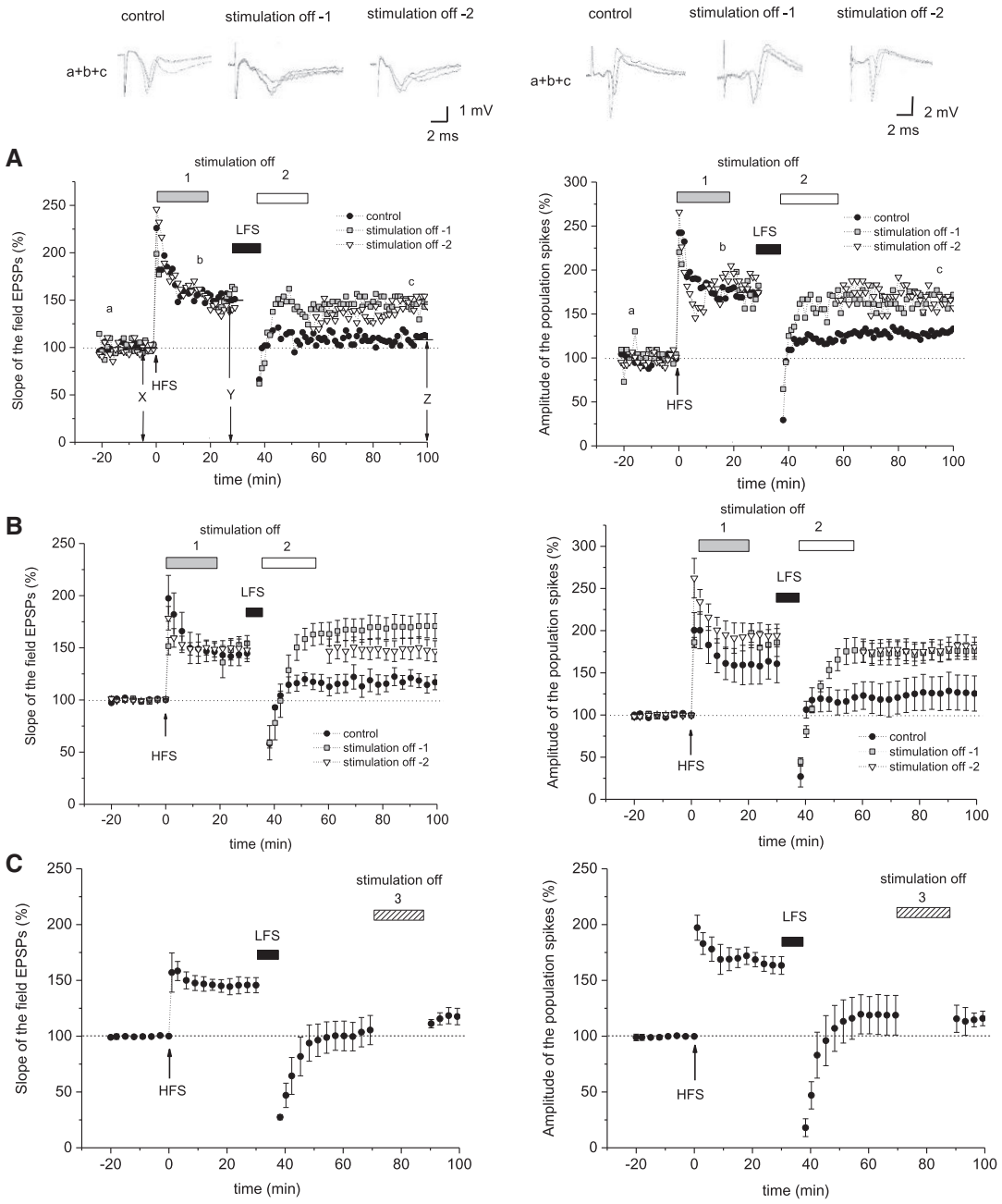

Figure 5. Effect of stopping test synaptic stimulation after HFS or LFS on depotentiation. Typical example (*A*) and summarized time course (*B*) for the change in the S-EPSP (*left* panel) or A-PS (*right* panel) in response to a single train of LFS (1000 pulses at 2 Hz) (horizontal black bar) applied at 30 min after HFS (100 pulses at 100 Hz) (arrow). The sample traces above the main panel were taken at the times indicated as **a**, **b**, and **c** in *A*. In the control, test synaptic stimulation was applied throughout the experiment (filled circles), while the horizontal gray or white bar represents, respectively, stopping test synaptic stimulation at 0.05 Hz for the 19-min period from 1 to 20 min after HFS (gray squares, stimulation off-1) or for the 20-min period from 0 to 20 min after the end of LFS (unfilled triangles, stimulation off-2). In (*B*), $n=7$ for the control and $n=6$ for the other two traces. (*C*) Summarized time course for the change in the S-EPSP (*left* panel) or A-PS (*right* panel) in response to a single train of LFS (1000 pulses at 2 Hz) (horizontal black bar) applied at 30 min after HFS (100 pulses at 100 Hz) (arrow). The horizontal white bar represents stopping test synaptic stimulation for the 20-min period at 30–50 min after HFS (stimulation off-3). $n=6$. X, Y, or Z in A represents the averaged value for the 10-min period immediately prior to HFS or LFS, the averaged value at 5–0 min immediately before LFS, and the stable level at 55–60 min after the end of LFS of the responses, respectively.

continued throughout the experiment (control, filled circles), test synaptic stimulation delivered just after LFS depressed the responses; these recovered toward a level below pre-LFS levels, reaching a plateau within 20 min. Table 1 shows the summarized results for seven experiments for the mean percentage reduction in LTP of the S-EPSP or A-PS (first row), which represents the magnitude of depotentiation in the responses. In hippocampal CA1 neurons, LFS (1000 pulses at 2 Hz) given at 30 min after the delivery of HFS (100 pulses at 100 Hz) caused a reduction in the LTP induced in the S-EPSP and A-PS (control in Figure 5A,B), while LFS itself did not induce a significant change in responses (control in Fig. 3). Therefore, these results indicate that the delivery of 100-Hz HFS

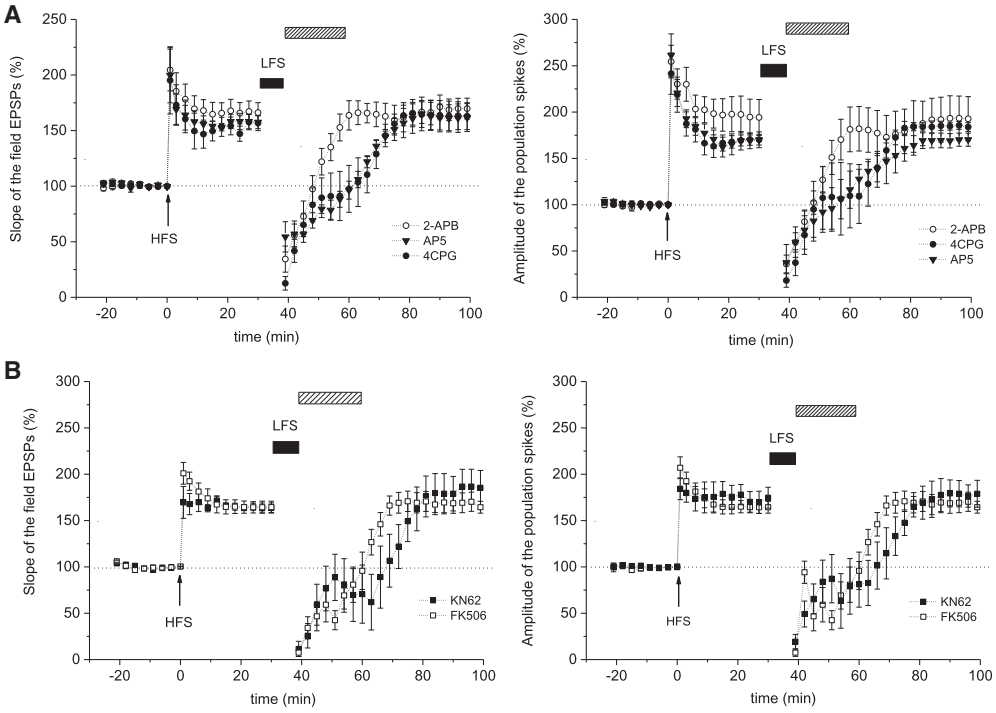

Figure 6. Involvement of NMDARs, mGluRs, group I mGluRs, IP_3Rs, CaMKII, or calcineurin activated after HFS in the induction of depotentiation. (*A*) Summarized results for the time course of depotentiation in the S-EPSP (*left* panel) or A-PS (*right* panel) when 50 μM AP5 ($n = 5$, unfilled circles), 100 μM S-4CPG ($n = 5$, filled circles), or 10 μM 2-APB ($n = 5$, filled triangles) was applied to the perfusate (hatched bar) in the presence of test synaptic stimulation during the 19-min period from 1 to 20 min after HFS (arrow) before the subsequent LFS (black bar). (*B*) Summarized results for the time course of depotentiation in the S-EPSP (*left* panel) or A-PS (*right* panel) when 10 μM KN-62 ($n = 6$, filled circles) or 1 μM FK506 ($n = 6$, unfilled circles) was applied to the perfusate (hatched bar) in the presence of test synaptic stimulation during the 10-min period from 1 to 11 min after the end of LFS (black bar).

has a preconditioning effect on LFS-induced depotentiation in hippocampal CA1 neurons.

The preconditioning effect of HFS on the depotentiation of hippocampal CA1 neurons involves processes that are disrupted by stopping the delivery of test synaptic stimulation at 0.05 Hz after HFS. As shown in a typical example (gray squares in Fig. 5A) or the summarized results for six experiments (gray squares in Fig. 5B), when test synaptic stimulation at 0.05 Hz was stopped for the 19-min period of 1 to 20 min after priming HFS (stimulation off-1), LTP was induced in the S-EPSP or A-PS, but depotentiation was not induced in CA1 neurons. In these cells, the mean magnitude of the S-EPSP or A-PS measured at 25–30 min after priming HFS was not different from the corresponding value for control depotentiation (second row in Table 1), showing that the LTP induced in these cells was not affected by stopping test synaptic stimulation for 19 min immediately after HFS. However, Table 1 shows that the mean magnitude of the S-EPSP or A-PS measured at 55–60 min after LFS and the mean percentage reduction in LTP in the S-EPSP or A-PS were significantly different from the corresponding values for control depotentiation (second row), indicating no induction, or significant attenuation, of depotentiation in the S-EPSP or A-PS. Since stopping test synaptic stimulation at 0.05 Hz immediately after HFS did not affect LTP induction in hippocampal CA1 neurons (Fig. 1), but inhibited depotentiation at CA1 synapses (Fig. 5A), we suggest that the delivery of 100-Hz HFS has a preconditioning effect on LFS-induced depotentiation at CA1 synapses and that this effect depends on test synaptic stimulation after HFS to CA1 synapses.

The induction of depotentiation in hippocampal CA1 neurons also depends on test synaptic stimulation immediately after LFS. As shown in a typical example (empty triangles in Fig. 5A) or the summarized results for six experiments (empty triangles in Fig. 5B), when test synaptic stimulation at 0.05 Hz was stopped for the 20-min period from 0 to 20 min after the end of LFS (stimulation off-2), LFS failed to induce depotentiation in the S-EPSP or A-PS. As shown in the third row of Table 1, the mean S-EPSP or A-PS measured at 25–30 min after HFS was not significantly different from the corresponding value for control depotentiation, whereas the mean S-EPSP or A-PS and the percentage reduction in LTP of the S-EPSP or A-PS measured at 55–60 min after LFS were significantly different from the corresponding values for control depotentiation. These results show that a preconditioning effect of HFS on depotentiation in hippocampal CA1 neurons involves processes that are disrupted by stopping test synaptic stimulation immediately after LFS. Since stopping test synaptic stimulation immediately after LFS did not affect synaptic transmission at 55–60 min after LFS at CA1 synapses (Fig. 3), but inhibited the depotentiation of hippocampal CA1 neurons (Fig. 5A), we conclude that the preconditioning effect of HFS on the LFS-induced depotentiation at CA1 synapses also depends on test synaptic stimulation for 20 min immediately after LFS.

Until when does preconditioning HFS affect the induction of depotentiation?

We studied the length of time in which preconditioning HFS continued to affect the induction of depotentiation in CA1 neurons. As shown by the summarized results for six experiments in Figure 5C, when test synaptic stimulation was stopped for 20 min from 30 to 50 min after the end of LFS (from 58 to 78

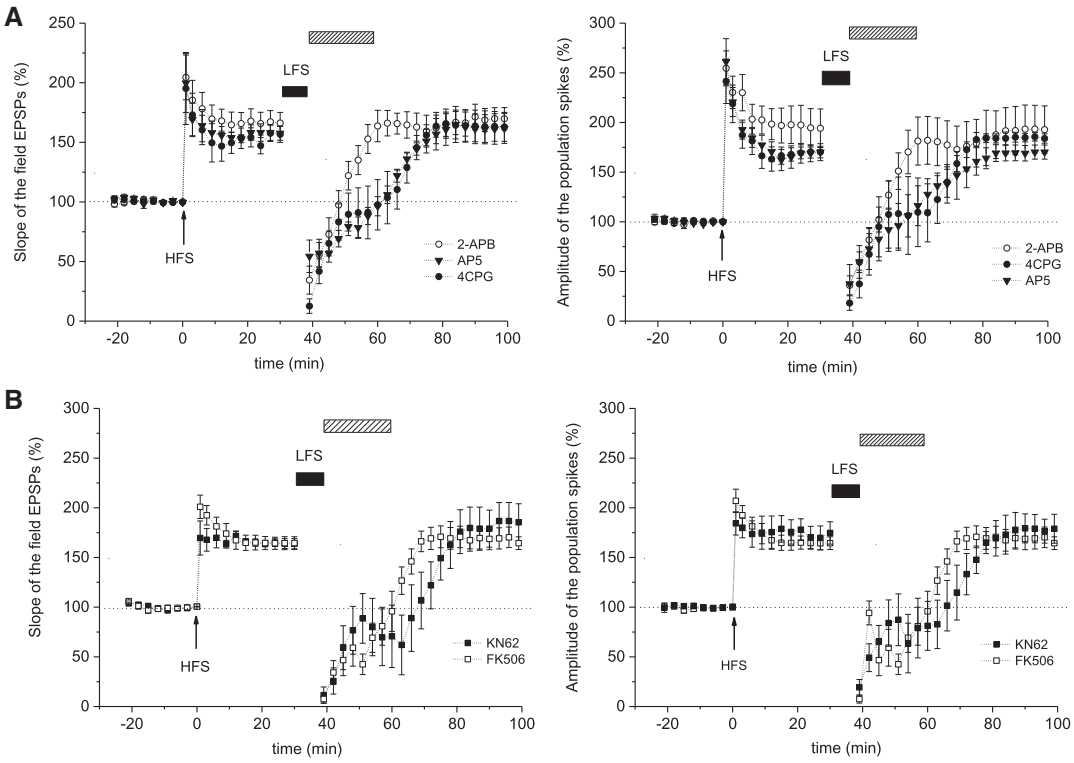

Figure 7. Effects of NMDARs, mGluRs, group I mGluRs, IP_3Rs, CaMKII, or calcineurin activated after LFS on the induction of depotentiation. (*A*) Summarized results for the time course of depotentiation in the S-EPSP (*left* panel) or A-PS (*right* panel) when 50 μM AP5 ($n=5$, unfilled circles), 100 μM S-4CPG ($n=5$, filled circles), or 2-APB ($n=7$, filled triangles) was applied to the perfusate (gray bar) in the presence of test synaptic stimulation during the 20-min period from 0 to 20 min after the end of LFS (black bar). (*B*) Summarized results for the time course of depotentiation in the S-EPSP (*left* panel) or A-PS (*right* panel) when 10 μM KN-62 ($n=6$, filled circles) or 1 μM FK506 ($n=6$, unfilled circles) was applied to the perfusate (hatched bar) in the presence of test synaptic stimulation during the 20-min period from 0 to 20 min after the end of LFS (black bar).

min after HFS) (stimulation off-3), depotentiation in the S-EPSP or A-PS was successfully induced at 55–60 min after the end of LFS in CA1 neurons. As shown in the bottom row of Table 1, the mean S-EPSP or A-PS measured at 25–30 min after HFS, the mean S-EPSP or A-PS measured at 55–60 min after LFS, and the percentage reduction in LTP of the S-EPSP or A-PS measured at 55–60 min after LFS were not significantly different from the corresponding values for control depotentiation. Thus, the depotentiation induced by LFS (1000 pulses at 2 Hz) was sensitive to the interval between priming HFS and the start of the halt of test synaptic stimulation, and was greater when this interval was <68 min. From the results shown in Figure 5, we conclude that the preconditioning effect of HFS on the LFS-induced depotentiation at CA1 synapses was maintained until 0–20 min after LFS (38–58 min after HFS), but ended at the 30- to 50-min period after the end of LFS (at 68–88 min after HFS).

Effect of NMDARs, mGluRs, or IP_3Rs activated immediately after priming HFS on depotentiation

In order to determine whether test synaptic stimulation delivered after priming HFS activated NMDARs, group I mGluRs, and/or IP_3Rs, which are involved in the induction of depotentiation in CA1 neurons, we examined whether the effect of stopping test synaptic stimulation for the 19-min period from 1 to 20 min after HFS could be replicated by perfusion with antagonists for these

Table 1. Effects of stopping the test synaptic stimulation at different times after HFS on depotentiation

		% change at 25–30 min after HFS		% change at 55–60 min after LFS		% reduction of LTP	
Stimulation stopped at	n	S-EPSP	A-PS	S-EPSP	A-PS	S-EPSP	A-PS
Control	7	143.8 ± 8.8	159.9 ± 22.1	116.2 ± 7.2	125.6 ± 20.8	60.0 ± 5.8	57.2 ± 8.0
1–20 min after HFS	6	155.4 ± 7.8	186.3 ± 16.7	171.4 ± 12.0**	176.8 ± 10.1*	−22.2 ± 13.6**	6.2 ± 5.5**
0–20 min after LFS	6	147.9 ± 8.1	194.2 ± 13.4	146.7 ± 10.1*	183.9 ± 4.7*	−9.4 ± 12.6**	−18.0 ± 3.5**
30–50 min after LFS	5	145.7 ± 6.8	163.4 ± 7.5	117.6 ± 7.3	115.9 ± 6.5	66.9 ± 11.4	77.1 ± 19.1

The table shows the mean percentage change in the S-EPSP and A-PS measured at 25–30 min after HFS or 55–60 min after LFS and the percentage reduction of LTP in the S-EPSP and A-PS measured at 55–60 min after LFS when test synaptic stimulation was either continued (Control, top row) or stopped during the 19-min period from 1 to 20 min after HFS (second row), 20-min period from 0 to 20 min after the end of LFS (third row), or 20-min period from 30 to 50 min after LFS (bottom row). Values are the mean ± S.E.M. *n*, number of experiments. *$P<0.05$, **$P<0.01$ (two-tailed Student's *t*-test) compared to the value for LFS-induced depotentiation in the control.

receptors. As shown in Figure 6A, when the slices were perfused with 50 μM AP5 ($n = 5$, unfilled circles) or 100 μM S-4CPG ($n = 5$, filled squares) for the same 19-min period after HFS in the presence of test synaptic stimulation of Schaffer collaterals, LTP was induced in the S-EPSP (left panel) and A-PS (right panel), but depotentiation was inhibited. As shown in Table 2, the mean values for the S-EPSP and A-PS at 25–30 min after HFS in the presence of these inhibitors were not significantly different from the corresponding values measured at 25–30 min after HFS for control LTP (filled circles in Fig. 1B) or control depotentiation (filled circles in Fig. 5B). However, in both cases, LFS at 30 min after priming HFS failed to induce depotentiation in the S-EPSP or A-PS; as shown in Table 2, the percentage reduction in LTP of the S-EPSP or A-PS measured at 55–60 min after LFS was significantly lower than the corresponding control value (Control in Table 1). These results show that test synaptic stimulation after priming HFS induces the coactivation of NMDARs and group I mGluRs in postsynaptic CA1 neurons and that this is required for the induction of depotentiation at CA1 synapses.

Figure 6A and Table 2 show that similar results were obtained when 10 μM 2-APB ($n = 5$, filled triangles) was used, indicating that test synaptic stimulation after priming HFS activates IP_3Rs and that this is also required for the induction of depotentiation in hippocampal CA1 neurons. Thus, for the induction of depotentiation in CA1 neurons, the effect of stopping test synaptic stimulation for the 19-min period of 1–20 min after priming HFS was replicated by perfusion with antagonists to NMDARs, group I mGluRs, or IP_3Rs. Since IP_3Rs act downstream from group I mGluRs in the signaling cascade in hippocampal neurons, we conclude that the mechanism of depotentiation at CA1 synapses involves the coactivation of NMDARs and IP_3Rs in postsynaptic neurons caused by test synaptic stimulation delivered after priming HFS. It is possible that an increase of the postsynaptic intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) during this period, as a result of Ca^{2+} influx through NMDARs and Ca^{2+} efflux through IP_3Rs into the cytosol of postsynaptic cells, is involved in the mechanism of LFS-induced depotentiation at CA1 synapses.

Activation of CaMKII and calcineurin after HFS is necessary for the induction of depotentiation

We hypothesized that the formation of Ca^{2+}/calmodulin complexes in hippocampal CA1 neurons due to a postsynaptic increase in $[Ca^{2+}]_i$ during the period immediately after priming HFS could activate either CaMKII or calcineurin in postsynaptic cells and induce depotentiation, while LTP induction does not depend on CaMKII activation during the 10-min period after HFS (100 pulses at 100 Hz) (filled bars (2) and circles in Fig. 2). We first studied the effects of CaMKII activation after priming HFS on the induction of depotentiation in CA1 neurons by applying HFS (100 pulses at 100 Hz) in a standard solution, then perfusing the slices with 10 μM KN-62 for the 10-min period from 1 to 11 min after priming HFS in the presence of test electrical stimulation at 0.05 Hz. As shown by the summarized results for six experiments in Figure 6B (filled squares), the delivery of LFS at 30 min after preconditioning HFS failed to induce depotentiation in the S-EPSP or A-PS in CA1 neurons. As shown in the upper row of Table 3, the mean value for the S-EPSP or A-PS at 25–30 min after HFS in slices perfused with a CaMKII inhibitor was not significantly different from the corresponding value measured at 25–30 min for control LTP (filled circles in Fig. 1B) or control depotentiation (filled circles in Fig. 5B and Control in Table 1). However, in these slices, the delivery of LFS at 30 min after priming HFS failed to induce depotentiation in the S-EPSP or A-PS; as shown in Table 3, the percentage reduction in the LTP of the S-EPSP or A-PS measured at 55–60 min after LFS was significantly lower than the corresponding control value (Control in Table 1).

We examined whether the activation of calcineurin immediately after priming HFS was involved in the mechanism of depotentiation in CA1 neurons. As shown in Figure 6B, when the slices ($n = 6$) were perfused with 1 μM FK506 for the 10-min period of 1 to 11 min after priming HFS in the presence of test synaptic inputs (unfilled circles), the delivery of LFS at 30 min after preconditioning HFS failed to induce depotentiation in the S-EPSP (left panel) and A-PS (right panel). As shown in the lower row of Table 3, the mean value for the S-EPSP or A-PS at 25–30 min after HFS in the presence of 1 μM FK506 was not significantly different from the corresponding value measured at 25–30 min for control LTP (filled circles in Fig. 1B) or control depotentiation (filled circles in Fig. 5B and Control in Table 1). However, in these cases, the percentage reduction in the LTP of the S-EPSP or A-PS measured at 55–60 min after LFS was significantly lower than the corresponding control value (Control in Table 1). On the basis of these results, we conclude that CaMKII and calcineurin activated immediately after priming HFS are required for the induction of depotentiation at CA1 synapses.

Effects of NMDARs, mGluRs, or IP_3Rs activated immediately after LFS on depotentiation

Since the delivery of test synaptic stimulation immediately after LFS was necessary for the induction of depotentiation in hippocampal CA1 neurons (stimulation off-2 in Fig. 5), we thought it possible that the coactivation of NMDARs and group I mGluRs and/or IP_3Rs due to test synaptic stimulation also occurred during the period immediately after LFS during the depotentiation of hippocampal CA1 neurons. Therefore, we examined whether the effect of stopping test synaptic stimulation for the 20-min period from 0 to 20 min after LFS could be replicated by perfusion with antagonists of these receptors. As shown in Figure 7A, when LFS was

Table 2. Effects of the application of an NMDAR, mGluR, or IP_3R antagonists for the 19-min period from 1 to 20 min after HFS on depotentiation

		% change at 25–30 min after HFS		% change at 55–60 min after LFS		% reduction of LTP	
Test reagent	n	S-EPSP	A-PS	S-EPSP	A-PS	S-EPSP	A-PS
AP5	5	144.4 ± 5.6	172.6 ± 13.1	149.7 ± 7.3**	194.8 ± 13.2**	−10.8 ± 8.4**	−15.5 ± 10.8**
S-4CPG	5	155.6 ± 6.3	150.3 ± 5.7	154.9 ± 6.1**	154.1 ± 8.2	0.2 ± 6.5**	−8.6 ± 10.7**
2-APB	5	153.6 ± 9.4	169.0 ± 5.2	155.8 ± 10.6	174.2 ± 11.1	−3.2 ± 3.8**	−2.0 ± 11.1**

The table shows the mean percentage change in the S-EPSP and A-PS measured at 25–30 min after HFS or at 55–60 min after LFS and the percentage reduction of LTP in the S-EPSP and A-PS measured at 55–60 min after LFS when 50 μM AP5, 100 μM S-4CPG, or 10 μM 2-APB was perfused from 1 to 20 min after HFS. *n*, number of experiments. A significant difference compared to the corresponding value for control depotentiation induced in the absence of drugs (Control in Table 1) is shown as $^{**}P < 0.01$ (two-tailed Student's *t*-test).

Table 3. Effects of the application of a protein kinase inhibitor or calcineurin inhibitor for the 10-min period from 1 to 11 min after HFS on depotentiation

		% change at 25–30 min after HFS		% change at 55–60 min after LFS		% reduction of LTP	
Test reagent	n	S-EPSP	A-PS	S-EPSP	A-PS	S-EPSP	A-PS
KN-62	6	158.9 ± 6.1	170.3 ± 12.3	150.0 ± 6.2**	156.6 ± 11.0	14.3 ± 10.3**	16.0 ± 4.4**
FK506	6	176.6 ± 13.2	163.5 ± 10.8	172.5 ± 17.5*	158.9 ± 16.2	16.1 ± 10.2**	11.6 ± 13.8*

The table shows the mean percentage change in the S-EPSP and A-PS measured at 25–30 min after HFS or 55–60 min after LFS and the percentage reduction of LTP in the S-EPSP and A-PS measured at 55–60 min after LFS when 10 μM KN-62 or 1 μM FK506 was perfused from 1 to 11 min after HFS. *n*, number of experiments. A significant difference compared to the corresponding value for control depotentiation induced in the absence of drugs (Control in Table 1) is shown as $*P < 0.05$; $**P < 0.01$ (two-tailed Student's *t*-test).

delivered in the standard perfusate, but 50 μM AP5 ($n = 5$, unfilled circles), 100 μM S-4CPG ($n = 5$, filled circles), or 10 μM 2-APB ($n = 7$, filled triangles) was applied for the 20-min period from 0 to 20 min after the end of LFS, the LFS-induced depotentiation in the S-EPSP (left panel) and A-PS (right panel) was attenuated. In these slices (first to third rows of Table 4), each percentage reduction of LTP in the S-EPSP or A-PS measured at 55–60 min after LFS was significantly lower than the corresponding value for control depotentiation (Table 1). These results suggest that, for the induction of depotentiation in CA1 neurons, the effect of stopping test synaptic stimulation for the 20-min period immediately after LFS could be replicated by perfusion with each antagonist of NMDARs, group I mGluRs, or IP_3Rs.

Application of the antagonists for NMDARs, group I mGluRs, or IP_3Rs to CA1 neurons for the 20-min period immediately after LFS had no effect on the level of responses measured at 55–60 min after LFS of naïve CA1 synaptic input pathways and test synaptic stimulation at 0.05 Hz delivered throughout the experiment (Fig. 4A). Therefore, we suggest that the preconditioning effect of HFS on the induction of depotentiation in hippocampal CA1 neurons involves the coactivation of NMDARs and IP_3Rs, the latter of which act downstream from group I mGluRs in the signaling cascade, in postsynaptic neurons during the 20-min period immediately after LFS.

Activation of CaMKII and calcineurin after LFS is necessary for depotentiation

In LFS-induced depotentiation at CA1 synapses, we thought it possible that the coactivation of NMDARs and IP_3Rs, which occurred immediately after LFS in postsynaptic CA1 neurons, could increase postsynaptic $[Ca^{2+}]_i$, leading to the formation of Ca^{2+}/calmodulin complexes and the activation of CaMKII and/or calcineurin in postsynaptic CA1 neurons. Thus, we studied the effects of CaMKII or calcineurin activated immediately after LFS on the induction of depotentiation in CA1 neurons by applying LFS in a standard solution, then perfusing the slices with 10 μM KN-62 ($n = 6$) or 1 μM FK506 ($n = 6$) for the 20-min period from 0 to 20 min after the end of LFS in the presence of test synaptic stimulation. As shown in Figure 7B, LFS-induced depotentiation in the S-EPSP (left panel) or A-PS (right panel) was attenuated by applying 10 μM KN-62 (filled squares) during the 20-min period immediately after LFS. In these slices (fourth row in Table 4), the mean magnitude of the S-EPSP and A-PS and the percentage reduction in the LTP of the S-EPSP or A-PS measured at 55–60 min after LFS were all significantly different from the corresponding control values. In addition, as shown in Figure 7B, LFS-induced depotentiation in the S-EPSP (left panel) and A-PS (right panel) was attenuated by applying 1 μM FK506 (unfilled squares) during the 20-min period immediately after LFS. In these slices (bottom row in Table 4), the mean magnitude of the S-EPSP and A-PS and percentage reduction in the LTP of the S-EPSP or A-PS measured at 55–60 min after LFS were also significantly different from the corresponding control values (Control in Table 1).

Since the application of CaMKII or calcineurin inhibitors to CA1 neurons for the 20-min period immediately after LFS did not affect the level of responses measured at 55–60 min after LFS of naïve CA1 synaptic input pathways and test electrical stimulation at 0.05 Hz delivered throughout the experiment (Fig. 4B), the results shown in Figure 7B indicate that the conditioning effect provided by priming HFS for the induction of depotentiation in CA1 neurons involves both CaMKII and calcineurin activated during the 20-min period immediately after LFS. The results shown so far indicate that that priming HFS triggers postsynaptic cellular events necessary for the depotentiation of CA1 neurons, including the activation of group I mGluRs and/or IP_3Rs, which is maintained by test synaptic inputs at 0.05 Hz, at least until 68 min after priming HFS. Although the role of CaMKII is still unclear in the

Table 4. Effect of the application of an NMDAR, mGluR, or IP_3R antagonist, protein kinase inhibitor, or calcineurin inhibitor for the 20-min period from 0 to 20 min after LFS on depotentiation

		% change at 25–30 min after HFS		% change at 55–60 min after LFS		% reduction of LTP	
Test reagent	n	S-EPSP	A-PS	S-EPSP	A-PS	S-EPSP	A-PS
AP5	5	157.6 ± 5.9	170.1 ± 8.6	161.8 ± 12.5**	170.2 ± 7.1	−7.1 ± 7.8**	−5.8 ± 7.2**
S-4CPG	5	156.7 ± 6.7	170.2 ± 5.8	163.0 ± 12.1**	183.9 ± 4.7*	−9.4 ± 12.6**	−18.0 ± 3.5**
2-APB	7	166.2 ± 8.9	194.2 ± 19.4	169.8 ± 9.4**	192.8 ± 21.9*	−4.2 ± 6.0**	4.1 ± 6.8**
KN-62	6	163.9 ± 5.1	174.7 ± 11.3	186.3 ± 18.4**	179.6 ± 13.0*	−26.2 ± 19.3**	−6.8 ± 9.6**
FK506	6	157.6 ± 6.6	164.4 ± 6.4	156.3 ± 10.5**	186.9 ± 10.5*	5.3 ± 11.3**	−5.24 ± 12.9**

The table shows the mean percentage change in the S-EPSP and A-PS measured at 25–30 min after HFS or 55–60 min after LFS and the percentage reduction of LTP in the S-EPSP and the A-PS measured at 55–60 min after HFS when 50 μM AP5, 100 μM S-4CPG, 10 μM 2-APB, 10 μM KN-62, or 1 μM FK506 was perfused from 0 to 20 min after the end of LFS. *n*, number of experiments. A significant difference compared to the corresponding value for control depotentiation induced in the absence of drugs (Control in Table 1) is shown as $*P < 0.05$ or $**P < 0.01$ (two-tailed Student's *t*-test).

mechanism of LFS-induced depotentiation in hippocampal CA1 neurons, we suggest that the activation of IP_3Rs by preconditioning HFS results in the activation of calcineurin after LFS in postsynaptic neurons, leading to the dephosphorylation of postsynaptic proteins and a decrease in the amplitude of LTP induced by priming HFS in hippocampal CA1 neurons.

Discussion

In the depotentiation of hippocampal CA1 neurons, LFS (1000 pulses at 2 Hz) given at 30 min after the delivery of HFS (100 pulses at 100 Hz) caused a reduction in LTP (control in Fig. 5), while 2-Hz LFS itself induced no significant change in responses (control in Fig. 3). In these neurons, LTP induction did not rely on the formation of depotentiation, but instead depended on the activity of postsynaptic neurons after priming HFS, since stopping test synaptic stimulation at 0.05 Hz for 20 min after priming HFS did not affect LTP induction (Fig. 1), but did disrupt the formation of depotentiation (Fig. 5A). In addition, stopping test synaptic stimulation at 0.05 Hz for 20 min immediately after LFS did not affect the synaptic responses in naïve synaptic pathways (Fig. 3), but did disrupt the formation of depotentiation (Fig. 5A). These results indicate that 100-Hz HFS has a conditioning effect for the induction of depotentiation, and this effect is maintained by test synaptic stimulation at 0.05 Hz for a 20-min period after LFS.

Since the effect of stopping test synaptic stimulation after HFS or LFS could be replicated by perfusion with AP5, S-4CPG, or 2-APB for the induction of depotentiation (Figs. 6A, 7A), we suggest that the preconditioning effect of HFS on the induction of depotentiation at CA1 synapses involves NMDARs, group I mGluRs, or IP_3Rs in postsynaptic neurons activated by test synaptic stimulation after priming HFS. Since the application of CaMKII or calcineurin inhibitors to CA1 neurons for the 10-min period immediately after HFS or 20-min period immediately after LFS inhibited the induction of depotentiation in the presence of test synaptic stimulation (Figs. 6B, 7B), we suggest that the conditioning effect provided by priming HFS for the induction of depotentiation at CA1 synapses involves CaMKII and calcineurin in postsynaptic cells, which are activated immediately after priming HFS or subsequent LFS.

Our previous study using hippocampal CA1 neurons from IP_3R1-deficient mice demonstrated that HFS (100 pulses at 100 Hz) induces normal LTP, but a train of LFS (1000 pulses at 1 Hz), delivered at 60 min after HFS, fails to induce depotentiation (Fujii et al. 2000). In CA1 neurons of mature guinea pigs, HFS (100 pulses at 100 Hz) given in the presence of 2-APB induces similar LTP to that seen in the absence of 2-APB and is maintained for at least 60 min (Taufiq et al. 2005; Fujii et al. 2016), while a train of LFS (1000 pulses at 2 Hz) delivered at 30 min after HFS in the presence of 2-APB induces significantly lower depotentiation than that seen in the absence of 2-APB (Sugita et al. 2016). These observations indicate that the conditioning effect provided by priming HFS for the induction of depotentiation at CA1 synapses involves the activation of IP_3Rs in postsynaptic neurons.

LTP induction at CA1 synapses requires sufficient depolarization of the postsynaptic membrane to activate NMDARs (Collingridge et al. 1988a, 1988b; Alford et al. 1993) and/or voltage-gated calcium channels (Ito et al. 1995) to increase postsynaptic $[Ca^{2+}]_i$ for the activation of CaMKII (Bliss and Collingridge 1993). In this study, we have shown the effect of CaMKII activated and autophosphorylated during or after HFS on LTP induction and the effect of CaMKII activated after HFS on the depotentiation of CA1 neurons by applying KN-62 to the perfusate during or after HFS (Figs. 2, 6B). While KN-62 and Ca^{2+}/calmodulin complexes bind competitively to the regulatory domain of CaMKII (Tokumitsu et al. 1990), KN-62 does not affect LTP induction that depends on the activity of CaMKII autophosphorylated during and/or after HFS (Bliss and Collingridge 1993). Therefore, we suggest that the preconditioning effect of HFS on the induction of depotentiation consists of IP_3Rs activated by HFS and CaMKII activated after HFS due to the binding of Ca^{2+}/calmodulin complexes to CaMKII after HFS, but does not include CaMKII autophosphorylated during and/or after HFS at CA1 synapses.

The effect of stopping test synaptic stimulation during the 20-min period after priming HFS or after the subsequent LFS on the induction of depotentiation (Fig. 5) could be replicated by perfusion with each antagonist of NMDARs, group I mGluRs, and IP_3Rs for the same period (Figs. 6A, 7A). This suggests that test synaptic stimulation sustains the preconditioning effect of HFS for the induction of depotentiation, causing the coactivation of NMDARs and group I mGluRs and/or IP_3Rs in postsynaptic CA1 neurons for a 20-min period after the subsequent LFS. Given that the increase of postsynaptic $[Ca^{2+}]_i$ is caused by the activation of NMDARs and/or mGluRs in CA1 neurons (Pin and Duvoisin 1995; Otani and Connor 1998; Skeberdis et al. 2001) and that IP_3Rs act downstream from group I mGluRs in the signaling cascade (Mikoshiba 1993), it is possible that the increase of postsynaptic $[Ca^{2+}]_i$ due to Ca^{2+} influx through NMDARs/channels and Ca^{2+} efflux through IP_3Rs caused by test synaptic stimulation after HFS is involved in the signaling mechanism of the preconditioning effect of HFS on LFS-induced depotentiation in CA1 neurons.

The activation of either CaMKII or calcineurin occurs downstream from the increase of postsynaptic $[Ca^{2+}]_i$ and Ca^{2+}/calmodulin complex formation in the signaling cascade of hippocampal synaptic plasticity in CA1 neurons (Bliss and Collingridge 1993; Bear and Abraham 1996; Griffith 2004). In this study, we demonstrated that CaMKII or calcineurin, either activated by test synaptic stimulation during a 10-min period after priming HFS or during a 20-min period after subsequent LFS, was involved in the mechanism of LFS-induced depotentiation at CA1 synapses (Figs. 6B, 7B). Thus, we suggest that the preconditioning effect of priming HFS on the induction of depotentiation involves CaMKII and calcineurin, with the activity of both enzymes being sustained by test synaptic stimulation, but also being modified by LFS and contributing to the dephosphorylation of postsynaptic proteins after LFS to induce depotentiation in hippocampal CA1 neurons. Since IP^3Rs activated by test synaptic stimulation after HFS did not affect LTP amplitude (Fig. 6A), while those activated after LFS caused a decrease in LTP amplitude (Fig. 7A), it is possible that the delivery of LFS modulates the activity of IP_3Rs to switch these two enzymes from the phosphorylation to dephosphorylation of AMPARs on postsynaptic neurons, decreases LTP amplitude after LFS, and induces depotentiation at CA1 synapses.

The delivery of a 2-Hz LFS train to CA1 synapses, which by itself does not induce NMDAR-dependent LTD in hippocampal CA1 neurons (Fujii et al. 2010), induces depotentiation after the prior activation of IP_3Rs at CA1 synapses (Sugita et al. 2016). Previous studies have demonstrated that LFS-induced depotentiation in CA1 neurons is blocked by the application, during LFS, of either the NMDAR antagonist AP5 (Fujii et al. 1991; Huang et al. 2001, Sugita et al. 2016) or IP_3R inhibitor 2-APB (Yamazaki et al. 2012; Sugita et al. 2016). This implies that the increase of postsynaptic $[Ca^{2+}]_i$ during a 2-Hz LFS train is a result of Ca^{2+} influx through NMDARs and release through IP_3Rs is involved in LFS-induced depotentiation in CA1 neurons. In hippocampal CA1 neurons, the homosynaptic LTD induced by 1-Hz LFS requires NMDAR activation and a moderate increase in postsynaptic $[Ca^{2+}]_i$, the latter triggering the activation of calcineurin (Linden 1994; Bear and Abraham 1996). In the mechanism of LTD in CA1 neurons, postsynaptic calcineurin dephosphorylates and inactivates inhibitor-1, allowing protein phosphatase 1 to act on Thr286 in the catalytic domain of CaMKII or Ser831 in the AMPAR GluA1 subunit

(Lisman 1994; Mulkey et al. 1994; Barria et al. 1997; Lee et al. 2000). Group I mGluRs are positively coupled to phospholipase C and their activation is required for the induction of LTD in hippocampal CA1 neurons (Palmer et al. 1997; Reyes-Harde and Stanton 1998). Taufiq et al. (2005) showed that IP_3R activation, which occurs downstream from group I mGluRs and phospholipase C in the signaling cascade, plays an important role in facilitating NMDAR-dependent LTD induced by 1-Hz LFS in hippocampal CA1 neurons.

Since IP_3R activation is increased by a decrease in the cytoplasmic levels of free Ca^{2+}/calmodulin complexes (Michikawa et al. 1999), the activation of CaMKII due to the binding of free Ca^{2+}/calmodulin complexes to the regulatory domain of its α-subunit (CaMKIIα) (Griffith 2004) and the activation of calcineurin due to the binding of free Ca^{2+}/calmodulin complexes to the catalytic domain of calcineurin (Ye et al. 2008; Baumgärtel and Mansuy 2015) may decrease the levels of free Ca^{2+}/calmodulin complexes and increase IP_3R activation in the dendritic spine of postsynaptic CA1 neurons. The results shown in Figures 6 and 7 indicate that the preconditioning effect of HFS on the induction of depotentiation consists of IP_3Rs, CaMKII, and calcineurin activated by test synaptic stimulation after HFS in postsynaptic CA1 neurons. Therefore, we think it possible that the activation of CaMKII and calcineurin due to the coactivation of NMDARs and IP_3Rs caused by test synaptic stimulation after priming HFS (Fig. 6A,B) plays a role in modulating IP_3R activation during and/or after 2-Hz LFS and contributes to the formation of NMDAR-dependent LTD after the delivery of 2-Hz LFS to CA1 synapses (Fig. 7A,B).

IP_3R-binding protein released with IP_3 (IRBIT) (Ando et al. 2003) binds to IP_3Rs and inhibits their activity by blocking the access of IP_3 to a common binding site (Ando et al. 2003, 2006). The phosphorylation of IRBIT is essential for its binding to IP_3Rs to prevent their activation when the concentration of IP_3 is low (Ando et al. 2006). Kawaai et al. (2015) recently demonstrated that IRBIT binds to the regulatory domain of CaMKIIα and inhibits its activity in the central nervous system. They also demonstrated that an excess of Ca^{2+}/calmodulin complexes resulted in the dissociation of IRBIT from the regulatory domain of CaMKIIα because IRBIT and Ca^{2+}/calmodulin complexes bind competitively to this domain (Kawaai et al. 2015). Therefore, we think it possible, in LFS-induced depotentiation in hippocampal CA1 neurons, that IRBIT phosphorylated by priming HFS remains phosphorylated after HFS or LFS and limits IP_3R activation and CaMKIIα activity by blocking the access of IP_3 to a common binding site of IP_3Rs and the binding of the Ca^{2+}/calmodulin complexes to CaMKIIα.

In this study, we have shown the effects of CaMKII activated after HFS on LTP induction and the formation of depotentiation in CA1 neurons by applying HFS (100 pulses at 100 Hz) in a standard solution, then perfused the slices with a CaMKII inhibitor during the 10-min period immediately after HFS in the presence of test synaptic stimulation at 0.05 Hz (Figs. 2, 6B). Thus, as illustrated in Figure 8A and B, we suggest the following signaling mechanism for depotentiation during and after HFS in postsynaptic CA1 neurons: the postsynaptic increase in $[Ca^{2+}]_i$ and free Ca^{2+}/calmodulin complex levels due to the coactivation of NMDARs/channels and group I mGluRs during the period of test synaptic stimulation after priming HFS stimulates CaMKII activation while IRBIT phosphorylated and released during and after HFS inhibits further activation of IP_3R or CaMKII after priming HFS (Kawaai et al. 2015). Therefore, HFS induces a state that maintains LTP, provided there is no subsequent LFS (Figs. 1, 5).

When LFS of 1000 pulses at 2 Hz was delivered to naïve CA1 synaptic pathways and test synaptic stimulation was delivered throughout the experiment, the activation of IP_3Rs during and after the application of a 2-Hz LFS train to CA1 synapses did not decrease responses (Fig. 4A; Fujii et al. 2010). However, since the

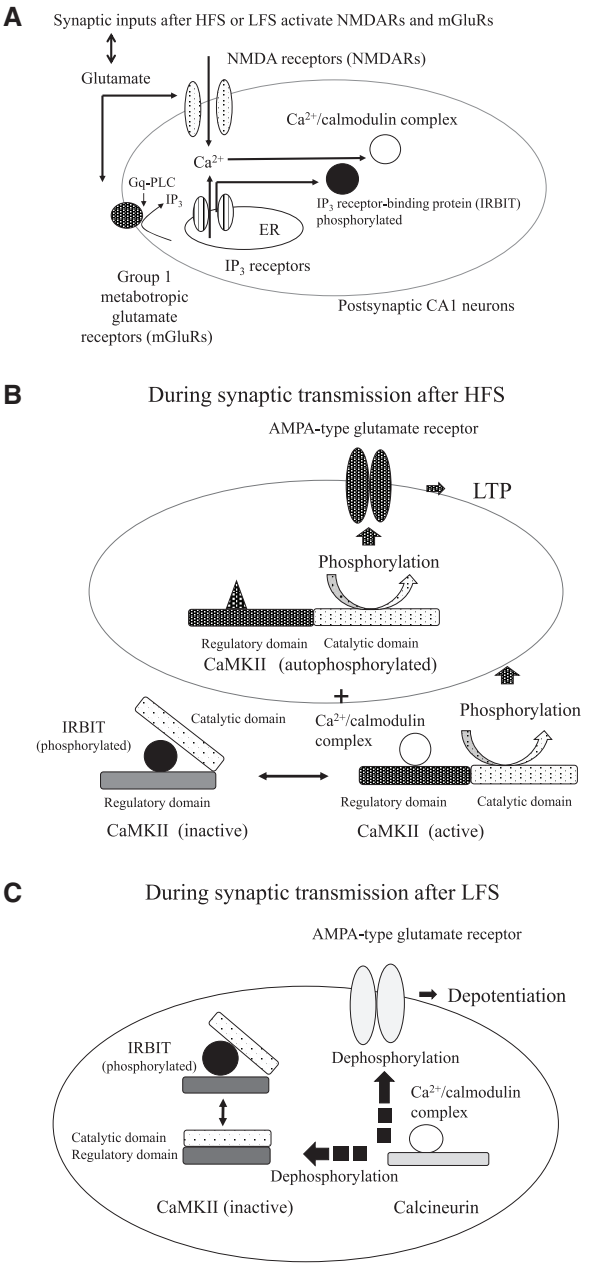

Figure 8. Schematic depiction of the mechanism of LFS-induced depotentiation in hippocampal CA1 neurons. (*A*) Synaptic inputs after HFS or LFS activate NMDARs and mGluRs on postsynaptic CA1 pyramidal neurons. The activation of IP_3Rs on the membrane of the ER occurs downstream from group I mGluRs and phospholipase C in the signaling cascade. The increase in $[Ca^{2+}]_i$ due to the coactivation of NMDARs and IP_3Rs induces an increase in the free cytoplasmic levels of Ca^{2+}/calmodulin complexes and IRBIT in the postsynaptic dendritic spines of CA1 neurons. (*B*) CaMKII autophosphorylated during and after priming HFS may continue to phosphorylate AMPA-type glutamate receptors on the dendritic spines of CA1 neurons to potentiate synaptic responses (to induce LTP), while IRBIT that is released from IP³Rs into the cytoplasm and is phosphorylated may block the binding of Ca^{2+}/calmodulin complexes to the regulatory domain of CaMKII and inhibit further CaMKII activation in postsynaptic CA1 neurons. CaMKII is active when Ca^{2+}/calmodulin complexes are bound to its regulatory domain. (*C*) During synaptic transmission after LFS, the levels of free Ca^{2+}/calmodulin complexes, which are not sequestrated by CaMKII in the presence of IRBIT, are increased to a point at which calcineurin is activated in the dendritic spines to dephosphorylate CaMKII and AMPARs at CA1 synapses, leading to a reduction in synaptic potentiation (depotentiation) in CA1 neurons.

activation of IP_3Rs during and after the LFS actually decreased them in depotentiation in hippocampal CA1 neurons (Fig. 7A; Sugita et al. 2016), it is possible that IRBIT phosphorylated by priming HFS remains phosphorylated during and after the 2-Hz LFS train and limits the activation of IP_3Rs at CA1 synapses. Stopping the test electrical stimulation of CA1 neurons during the 20-min period from 0 to 20 min after the end of the 2-Hz LFS train significantly attenuated the induction of depotentiation in CA1 neurons (Fig. 5), and this effect could be replicated by perfusion with 50 µM AP5, 100 µM S-4CPG, 10 µM 2-APB, or 2 µM FK506 (Fig. 7). In our previous study of LFS-induced depotentiation at CA1 synapses (Sugita et al. 2016), we suggested that the activation of calcineurin that occurs during LFS is maintained after LFS by test synaptic inputs applied to CA1 synapses. Therefore, we suggest the following signaling mechanism for depotentiation during and after LFS in postsynaptic CA1 neurons (Fig. 8C): the postsynaptic increase of $[Ca^{2+}]_i$ due to the coactivation of NMDARs and group I mGluRs, caused by the delivery of LFS and/or test synaptic stimulation after LFS, might stimulate calcineurin activation in postsynaptic CA1 neurons. Calcineurin has a high affinity for Ca^{2+}/calmodulin complexes (Meyer et al. 1992; Ye et al. 2008). Since IRBIT binds to the regulatory domain of CaMKIIα and inhibits CaMKII kinase activity and since IRBIT and Ca^{2+}/calmodulin complexes bind competitively to the regulatory domain of CaMKIIα (Kawaai et al. 2015), after LFS, the levels of free Ca^{2+}/calmodulin, which is not sequestrated by CaMKII in the presence of IRBIT, may be increased to a point at which calcineurin is activated in the dendritic spines, resulting in the dephosphorylation of AMPARs in postsynaptic cells and the reduction of LTP at CA1 synapses.

Recently, Park et al. (2019) studied depotentiation induced by the LFS train (2 Hz for 10 min) in adult rat hippocampal CA1 neurons and found a sensitive form of LTP to the effect of LFS and the effective timing of LFS after LTP induction. In their study, LTP was induced by a compressed theta burst stimulation (TBS) and a spaced TBS induction protocol where the only difference was in the interepisode interval of three bursts (10 sec vs.10 min). They showed a pronounced effect of LFS on the LTP induced by the compressed TBS when the timing between the induction of LTP and the delivery of LFS was from between 5 to 60 min and suggested that a type of transcriptionally independent form of LTP induced by the compressed TBS is sensitive to depotentiation. Since the depotentiation of CA1 neurons is significantly attenuated when the interval between HFS and LFS is 60 min or longer (Fujii et al. 1991; Sugita et al. 2016), the depotentiation of CA1 neurons is considered to be sensitive to the transcriptionally independent form of LTP. Angiotensin II (ANG II) stimulates the renal tubular reabsorption of NaCl by activating Na^+/H^+ exchanger type 3 (NHE3). In cultured opossum kidney proximal tubule cells, the activation of NHE3 by ANG II is mediated by the binding of IRBIT to NHE3, a process stimulated by CaMKII (He et al. 2010). The addition of ANGII to this cell line increases the binding of IRBIT to NHE3 after 5 min, but the bound IRBIT is released after 45 min, and at least 15 min of ANG II treatment is required to increase NHE3 activity and surface expression (He et al. 2010). Since the depotentiation of CA1 neurons is significantly attenuated when the interval between HFS and LFS is 60 min or longer (Fujii et al. 1991; Sugita et al. 2016) or when the interval between priming HFS and the start of the halt of test synaptic stimulation is 68 min or longer (Fig. 5C), we think it possible that IRBIT that is phosphorylated and released from IP_3Rs during and/or after HFS inhibits the further activation of CaMKII during a period of <68 min after HFS in postsynaptic CA1 neurons.

Concluding remarks

From the results of the present study, we suggest that the postsynaptic cellular events involved in the induction of LFS-induced depotentiation are as follows: (1) depotentiation triggered by priming HFS involves the coactivation of NMDARs and group I mGluRs, which is sustained by test synaptic stimulation after HFS (Fig. 8A); (2) coactivation of NMDARs and mGluRs caused by test synaptic stimulation induces Ca^{2+} influx through NMDARs/channels, Ca^{2+} efflux due to Ca^{2+}-induced Ca^{2+} release from intracellular stores, and Ca^{2+} efflux through IP_3Rs in the dendrites of postsynaptic CA1 neurons (Fig. 8A); (3) an increase of postsynaptic $[Ca^{2+}]_i$ due to Ca^{2+} influx through NMDARs/channels and Ca^{2+} efflux through IP_3Rs results in the formation of free Ca^{2+}/calmodulin complexes that activate CaMKII, while IRBIT released from IP_3Rs due to IP_3R activation inhibits CaMKII activation further in the dendritic cytoplasm of postsynaptic CA1 neurons (Fig. 8B); (4) in the presence of IRBIT, which binds to the regulatory domain of CaMKIIα and inhibits its kinase activity, free Ca^{2+}/calmodulin complexes are not sequestrated by CaMKII; (5) the moderate increase in the levels of $[Ca^{2+}]_i$ caused by the coactivation of NMDARs and IP_3Rs during and/or after the 2-Hz LFS results in an increase in the levels of free Ca^{2+}/calmodulin complexes in postsynaptic CA1 neurons; and (6) the levels of free Ca^{2+}/calmodulin complexes, which are not sequestrated by CaMKII in the presence of IRBIT, are increased to a point at which calcineurin is activated in the dendritic spines, resulting in the dephosphorylation of AMPARs in postsynaptic CA1 neurons and the reduction of LTP at CA1 synapses (Fig. 8C). Therefore, we conclude that sustained synaptic activity after LTP induction, which continuously stimulates group I mGluRs and/or IP_3Rs in postsynaptic CA1 neurons, is essential for LFS-induced depotentiation in CA1 neurons. In LFS-induced LTP suppression at CA1 synapses, the coactivation of NMDARs and group I mGluRs caused by sustained synaptic activity after priming LFS results in the activation of IP_3Rs, which leads to the failure of LTP induction (Fujii et al. 2016). Thus, in hippocampal CA1 neurons, sustained synaptic activity after priming HFS or LFS, which continuously activates postsynaptic IP_3Rs, determines the direction of LTP expression after the subsequent application of LFS or HFS.

Materials and Methods

Ethics approval

The animals used were maintained and handled according to the guidelines of the Animal Care and Use Committee of Yamagata University School of Medicine.

Slice preparation

Male Hartley guinea pigs (3–6 wk old; Funabashi Farm Co.) were decapitated and the hippocampi were removed rapidly and cut into 500 µm-thick transverse slices. The slices were preincubated for a minimum of 1 h at 30°C in a 95% O_2/5% CO_2 atmosphere in a standard solution ([mM] NaCl, 124; KCl, 5.0; NaH_2PO_4, 1.25; $MgSO_4$, 2.0; $CaCl_2$, 2.5; $NaHCO_3$, 22.0; and D-glucose, 10.0, pH ~7.4 at 30°C) before being placed in a 1-mL recording chamber and completely submerged in standard solution perfused continuously at a rate of 2–3 mL/min; the temperature in the recording chamber was maintained at 30°C–32°C.

Electrophysiology

A bipolar stimulating electrode was placed in the stratum radiatum to stimulate the input pathways to the CA1 neurons. One recording electrode was positioned in the stratum radiatum and another in the pyramidal cell body layer of the CA1 region to record field EPSPs and population spikes (PSs), respectively, and a test electrical stimulus with a pulse duration of 0.1 msec was applied every 20 sec (test synaptic stimulation). The slope of field EPSPs (S-EPSP) and the amplitude of PSs (A-PS) were measured and plotted automatically. At the beginning of each experiment, the strength of the

stimulus pulse was adjusted to elicit PSs with an amplitude of 40%–60% of maximal and was fixed at this level. After checking the stability of the S-EPSP and A-PS for more than 15 min, a conditioning stimulus of tetanus or LFS was delivered to induce synaptic plasticity at CA1 neurons.

To induce LTP, HFS consisting of 100 pulses at 100 Hz (tetanus) was used. To induce depotentiation, a train of LFS consisting of 1000 pulses at 2 Hz was applied at 30 min after HFS delivery. The mean value of the S-EPSP or A-PS during the 10-min period immediately before HFS delivery was defined as the 100% level, while the other responses were expressed as a mean percentage ± standard error of the mean (S.E.M.) of this control level. To evaluate the control effects of LFS on the synaptic transmission of naïve synaptic pathways, the mean value of the S-EPSP or A-PS during the 10-min period immediately before LFS delivery was defined as the 100% level, while the other responses were expressed as a mean percentage ± S.E.M. of this control level.

Changes in responses after HFS or LFS were calculated as follows: (i) the percentage change in the responses after HFS was calculated as $(Y/X) \times 100$; (ii) the percentage change in the responses after LFS was calculated as $(Z/X) \times 100$; and (iii) the percentage reduction in LTP after LFS (depotentiation) was calculated as $(Y - Z)/(Y - X) \times 100$, where X is the averaged value for the 10-min period immediately prior to HFS or LFS, Y is the averaged value at 5–0 min immediately before LFS, and Z is the stable level at 55–60 min after the end of LFS (left panel in Fig. 5A). Using the equation given in (iii), 100 or 0% indicate a complete reduction to the pre-HFS control level or no induction of depotentiation, respectively.

When the delivery of test synaptic stimulation at 0.05 Hz was halted for the 19-min period from 1 to 20 min after HFS delivery, for the 20-min period from 0 to 20 min after the end of LFS, or the 20-min period from 30 to 50 min after the end of LFS, the mean magnitude of the S-EPSP or A-PS was not measured during these periods and was omitted from the time course figures (Figs. 1, 3, 5).

S-4-Carboxyphenylglycine (S-4CPG) was purchased from Tocris Cookson Ltd., while 2-APB, D, L-2-amino-5-phosphonovalerate (AP5), 1-[N, O-bis(5-isoquinolinesulfonyl)-N-methyl-L-tryosyl]-4-phenylpiperazine (KN-62), and FK506 (tacrolimus) were purchased from Sigma. Each of these test reagents was added to the perfusate when test synaptic stimulation at 0.05 Hz was continued throughout the experiment. AP5, 2-APB, or S-4CPG was applied for the 19-min period from 1 to 20 min after HFS or the 20-min period from 0 to 20 min after the end of LFS. KN-62 was applied for the 10-min period from 9 min before to 1 min after HFS, the 10-min period from 1 to 11 min after HFS, or the 20-min period after LFS. FK506 was applied for the10-min period from 1 to 11 min after HFS or the 20-min period after LFS.

Statistical analysis

The results were analyzed for statistical significance using the two-tailed Student's *t*-test, taking a *P*-value <0.05 as significant.

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