Research

Involvement of IP3 receptors in LTP and LTD induction in guinea pig hippocampal CA1 neurons

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The role of inositol 1, 4, 5-trisphosphate receptors (IP3Rs) in long-term potentiation (LTP) and long-term depression (LTD) was studied in CA1 neurons in guinea pig hippocampal slices. In standard solution, short tetanic stimulation consisting of 15 pulses at 100 Hz induced LTP, while three short trains of low-frequency stimulation (LFS; 200 pulses at 1 Hz) at 18-min intervals or one long train of LFS (1000 pulses at 1 Hz) induced stable LTD in both the slope of the field EPSP (S-EPSP) and the amplitude of the population spike (A-PS). Bath application of 2-aminoethoxydiphenyl borate (2-APB), an IP3R antagonist, or of N-methyl-D-aspartate (NMDA) antagonists (MCPG), a wide-spectrum metabotropic glutamate receptor antagonist, during weak tetanic stimulation significantly increased the magnitude of the LTP in both the S-EPSP and A-PS. Three short trains of LFS or one long train of LFS delivered in the presence of 2-APB or MCPG did not induce LTD, but elicited LTP. Based on these results, we conclude that, in hippocampal CA1 neurons, IP3Rs play an important role in synaptic plasticity by attenuating LTP and facilitating LTD.

In the hippocampus, long-term potentiation (LTP) and long-term depression (LTD) are thought to play important roles in learning and memory. Activity-dependent LTP is the persistent enhancement of synaptic transmission induced by brief high-frequency afferent stimulation (HFS) (Bliss and Lømo 1973), and it is generally believed to be triggered by the influx of calcium ions (Ca2+) into the post-synaptic neuron through channels coupled to N-methyl-D-aspartate receptors (NMDARs) (Bliss and Collingridge 1993). Furthermore, LTD involves the depression of a response in a naïve pathway by low-frequency (1–5 Hz) stimulation (LFS) and is blocked by NMDA antagonists (Bear and Abraham 1995). Thus, activation of NMDARs during HFS or LFS is an essential factor in the induction of activity-dependent LTP or LTD in CA1 neurons.

Activation of both metabotropic glutamate receptors (mGluRs) and NMDAR/Ca2+ channels is necessary for the induction of homosynaptic LTD in hippocampal CA1 neurons (Bashir et al. 1993; Bliss and Collingridge 1993), and we recently showed cooperativity between these receptors in LTP induction at CA1 synapses (Fujii et al. 2003). However, although homosynaptic LTD induced by LFS in hippocampal CA1 neurons has been reported to require the activation of both NMDAR/Ca2+ channels and mGluRs (Dudek and Bear 1992; Mulkey and Malenka 1992; Linden 1994), it is still controversial whether both receptor types must be coactivated. Oliet et al. (1997) showed the coexistence of two mechanistically distinct forms of LTD in hippocampal CA1 neurons in juvenile rats, one of which is dependent on the activation of T-type voltage-gated Ca2+ channels, group I mGluRs, and protein kinase C (PKC), while the other is dependent on the activation of NMDARs. Other studies have shown that homosynaptic LTD in CA1 neurons induced by activation of mGluRs may not require activation of NMDARs (Otani and Connor 1998; Palmer et al. 1997; Fujii et al. 2003).

Stimulation of group I mGluRs in hippocampal CA1 neurons activates phospholipase C (PLC), which hydrolyzes the inositol lipid precursor into the post-synaptic plasma membrane into inositol 1, 4, 5-trisphosphate (IP3) and diacylglycerol (DAG); the former opens IP3 receptor channels, while the latter activates PKC (Ben-Ari et al. 1992; Nakanishi 1992). In hippocampal CA1 neurons, DAG or PKC is suggested to be involved in the induction of activity-dependent LTD (Kato et al. 1991; Abeliovich et al. 1993).

The IP3 receptor (IP3R) acts as an IP3-gated Ca2+ release channel in a variety of cells (Berridge 1993; Mikoshi 1993; Furuiuchi et al. 1994). The type 1 IP3 receptor (IP3R1) is the major neuronal member of the IP3R family in the central nervous system and is present at high levels in cerebellar Purkinje cells and hippocampal CA1 neurons (Furuiuchi et al. 1993). Recently, using IP3R1-lacking mice produced by gene targeting (Matsumoto et al. 1996), we investigated the role of IP3R1 in LTP induction in hippocampal CA1 neurons and demonstrated that LTD induction was facilitated in these mice (Fujii et al. 2000). We concluded that this receptor plays an important role in suppressing LTP in hippocampal CA1 neurons.

Since IP3R activation occurs downstream of mGluR activation in the signaling cascade in hippocampal CA1 neurons, it is possible that activation of IP3Rs also plays an essential role in LTD induction at CA1 synapses. In fact, PLC has been shown to be required for the formation of activity-dependent LTD in hippocampal CA1 neurons (Reyes-Harde and Stanton 1998). It is therefore possible that IP3R-dependent signaling pathways are involved in activity-dependent LTD. However, previous studies have shown that LTD induced by a 1-Hz LFS train in CA1 neurons of IP3R1-lacking mice is not significantly affected (Fujii et al. 2000; Nagase et al. 2003).

It is theoretically possible that the above LTD experiments using IP3R1-lacking mice failed to show involvement of IP3Rs in CA1 synaptic plasticity because some other molecular mechanism for LTD induction might have been switched on in the hippocampal CA1 neurons of these mice during development to...
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compensate for the IP3R1 deficiency. In the present study, we therefore examined the effects of acute exposure of hippocampal slices to either 2-aminothoxydiphenyl borate (2-APB), an antagonist for both IP3Rs and store-operated calcium (SOC) channels (Maruyama et al. 1997; Peppiatt et al. 2003), or to α-methyl-4-carboxyphenylglycine (MCPG), an mGluR antagonist, on LTP and LTD induction in CA1 neurons.

Results

Effect of 2-APB on LTP

We examined the effect of 3–10 µM 2-APB on the configuration and amplitude of the baseline responses to a single stimulus and found that the application of 2-APB at concentrations up to 10 µM did not have any significant effect. Figure 1A shows the summarized time-course for the change in the slope of the field EPSP (S-EPSP) and the amplitude of the population spike (A-PS) during the 50-min period following 10-min application of 10 µM 2-APB (Fig. 1A), and sample strength-response curves (SRC) recorded during, and 35–45 min after, application of 10 µM 2-APB (Fig. 1A). These data show that 2-APB did not affect either the S-EPSP or the input-output function of CA1 synaptic responses. The S-EPSP or A-PS measured 35–45 min after wash-out of 3 µM 2-APB were, respectively, 101.2% ± 4.2% and 98.8% ± 3.5% of the pre-perfusion control levels (n = 5, data not shown); and the corresponding values measured 35–45 min after wash-out of 10 µM 2-APB were, respectively, 99.8% ± 3.3% and 99.9% ± 3.7% of the pre-perfusion control levels (n = 5, Fig. 1A). The threshold intensity for eliciting the field EPSP (f-EPSP) in the 10-min period during, or at 35–45 min after, application of 10 µM 2-APB was 0.17 ± 0.02 mA and 0.17 ± 0.01 mA, respectively (n = 7). In addition, the threshold intensity for eliciting the population spike was not significantly different during, or at 35–45 min after, application of 10 µM 2-APB (0.19 ± 0.02 and 0.19 ± 0.01 mA, respectively; n = 7). A 2-APB concentration of 3 µM or 10 µM was therefore used in the following experiments.

In hippocampal CA1 neurons, a short tetanus (T10: 10 pulses at 100 Hz) induced a robust LTP in the presence of 2-APB, but not in standard solution. Figure 1, B and C, shows examples of the time-course of the change in the S-EPSP (panel 1), samples of f-EPSP wave forms (panel 2), and strength-response curves (SRCs) (panel 3) in response to two successive short tetani of 10 pulses (T10) and 15 pulses (T15) at 100 Hz given in standard medium (Fig. 1B) or in the presence of 10 µM 2-APB (Fig. 1C). In standard solution, T10 evoked short-term potentiation, which fell back close to the pre-tetanic level within 20 min (Fig. 1B), whereas in the presence of 10 µM 2-APB, it elicited a marked increase in the S-EPSP that was maintained for >45 min (Fig. 1C). Although T15 in the absence or the presence of 10 µM 2-APB induced stable LTP in the S-EPSP (Fig. 1B, B1, B2, B3) and A-PS (Fig. 1C, C1, C2, C3), the application of 10 µM 2-APB during T10 and T15 induced clear changes in the configuration of the f-EPSP (Fig. 1B, B3, C2). These results suggest that 10 µM 2-APB facilitates LTP induction at CA1 synapses.

Figure 2 summarizes the time-course of the change in the S-EPSP (Fig. 2A) or A-PS (Fig. 2B) in response to two successive short tetani of T10 and T15 given in standard medium or in the presence of 3 µM or 10 µM 2-APB. In standard solution, T10 evoked short-term potentiation, which fell gradually back almost to pre-tetanic levels within 20 min, while T15 induced stable LTP in both the S-EPSP and A-PS (empty circles, Fig. 2A, B). The mean magnitude of the S-EPSP measured 35–45 min after T10 or T15 was, respectively, 112.5% ± 4.5% and 125.4% ± 6.9% (n = 9) of the pre-T10 values; the corresponding values for the A-PS were 106.2% ± 4.4% and 123.5% ± 7.0% (n = 8). These results show that T10 is just below, and T15 just above, the threshold for inducing LTP in hippocampal CA1 neurons.

In the presence of 3 µM 2-APB, T10 induced a small LTP in both the S-EPSP and A-PS (filled circles, Fig. 2A, B). The mean magnitude of the LTP in the S-EPSP or A-PS was, respectively, 127.9% ± 5.8% (n = 10) and 115.8% ± 2.4% (n = 10) of the pre-T10 control values, both of which were significantly higher (P < 0.05, two-tailed Student’s t-test) than the corresponding values of 112.5% ± 4.5% and 106.2% ± 4.4% in standard medium. Delivery of T15 further increased the LTP of the S-EPSP or A-PS to 160.1% ± 12.2% (n = 10) and 135.8% ± 4.2% (n = 10) of the pre-T10 control values, respectively, showing that the LTP in the S-EPSP was significantly higher (P < 0.05) than the corresponding value of 125.4% ± 6.9% in standard medium, whereas the LTP in the A-PS was not significantly different from the value of 123.5% ± 7.0% in standard medium.

In the presence of 10 µM 2-APB, T10 induced a robust LTP in both the S-EPSP and A-PS (filled triangles, Fig. 2A, B). The mean magnitude of the LTP in the S-EPSP or A-PS was, respectively, 145.0% ± 6.6% (n = 6) and 136.1% ± 7.2% (n = 6) of pre-T10 levels.
values, both of which were significantly higher ($P < 0.01$) than the corresponding values in standard medium. Delivery of T$_{15}$ further increased the magnitude of the LTP in the S-EPSP or A-PS to $185.2\% \pm 10.2\%$ (n = 6) and $168.8\% \pm 14.7\%$ (n = 6) of the pre-T$_{10}$ values, respectively, both of which were significantly ($P < 0.01$) higher than the values in standard medium. These results show that 10 µM 2-APB facilitates LTP induction in both the S-EPSP and A-PS after a T$_{10}$ or T$_{15}$ short tetanus without any significant effect on baseline responses.

In contrast, a standard tetanus (T$_{100}$; 100 pulses at 100 Hz) applied in the presence of 10 µM 2-APB induced an LTP similar to that seen in the absence of 2-APB. The mean magnitude of the control LTP in the S-EPSP or A-PS (empty circles, Fig. 3A,B) was, respectively, $156.1\% \pm 4.8\%$ (n = 6) and $161.3\% \pm 6.9\%$ (n = 6) of the pre-T$_{100}$ values; the corresponding values for the LTP induced in the presence of 10 µM 2-APB (empty triangles, Fig. 3A,B) were $156.5\% \pm 16.3\%$ (n = 6) and $161.3\% \pm 6.9\%$ (n = 6), with no significant difference between the groups. This shows that 10 µM 2-APB does not affect LTP induced in CA1 neurons by a standard tetanus.

We then examined whether 10 µM 2-APB directly affected the function of NMDARs in CA1 neurons. For this purpose, we performed whole-cell recordings on CA1 pyramidal cells and recorded NMDAR-mediated excitatory postsynaptic currents (EPSCs) in the presence of 20 µM 6, 7-dinitroquinoxaline-2,3-dione (DNQX), a non-NMDAR antagonist, and 10 µM bicuculline, a GABA$_A$ receptor antagonist. The pyramidal cells were voltage-clamped at $-30$ to $-40$ mV, and test stimuli were delivered at a frequency of 0.033 Hz. An example of the time-course of the NMDAR-mediated EPSCs is shown in Figure 4A; this was confirmed as an NMDAR response by blocking with DL-2-amino-5-phosphonovalerate (AP5; 50 µM). The summarized results from seven slices, shown in Figure 4B, showed that 10 µM 2-APB did not have any significant effect on post-synaptic NMDAR currents in response to a single CA1 synaptic input. Thus, it seems unlikely that 2-APB applied during a short tetanus enhances NMDAR activation during tetanus and facilitates LTP induction in hippocampal CA1 neurons.

**Effect of 2-APB on LTD**

Figure 5, A and B, shows the summarized time-course for the LTD induced in the absence or the presence of 10 µM 2-APB. Delivery of short LFSs (three 200 pulses of 1 Hz at 18-min intervals) in the absence of 2-APB produced a robust LTD in the S-EPSP (empty circles, Fig. 5A) and A-PS (empty squares, Fig. 5B). The mean magnitude of the LTD in the S-EPSP or A-PS measured during the 35–45 min after the end of the third LFS was, respectively, $78.9\% \pm 5.5\%$ (n = 8) and $83.0\% \pm 3.5\%$ (n = 8) of the pre-LFS levels. In contrast, the sequential delivery of short LFSs in the presence of 10 µM 2-APB resulted in induction of LTD in the
The S-EPSP and A-PS measured 35–45 min after the end of the third LFS were, respectively, 143.2% (n = 6) and 203.0% (n = 6) of the pre-LFS levels, both of which were significantly higher (P < 0.001 and P < 0.05) than the values in standard medium. These results show that 10 µM 2-APB applied during short LFSs attenuates LTD formation and facilitates LTP induction in both the S-EPSP and A-PS.

Effect of MCPG on LTP and LTD
Since IP3R activation occurs downstream of mGluR activation in the signaling cascade in hippocampal CA1 neurons, the above results suggested that activation of group I mGluRs during HFS attenuates LTP induction, while their activation during LFS facilitates LTD induction. We therefore examined the effects of a broad-spectrum mGluR antagonist, MCPG, on LTP and LTD induction.

Figure 3 shows the effect of MCPG at two different concentrations (200 µM and 500 µM) on LTP induced in CA1 neurons by a standard tetanus (T100; 100 pulses at 100 Hz). In standard solution, T100 induced a robust LTP in both the S-EPSP and A-PS (empty circles, Fig. 3A,B). The mean magnitude of the LTP in the S-EPSP or A-PS was, respectively, 155.3 ± 3.6% (n = 10) and 145.5 ± 4.1% (n = 10) of the pre-T100 control values. In contrast, delivery of T100 in the presence of 200 µM or 500 µM MCPG failed to induce LTP in hippocampal CA1 neurons. In the presence of 200 µM MCPG, T100 induced short-term potentiation, which fell gradually back almost to pre-tetanic levels within 35 min (filled triangles, Fig. 3A,B). The mean magnitude of the S-EPSP measured 35–45 min after T100 was 107.4% ± 3.6% (n = 5) of the pre-T100 values; the corresponding values for the A-PS were 109.5% ± 7.5% (n = 5), both of which were significantly lower (P < 0.01) than the corresponding LTP values in standard medium. In the presence of 500 µM MCPG, similar results were seen (filled squares, Fig. 3A,B). The S-EPSP or A-PS 35–45 min after T100 were 104.4% ± 8.3% and 100.0% ± 6.8% (n = 4) of the pre-T100 control values, respectively, both significantly lower (P < 0.01) than the corresponding LTP values in standard medium, whereas the S-EPSP or A-PS 35–45 min after T100 in the presence of either 200 µM or 500 µM MCPG were not significantly different from the corresponding values in standard medium. These results show that MCPG at a concentration of 200 µM or 500 µM inhibited activation of mGluRs in hippocampal CA1 neurons during a 100-pulse tetanus and blocked the LTP induction in both the S-EPSP and A-PS.

However, 200 µM MCPG facilitated the LTP induced with a two short tetani protocol. As shown in the summarized LTP time-course plots in Figure 6A, two successive short tetani given in the presence of 200 µM MCPG induced a robust LTP in the S-EPSP and A-PS. The mean magnitude of the LTP in the S-EPSP or A-PS induced by T10 at 35–45 min after T10 was, respectively, 141.2% ± 11.2% and 126.3% ± 5.7% (n = 4 for both) of the pre-T10 control values, both significantly higher (P < 0.05 or P < 0.01) than the corresponding values in standard medium (empty circles, Fig. 2A,B). The subsequent delivery of T15 further increased the magnitude of the LTP in the S-EPSP or A-PS, respectively, to 217.5% ± 39.5% and 172.3% ± 10.0% (n = 4 for both) of the pre-T10 values.
of the pre-T<sub>10</sub> control values, both significantly (<i>P</i> < 0.05 or <i>P</i> < 0.01) higher than the corresponding values in standard medium (empty circles, Fig. 2A,B). These results show that 200 µM MCPG applied during a short tetanus of 10 or 15 pulses inhibits the activation of mGluRs and facilitates LTP induction in both the S-EPSP and A-PS.

Furthermore, the sequential delivery of short LFSs (200 pulses at 1 Hz) in the presence of 200 µM MCPG resulted in induction of LTP in the S-EPSP and A-PS (Fig. 6B). The S-EPSP or A-PS measured 35–45 min after the third LFS were, respectively, 123.2% ± 13.7% (n = 5) and 113.4% ± 24.0% (n = 4) of the pre-LFS control values, the former being significantly higher (<i>P</i> < 0.01) than the corresponding value in standard medium (empty circles, Fig. 5A).

We also examined the effect of 500 µM MCPG on LTD induced by a LFS train of 1000 pulses at 1 Hz. In standard solution, LTD was induced by a long LFS train of 1000 pulses at 1 Hz. The S-EPSP and A-PS at 35–45 min after the end of the LFS train were, respectively, 92.5% ± 4.9% and 92.0% ± 4.0% (n = 10 for both) of the pre-LFS values (data not shown). However, when the LFS train was applied in the presence of 500 µM MCPG, LTD was not induced in CA1 neurons. The S-EPSP or A-PS measured 35–45 min after the end of LFS were, respectively, 136.6% ± 13.2% and 121.5% ± 16.1% (n = 7 for both) of the pre-LFS control levels, both significantly higher (<i>P</i> < 0.01 or <i>P</i> < 0.05) than the values for the corresponding LTD in standard medium (data not shown). We therefore conclude that, in hippocampal CA1 neurons, LTD requires activation of mGluRs during 1-Hz LFS. The failure of LTD induction and the induction of LTP due to the blockade of mGluRs during LFS facilitates LTD.

Discussion

The present study demonstrated that, in hippocampal CA1 neurons, LTP induction was facilitated by 10 µM 2-APB or 200 µM MCPG when these reagents were applied to slices during a short tetanus (10 or 15 pulses at 100 Hz) (Figs. 2, 6A). Since IP3R activation occurs downstream of mGluR activation in the signaling cascade in hippocampal CA1 neurons (Berridge 1993), the facilitation of LTP by a short tetanus revealed in the presence of 2-APB (Fig. 2) indicates that MCPG modification of LTP induction (Fig. 6A) is due to the blockade of IP3Rs at CA1 synapses. We therefore conclude that, in hippocampal CA1 neurons, IP3Rs play an important role in synaptic plasticity and that their activation during a short tetanus attenuates LTP. This activity-dependent LTP is generally believed to be triggered by the influx, during HFS, of calcium ions into the post-synaptic neuron through channels coupled to NMDARs (Bliss and Collingridge 1993). It is therefore possible that, in these cells, an increase in the [Ca<sup>2+</sup>] mediated by Ca<sup>2+</sup> efflux from internal stores and an increase due to Ca<sup>2+</sup> influx through NMDARs play opposite roles in LTP induction.

Two distinct calcium-releasing sites on the surface of the internal stores of the endoplasmic reticulum have been identified, these being the ryanodine receptor (RyR), which is associated with a calcium-sensitive calcium store (SOC channels), and the IP3R, which is associated with an IP3-dependent calcium store. Hippocampal CA1 neurons contain high levels of the type 3 RyR (RyR3), but little type 1 or type 2 RyR (Furuchi et al. 1994). RyR3-deficient mice produced by gene targeting exhibit facilitation of the LTP induced by a short tetanus (10 pulses at 100 Hz) at hippocampal CA1 synapses (Futatsugi et al. 1999), suggesting that calcium-induced calcium release, as a result of Ca<sup>2+</sup> influx during/after tetanus, contributes to the suppression of LTP in-
dution in hippocampal CA1 neurons. Our previous study in hippocampal CA1 neurons in IP3R-lacking mice demonstrated that LTP induction by a short tetanus (10 pulses at 100 Hz) was facilitated (Fujii et al. 2000). These results suggest that Ca\(^{2+}\) efflux through SOC channels or the IP3R1 during and after a short tetanus plays a role in suppressing synaptic potentiation in hippocampal CA1 neurons.

Furthermore, the present study demonstrated that 2-APB facilitated LTP induced by a short tetanus at hippocampal CA1 synapses (Fig. 2). Since 2-APB did not directly affect EPSCs elicited through NMDARs by a single synaptic input (Fig. 4), it is unlikely that 2-APB modulation of LTP induction was due to enhancement of NMDAR activation during a short tetanus. Thus, it is possible that the activation of SOC channels or IP3Rs during a short tetanus plays an important role in limiting LTP induction at CA1 synapses.

A standard tetanus (100 pulses at 100 Hz) given in the presence of 2-APB induced a similar LTP to that seen in the absence of 2-APB, both being maintained for at least 60 min (Fig. 3). This may indicate that the activation of SOC channels or IP3Rs during a standard tetanus cannot block LTP induction at CA1 synapses. LTP induction in hippocampal CA1 neurons requires sufficient depolarization of the post-synaptic membrane to activate NMDAR/Ca\(^{2+}\) channels, and this depolarization depends on input activity (Collingridge et al. 1988a,b). Furthermore, an increase in [Ca\(^{2+}\)] (through VGCCs, which also depends on input activity, is thought to contribute to LTP induction (Ito et al. 1995). Thus, it is possible that SOC channels or IP3Rs activated during a standard tetanus cannot antagonize the large Ca\(^{2+}\) influx through NMDAR/Ca\(^{2+}\) channels or VGCCs, which is the main force triggering LTP formation.

In hippocampal CA1 neurons, LTD is increasingly recognized to have at least three variants: NMDARs-dependent LTD, a calcium-sensitive calcium store-dependent LTD, and mGlurS-dependent LTD. The homosynaptic LTD induced by LFS at 1 Hz also appears to require activation of NMDARs and an increase in the postsynaptic [Ca\(^{2+}\)], which triggers activation of Ca\(^{2+}\)-dependent second messenger systems (Linden 1994), followed by calcium-independent signaling steps (Bear and Abraham 1995) and dephosphorylation of the GluR1 subunit of \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (Lee et al. 1998, 2000). In RyR3-deficient mice, a train of LFS (900 pulses at 1 Hz) does not induce LTD (Futatsugi et al. 1999). In addition, the results of the present study demonstrated that acute exposure to MCPG or 2-APB not only attenuated LTD, but also induced LTD (Figs. 2, 6) in CA1 synapses. It is therefore possible that, in hippocampal CA1 neurons, an increase in the [Ca\(^{2+}\)], mediated by Ca\(^{2+}\) efflux from internal stores via IP3Rs contributes to LTD induction.

In CA1 neurons, MCPG or 2APB applied during 1-Hz LFS blocked LTD induction and induced LTD; in contrast, when applied during 100-Hz HFS (10 or 15 pulses), they facilitated LTD induction (Figs. 2, 4, 6). Since the only procedural difference between the induction of LTD or LFS was the frequency of stimulation (HFS or LFS), this indicates that the LTD facilitation and LTD attenuation seen in hippocampal CA1 neurons in the presence of 2-APB or MCPG are mechanistically similar forms of synaptic plasticity, and that synaptic transmission is readily potentiated by input activity and maintained due to blockade of Ca\(^{2+}\) efflux from internal stores at CA1 synapses.

We have previously shown that both a short LFS (200 pulses at 1 Hz) and a long LFS (1000 pulses at 1 Hz) induce a robust LTD in hippocampal slices from mice lacking IP3Rs (Fujii et al. 2000). In contrast, acute exposure of hippocampal slices from normal mice to 10 \(\mu\)M 2-APB inhibits both chemically induced LTD (Fujii et al. 2003, 2004) and activity-dependent LTD (Figs. 4, 6) at CA1 synapses. These results suggest that some other molecular mechanism for LTD induction might be switched on in the hippocampal CA1 neurons of IP3R-deficient mice during development to compensate for the IP3R1 deficiency.

**Materials and Methods**

**Slice preparation**

The animals used were maintained and handled in accordance with the guidelines of the Animal Care and Use Committee of the Yamagata University School of Medicine.

Male Hartley guinea pigs (250–350 g) were killed by decapitation. The hippocampus was rapidly removed and cut into 500-\(\mu\)m thick transverse slices using a rotary slicer (Dosaka DK-7700). The slices were preincubated at 30–32°C in a 95% O\(_2\)/5% CO\(_2\) atmosphere in standard solution (in mM: 124 NaCl, 5.0 KCl, 1.25 Na\(_2\)HPO\(_4\), 2.0 MgSO\(_4\), 2.5 CaCl\(_2\), 22.0 NaHCO\(_3\), and 10.0 D-glucose, pH 7.38–7.42) for a minimum of 2 h, then one slice was placed in a 1-mL recording chamber and continuously perfused with standard solution at a rate of 2–3 mL/min. The temperature in the recording chamber was maintained at 30–32°C.

**Electrophysiological recordings**

A bipolar stimulating electrode was placed in the stratum radiatum to stimulate the input pathways to the CA1 neurons, and two recording electrodes were positioned in the stratum radiatum and the pyramidal cell body layer of the CA1 region. At the beginning of each experiment, the strength of the stimulus pulse (100-\(\mu\)sec duration) was adjusted to elicit a fEPSP, recorded at the stratum radiatum, with an initial slope 40%–60% of maximal, and was then fixed at this level. The f-EPSP and the A-PS were recorded in CA1 neurons, test stimuli to Schaffer collaterals/commissural afferents being delivered at 0.05 Hz.

After recording stable baseline responses for \(\geq10\) min, LTP was elicited by applying two short tetani of HFS at 100 Hz consisting of 10 pulses (\(T_{100}\)) and 15 pulses (\(T_{150}\)) separated by an interval of 45 min, or by applying a single tetanus consisting of 100 pulses (\(T_{100}\)). In LTD experiments, either three trains of short LFS (200 pulses at 1 Hz) separated by an interval of 18 min or one train of standard LFS (1000 pulses at 1 Hz) were applied. To evaluate changes in the response, the mean value of the S-EPSP or A-PS in the 10-min period before the delivery of the tetanus or LFS to naive slices was defined as the control level (100%), and mean responses during the 35- to 45-min period after the tetanus or LFS were expressed as a percentage of the control level. LTP in the S-EPSP or A-PS was defined as a value \(>120\%\) of the baseline value. Test drugs were applied for the entire period from 10 min before to 3 min after the tetanus or LFS, then replaced by standard solution, and their effect was evaluated by comparing the S-EPSP and A-PS at 35–45 min after the end of the tetanus or LFS in the presence or absence of the drug.

For whole-cell recordings, pyramidal cells were visualized using a 40\(\times\) water-immersion objective and differential interference contrast system under infrared light (Nikon). Patch electrodes were pulled from borosilicate glass using a micropipette puller. The pipettes had a resistance of 3–7 M\(\Omega\) after being filled with pipette solution containing 117 mM Cs-methanesulfonate, 10 mM HEPES, 0.5 mM EGTA, 5 mM TEA-Cl, 2.8 mM NaCl, 2.5 mM Mg-ATP, 0.3 mM Na-GTP, and 5 mM QX-314 (pH 7.3 with NaOH). NMDAR-mediated synaptic currents were recorded following the delivery of test stimuli at 0.033 Hz in the presence of antagonists of non-NMDA and GABA\(_{A}\) receptors (20\(\mu\)M DNQX and 10\(\mu\)M bicuculline). The recorded pyramidal cells were voltage-clamped at −30 to −40 mV. Current signals were amplified (Axopatch-1D amplifier, Axon Instruments Inc.), filtered (2–5 kHz), digitized at 10 kHz, and stored on a computer.

MCPG and 2-APB were obtained from Tocris Cookson Ltd., and the other drugs were obtained from Sigma. All values are given as the mean ± SEM. The results were analyzed for statistical significance (\(P < 0.05\) or \(P < 0.01\)) using the two-tailed Student’s t-test.
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