Research

Synaptic plasticity in hippocampal CA1 neurons of mice lacking inositol-1,4,5-trisphosphate receptor-binding protein released with IP_3 (IRBIT)

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In hippocampal CA1 neurons of wild-type mice, a short tetanus (15 or 20 pulses at 100 Hz) or a standard tetanus (100 pulses at 100 Hz) to a naive input pathway induces long-term potentiation (LTP) of the responses. Low-frequency stimulation (LFS; 1000 pulses at 1 Hz) 60 min after the standard tetanus reverses LTP (depotentiation [DP]), while LFS applied 60 min prior to the standard tetanus suppresses LTP induction (LTP suppression). We investigated LTP, DP, and LTP suppression of both field excitatory postsynaptic potentials and population spikes in CA1 neurons of mice lacking the inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R)-binding protein released with IP_3 (IRBIT). The mean magnitudes of LTP induced by short and standard tetanus were not different in mutant and wild-type mice. In contrast, DP and LTP suppression were attenuated in mutant mice, whereby the mean magnitude of responses after LFS or tetanus were significantly greater than in wild-type mice. These results suggest that, in hippocampal CA1 neurons, IRBIT is involved in DP and LTP suppression, but is not essential for LTP. The attenuation of DP and LTP suppression in mice lacking IRBIT indicates that this protein, released during or after priming stimulations, determines the direction of LTP expression after the delivery of subsequent stimulations.

The inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R) acts as an IP_3-gated Ca^{2+} release channel in a variety of cells (Berridge 1993; Mikoshiba 1993). The type 1 IP_3 receptor (IP_3R1) is the major neuronal member of the IP_3R family in the central nervous system and is predominantly enriched in cerebellar Purkinje cells and hippocampal CA1 neurons (Furuichi et al. 1993). Stimulation of group I metabotropic glutamate receptors (mGluRs) on hippocampal neurons activates phospholipase C (PLC), which hydrolyses the inositol lipid precursor in the postsynaptic plasma membrane to form IP_3 and diacylglycerol, the former opening IP_3R channels and the latter activating protein kinase C (PKC) (Ben-Ari et al. 1992; Nakanishi 1992). Ca^{2+} released through IP_3Rs generates a Ca^{2+} wave in the cell, resulting in an increase in the diffusion coefficient of Ca^{2+} in the cell and in the life-time of Ca^{2+} in the cytoplasm (Miyazaki et al. 1992; Mikoshiba 1993).

IP_3R binding protein released with IP_3 (IRBIT), originally identified as a molecule that interacts with IP_3Rs (Ando et al. 2003), binds to IP_3Rs and inhibits their activity by blocking access of IP_3 to a common binding site (Ando et al. 2003, 2006). Phosphorylation of IRBIT is essential for its activation-inhibiting binding to IP_3Rs when the concentration of IP_3 is low (Ando et al. 2006). Using mice lacking IRBIT (Park et al. 2013), Kawaai et al. (2015) demonstrated that, in the central nervous system, IRBIT binds to Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα) and inhibits its activity by blocking the binding of the Ca^{2+}/calmodulin complex to the regulatory domain of CaMKIIα.

Several types of activity-dependent synaptic plasticity are exhibited by hippocampal CA1 neurons. One of these, long-term potentiation (LTP; i.e., persistent synaptic enhancement induced by a brief period of high-frequency electrical stimulation [HFS] of afferents) (Bliss and Lømo 1973; Bliss and Gardner-Medwin 1973), is assumed to be responsible for important processes involved in the cellular basis of learning and memory (Bliss and Collingridge 1993). Another, long-term depression (LTD), involves the depression by low-frequency afferent stimulation (LFS) of a response in a naive pathway (Dudek and Bear 1992; Bear and Abraham 1995). We have reported two further types of synaptic plasticity in these neurons: depotentiation (DP), in which a train of LFS (1000 pulses at 1 Hz) given 60 min after the delivery of HFS (100 pulses at 100 Hz) causes LTP reversal (Fujii et al. 1991; Bashir and Collingridge 1994), and LTP suppression, in which a train of LFS (1000 pulses at 1 Hz) given 60 min prior to the delivery of HFS (100 pulses at 100 Hz) suppresses LTP induction (Fujii et al. 1991, 1996).

The HFS-induced LTP at CA1 synapses is generally believed to be triggered by increases in postsynaptic intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ (Ascher and Nowak 1988) caused by Ca^{2+} influx through N-methyl-D-aspartate (NMDA) receptors and voltage-dependent Ca^{2+} receptor channels, which activates CaMKII in postsynaptic neurons (Alford et al. 1993; Bliss and Collingridge 1993) to phosphorylate α-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptors during LTP induction (Barria et al. 1997). Using IP_3R1-lacking mice produced by gene targeting (Matsumoto et al. 1996), we previously investigated the role of IP_3R1s in LTP in hippocampal CA1 neurons (Fujii et al. 2000). LTP induction in these mice was facilitated at CA1 synapses, whereby the mean magnitude of the responses after delivery of a short

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tetanus (10 pulses at 100 Hz) was significantly greater than in wild-type mice, but was blocked in the presence of the NMDA receptor/Ca^{2+} channel antagonist D,L-2-amino-5-phosphonovalerate (10 μM). We suggested it was possible that increased postsynaptic $[Ca^{2+}]_i$ caused by Ca^{2+} efflux through IP_3-gated channels attenuated the HFS-LTP induction in hippocampal CA1 neurons (Fujii et al. 2000). As IRBIT is thought to prevent the activation of IP_3-gated Ca^{2+} release channels or to suppress CaMKII activity during or after LTP induction, we now investigated whether IRBIT attenuates or facilitates LTP induction in hippocampal CA1 neurons using mice lacking IRBIT (Park et al. 2013).

Yoshioka et al. (2010) reported that lack of postsynaptic IP_3R1s results in a slower decay in the transient $[Ca^{2+}]_i$ in CA1 pyramidal neurons and in an attenuation of GABAergic inhibition on postsynaptic CA1 pyramidal neurons, leading to the enhancement of LTP induction in hippocampal CA1 neurons. Bannai et al. (2015) reported that slow mGluR signaling activates IP_3-R-dependent Ca^{2+} release channels and PKC to promote clustering of α-aminobutyric acid A receptors ($GABA_A$Rs) on the postsynaptic neuronal membrane. Thus, we also investigated whether IP_3-R-dependent Ca^{2+} release during or after HFS increases GABAergic inhibition on postsynaptic CA1 neurons to attenuate LTP induction at CA1 synapses of wild-type mice. We then examined whether IRBIT is involved in the mechanism of IP_3R-dependent GABAergic inhibition of LTP induction in CA1 neurons using mutant mice.

We also examined paired-pulse inhibition (PPI) and paired-pulse facilitation (PPF) in hippocampal slices from mice lacking IRBIT to determine whether this alters the activity of local circuit interneurons and whether it changes presynaptic transmitter release in the CA1 region. A decrease in PPI or PPF indicates an increase in GABAergic synaptic transmission in the local inhibitory circuit in the CA1 area (Lynch et al. 1981; Davies et al. 1990; Freund and Busaki 1996) or a decrease in the probability of release of neurotransmitter from presynaptic terminals of CA1 synapses (Manabe et al. 1993; Gerges et al. 2003), respectively. Previous studies showed that activation of postsynaptic IP_3Rs increases $GABA_A$R activity in CA1 neurons (Yoshioka et al. 2010) or reported that activation of presynaptic IP_3Rs increases presynaptic transmitter release and regulates short-term plasticity induced at CA1 synapses (Singh et al. 2021). Thus, we examined PPI and PPF induced in hippocampal slices of mice lacking IRBIT to investigate whether IRBIT is involved in the mechanism of local circuit interneurons or the presynaptic transmitter release in the CA1 region.

We previously demonstrated (Fujii et al. 2000) that DP and LTP suppression at CA1 synapses is attenuated in mice lacking IP_3R1. As DP and LTP suppression in CA1 neurons are significantly attenuated when the interval between HFS and LFS is >60 min, we suggested that DP and LTP suppression in CA1 neurons might depend on the mechanism activated during the period after the priming stimulations (Fujii et al. 1991, 1996; Sugita et al. 2016). We further demonstrated that halting the test electrical stimulation of CA1 synaptic inputs after the priming LFS or HFS significantly attenuated the suppression of LTP induction (Fujii et al. 2016) and DP induction (Fujii et al. 2020). The effect of halting electrical stimulation on LTP suppression and DP induction could be mimicked by perfusing with an IP_3Rs antagonist or a CaMKII inhibitor (Fujii et al. 2016, 2020). As IRBIT is essential for the binding to IP_3Rs that prevents its activation and as IRBIT is available for binding to CaMKIIα as a CaMKIIα kinase inhibitor, we think it is possible that phosphorylated IRBIT, which is released from IP_3Rs during and/or after the priming LFS or HFS, inhibits further activation of CaMKII during a certain period after priming stimulations and induces LTP suppression or DP induction in CA1 neurons. Therefore, in the present study, we also investigated the involvement of IRBIT in DP and LTP suppression in hippocampal CA1 neurons of mice lacking IRBIT.

Results

LTP in hippocampal CA1 neurons of mice lacking IRBIT

At hippocampal CA1 neuronal synapses, a tetanus of 15 or 20 pulses at 100 Hz induced robust LTP, while a short tetanus of 10 pulses at 100 Hz failed to do so, in slices from both wild-type and IRBIT (−/−) mutant mice. Figure 1 shows sample wave forms and the summarized results of the time-course of LTP in slices from the wild-type and mutant mice. In CA1 neurons from both wild-type and mutant mice, a short tetanus of 10 pulses evoked only short-term potentiation in the S-EPSPs and A-PSs, which then fell gradually to nearly pretetanic, baseline levels within 40–50 min. The summarized results show there was small potentiation in either the S-EPSPs or A-PSs of either genotype. Sixty minutes after the tetanus, the average S-EPSPs was 108.3% ± 8.4% of baseline levels in the wild-type slices and 110.9% ± 6.1% in the mutant slices, both not significantly different from the pre-HFS control levels; the average A-PSs was 114.4% ± 4.9% of baseline in slices from the wild-type mice and 112.7% ± 6.0% in mutant mice, both significantly larger ($P < 0.05$) than pre-HSF control levels. Neither the S-EPSP nor the A-PS values were significantly different between wild-type and mutant mice, indicating that a tetanus of 10 pulses failed to induce stable LTP at CA1 synapses in slices of either genotype.

On the other hand, as the summarized results show (Fig. 1), a tetanus of 15 pulses evoked a marked increase in both S-EPSPs and A-PSs that was maintained for >1 h at CA1 synapses in slices of mice of both genotypes. Sixty minutes after the tetanus, the average S-EPSPs in wild-type and mutant mice were 132.3% ± 7.2% and 138.8% ± 6.0% of baseline levels, respectively; while the average A-PSs were 124.7% ± 10.0% and 135.3% ± 7.1% of baseline, respectively. None of these values were significantly different between wild-type and mutant mice, indicating that a tetanus of 15 pulses induced LTP of approximately the same magnitude at CA1 synapses in mice of both genotypes.

Furthermore, the summarized results show that a tetanus of 20 pulses induced robust LTP in both the S-EPSPs and A-PSs at CA1 synapses of wild-type and mutant mice. Sixty minutes after the tetanus, the average S-EPSPs were 151.3% ± 8.6% and 143.5% ± 5.5% of baseline levels and the average A-PSs were 139.8% ± 8.3% and 151.0% ± 8.4% of baseline levels in slices from the wild-type and mutant mice, respectively. Neither the S-EPSPs nor the A-PSs were significantly different between wild-type and mutant mice, suggesting that LTP induction is not enhanced at CA1 synapses in mice lacking the IRBIT, and that the threshold of the short tetanus is around 15 pulses for LTP induction in hippocampal CA1 neurons of both genotypes.

In slices from mice lacking IR_3R1s, a previous study showed that robust LTP was induced in the S-EPSPs by HFS (10 pulses at 100 Hz) (Fujii et al. 2000). In the present study, we examined the effects of acute exposure of hippocampal slices to 2-APB, an antagonist for both IP_3Rs and store-operated calcium (SOC) channels (Bootman et al. 2002; Peppiatt et al. 2003) on LTP induction in hippocampal CA1 neurons. Taufiq et al. (2005) showed in guinea pig hippocampal CA1 neurons that stable LTP was induced in the S-EPSPs and A-PSs by a tetanus (10 pulses at 100 Hz) given in the presence of 200 μM α-methyl-4-carboxyphenylglycine (MCPG), a wide-spectrum mGluR antagonist, or 10 μM 2-APB. Since IP_3R activation occurs downstream from mGluR activation in the signaling cascade in hippocampal CA1 neurons (Mikoshiba 1993) and since 2-APB is thought as a membrane-penetrable antagonist for IP_3Rs and SOC channels, inhibits IP_3-induced Ca^{2+} release (IICR) and calcium-induced calcium release (CICR) from the endoplasmic reticulum in the cells (Maruyama et al. 1997, Prakriya and Lewis 2015), the above results suggested that activation of IP_3Rs or SOC channels during HFS attenuates LTP induction. In the present

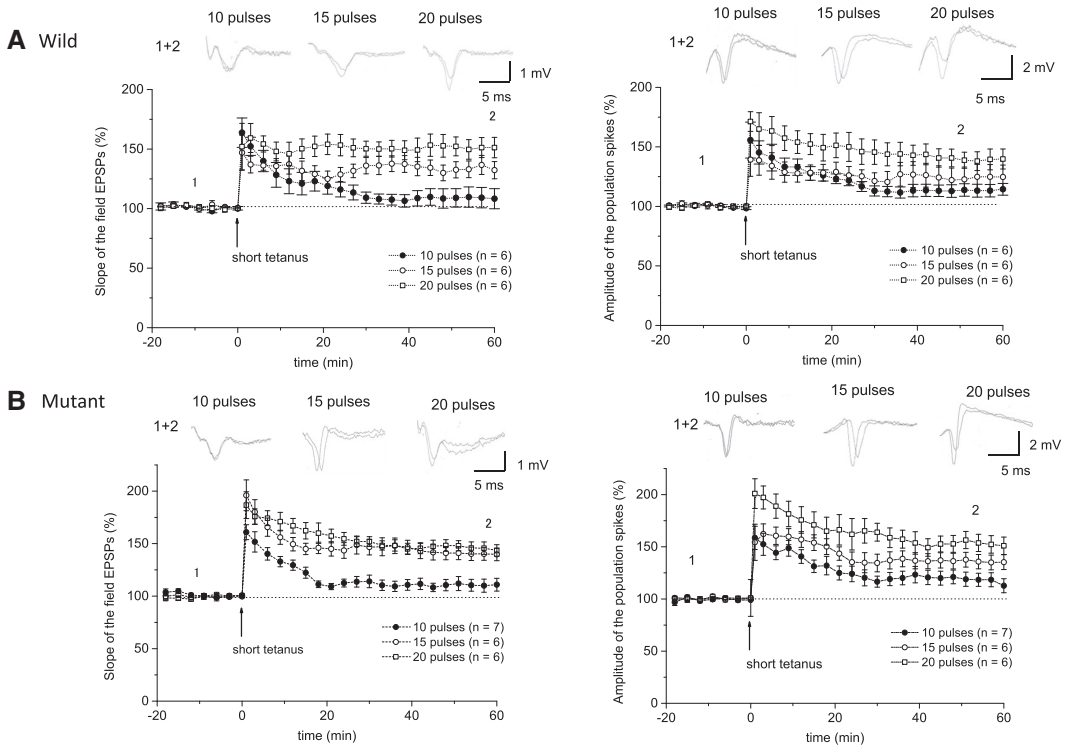

Figure 1. Effects of short tetanic stimuli at a threshold level on LTP induction in CA1 neurons of hippocampal slices from wild-type and mutant mice. Summarized time-course data from six slices in six mice for long-term changes in average S-EPSPs (*left*) and A-PSs (*right*) induced by 10-pulse tetanus (filled circles), 15-pulse tetanus (empty circles) or 20-pulse tetanus (empty squares) in slices of wild-type (*A*) or mutant mice lacking IRBIT (*B*). Sample traces were taken at the indicated times (1 and 2) on each of the time courses. In CA1 neurons of both genotypes of mice, 15 pulses at 100 Hz was the threshold for LTP induction.

study, as shown in Figure 2A, robust LTP was induced in the S-EPSPs and A-PSs in both wild-type and IRBIT (−/−) mutant mice by a tetanus (10 pulses at 100 Hz) delivered in the presence of 10 μM 2-APB. The average S-EPSPs (149.2% ± 9.6%) and A-PSs (149.3% ± 8.8%) in wild-type mice measured 60 min after the short tetanus were significantly ($P < 0.01$) higher than the control LTP levels induced in the standard solution (Fig. 2A). Similarly, 10 μM 2-APB facilitated the LTP induced in mutant mice by a short tetanus in the S-EPSPs (165.6% ± 9.8%) and A-PSs (160.0% ± 12.1%), and both were significantly ($P < 0.01$) higher than the control LTP levels induced in the standard solution (Fig. 2A) at 60 min. Because LTP induction was facilitated at CA1 synapses in mice of both genotypes when the tetanus was applied in the presence of 2-APB, the lack of IRBIT at CA1 neurons appears not to be involved in the effects of the antagonists for IICRs or CICRs on the facilitation of LTP induction seen in hippocampal CA1 neurons of mutant mice.

We also previously found that a lack of IP_3R1s attenuates the activity of the CA1 local inhibitory circuit, leading to a facilitation of LTP induction in CA1 neurons (Fujii et al. 2000; Yoshioka et al. 2010). Here, we showed that a short tetanus of 20 pulses at 100 Hz induced stable LTP in CA1 neurons of mice lacking IRBIT (Fig. 1). We then examined whether this effect might involve the attenuation of activity of the CA1 local inhibitory circuit. As shown in Figure 2B, a short tetanus of 20 pulses at 100 Hz failed to induce LTP at CA1 synapses of both genotypes when the tetanus was delivered in the presence of 1 μM muscimol, a $GABA_A$ receptor agonist. In wild-type slices, the average S-EPSPs (110.5% ± 5.2%) and A-PSs (110.3% ± 2.1%) measured 60 min after the short tetanus were both significantly ($P < 0.01$) lower than the control LTP level induced in the standard solution (Fig. 1). Muscimol also attenuated LTP induced by the short tetanus in the S-EPSPs (110.5% ± 4.8%) or A-PSs (109.0% ± 4.9%) of mutant mice, both of which were significantly ($P < 0.01$) lower than the control LTP induced in the standard solution (Fig. 1). These results indicate that the lack of IRBIT at CA1 synapses did not affect the effects of the $GABA_A$ receptor agonist on the attenuation of LTP induction in mice of either genotype. Thus, we conclude that the lack of IRBIT at CA1 neurons is not involved in the effects of the $GABA_A$ receptor agonist on the attenuation of LTP induction seen in hippocampal CA1 neurons of mutant mice.

Activity of CA1 inhibitory interneurons of mice lacking IRBIT

PPSs applied to the input pathway of the CA1 neuron induced either inhibition or facilitation of the PSs elicited by the second of the paired stimuli (Fig. 3A,B). In wild-type and mutant mice, pairs of stimuli delivered at an interval of 10 msec induced PPI, the second A-PS being smaller than the first, whereas those delivered at an interval of 30 msec induced PPF, the second A-PS being larger than the first.

PPSs delivered at an interval of either 10 or 30 msec in standard perfusate resulted PPI and PPF in slices from mice of both genotypes. The paired-pulse ratios of the A-PSs following 10-msec PPS (0.63 ± 0.04) or 30-msec PPS (1.34 ± 0.05), in slices from mice lacking IRBIT ($n = 16$), were not significantly different from those in wild-type mice ($n = 12$; 0.65 ± 0.03 and 1.37 ± 0.08, respectively) (data not shown), indicating that PPI and PPF induced in the standard solution were not affected by the lack of IRBIT in hippocampal CA1 neurons. These results suggest that IRBIT in CA1 neurons is not involved in either activity of the local inhibitory circuit in

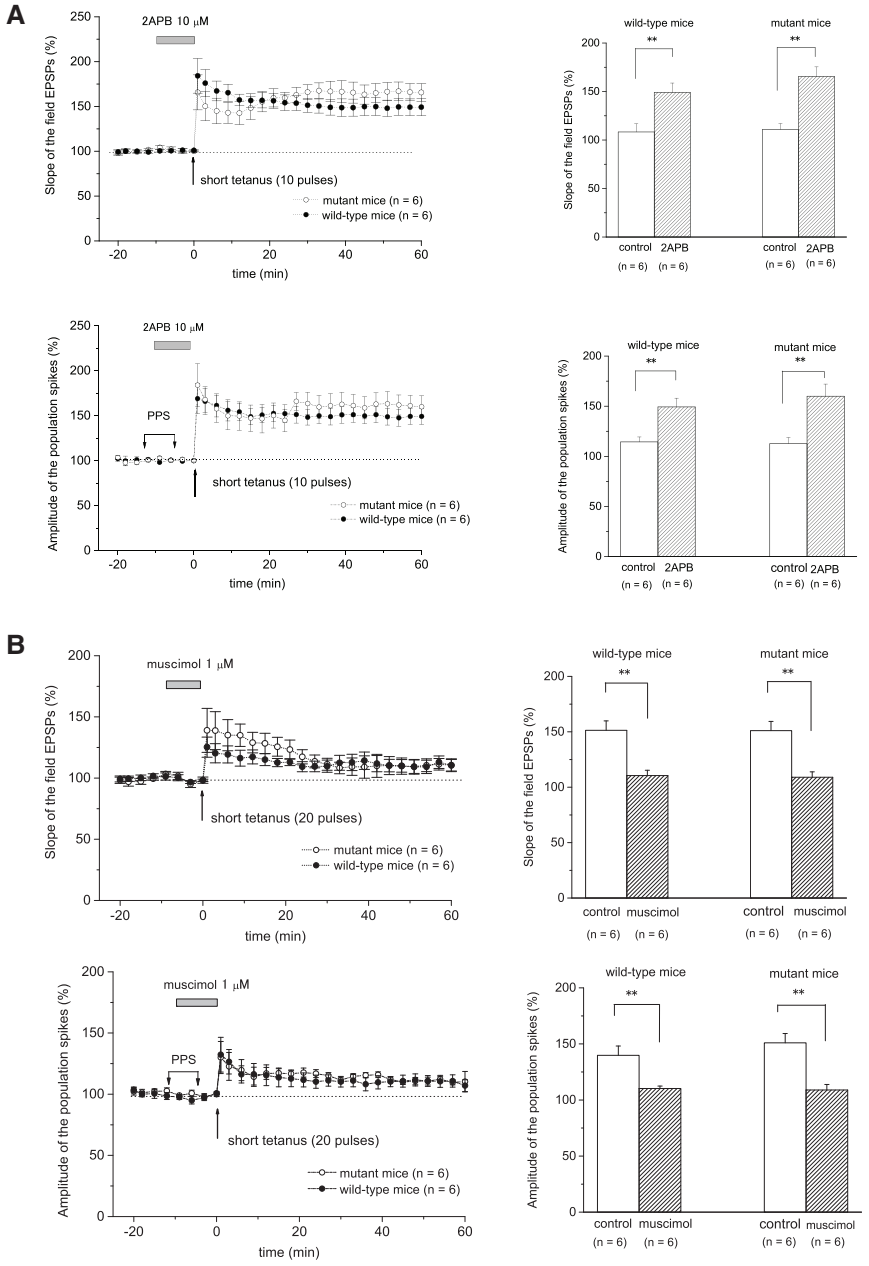

Figure 2. (*A*) Effects of an IP_3R antagonist on LTP induced by a 10-pulse tetanus in hippocampal CA1 neurons of wild-type and mutant mice. (*Left* panels) Summarized time-course data for LTP in S-EPSPs (*top* panel) and A-PSs (*bottom* panel) induced by a 10-pulse tetanus given in the presence of 10 μM 2-APB (horizontal bars). The short tetanus (10 pulses at 100 Hz, upward arrows) induced LTP in slices from wild-type (closed circles) and IRBIT (−/−) mutant (empty circles) mice. Paired-pulse stimulations (PPSs) were delivered before or during drug perfusion (small arrows). (*Right* panels) Average S-EPSPs (*top* panels) and A-PSs (*bottom* panels) measured 60 min after a 10-pulse tetanus given in the standard solution (empty bars, control) or in the presence of 2-APB (hatched bars). (**) $P < 0.01$ (significant differences between mutant and wild-type mice). In 10 μM 2-APB, a 10-pulse tetanus that is subthreshold for LTP induction in standard solution (control) successfully induced stable LTP in CA1 neurons of both genotypes of mice. (*B*) Effects of a $GABA_AR$ agonist on LTP induced by a 20-pulse tetanus in hippocampal CA1 neurons of wild-type and mutant mice. (*Left* panels) Summarized time-course data for long-term changes in S-EPSPs (*top* panel) and A-PSs (*bottom* panel) induced by a 20-pulse tetanus given in the presence of 1 μM muscimol (horizontal bars). The short tetanus (20 pulses at 100 Hz, upward arrows) failed to induce LTP in slices from wild-type (closed circles) and mutant (empty circles) mice. Paired-pulse stimulations (PPSs) was delivered before and during drug perfusion (small arrows). (*Right* panels) Average S-EPSPs (*top* panels) and A-PSs (*bottom* panels) measured 60 min after a 20-pulse tetanus given in the standard solution (empty bars, control) or in the presence of 2-APB (hatched bars). In 1 μM muscimol, a 20-pulse tetanus that induced stable LTP in the standard solution (control) failed to induce LTP in CA1 neurons of both genotypes of mice. (**) $P < 0.01$ (significant differences between mutant and wild-type mice).

the CA1 area or in the mechanism of presynaptic transmitter releases at CA1 synapses.

We then delivered PPSs in the presence of 10 μM 2-APB to CA1 synaptic inputs in wild-type and mutant mice (Fig. 3A) and observed changes in PPI and PPF induced in postsynaptic CA1 neurons (Fig. 3A). PPSs delivered at an interval of 10 or 30 msec in standard perfusate resulted in paired-pulse ratios of the A-PSs in slices from mutant mice not significantly different from those in wild-type mice. In mice of both genotypes, the paired-pulse ratios of the A-PSs for PPI recorded in 10 μM 2-APB were significantly higher ($P < 0.01$) than those recorded in the standard solution (left column of Fig. 3A) while those for PPF were not different from those recorded in the standard solution (right column of Fig. 3A). From these results, we suggest that the activation of postsynaptic IP_3-gated calcium release channels enhances an increase in GABAergic synaptic transmission in the local inhibitory circuit in the CA1 area but does not affect the probability of release of neurotransmitter from presynaptic terminals of CA1 synapses in mice of both genotypes.

We also delivered PPSs in the presence of 1 μM muscimol to CA1 synaptic inputs of wild-type and mutant mice (Fig. 2B) and observed changes in PPI and PPF (Fig. 3B). PPSs delivered in standard perfusate resulted in paired-pulse ratios of the A-PSs in slices from mutant mice not significantly different from those in wild-type mice. The paired-pulse ratios of the A-PSs for PPI recorded in the presence of muscimol were significantly lower ($P < 0.01$) than those for PPI recorded in the standard solution in both types of mice (left column of Fig. 3B), while those for PPF were not different from those recorded in the standard solution in both types of mice (right column of Fig. 3B). Because the lack of IRBIT in CA1 neurons does not affect the effects of a $GABA_AR$ antagonist on PPI, we conclude that IRBIT is not involved in the mechanism of the local inhibitory neuronal circuits in the CA1 region.

Depotentiation of LTP in hippocampal CA1 neurons of mice lacking IRBIT

A standard tetanus induced robust LTP in slices from both wild-type and mutant mice (Fig. 4A). To statistically evaluate the LTPs, we measured the S-EPSPs (Fig. 4A, left panel) and A-PSs (Fig. 4A, right panel) 20, 60, and 140 min after tetanus in mutant and wild-type mice. In the wild-type mice, the S-EPSP values were

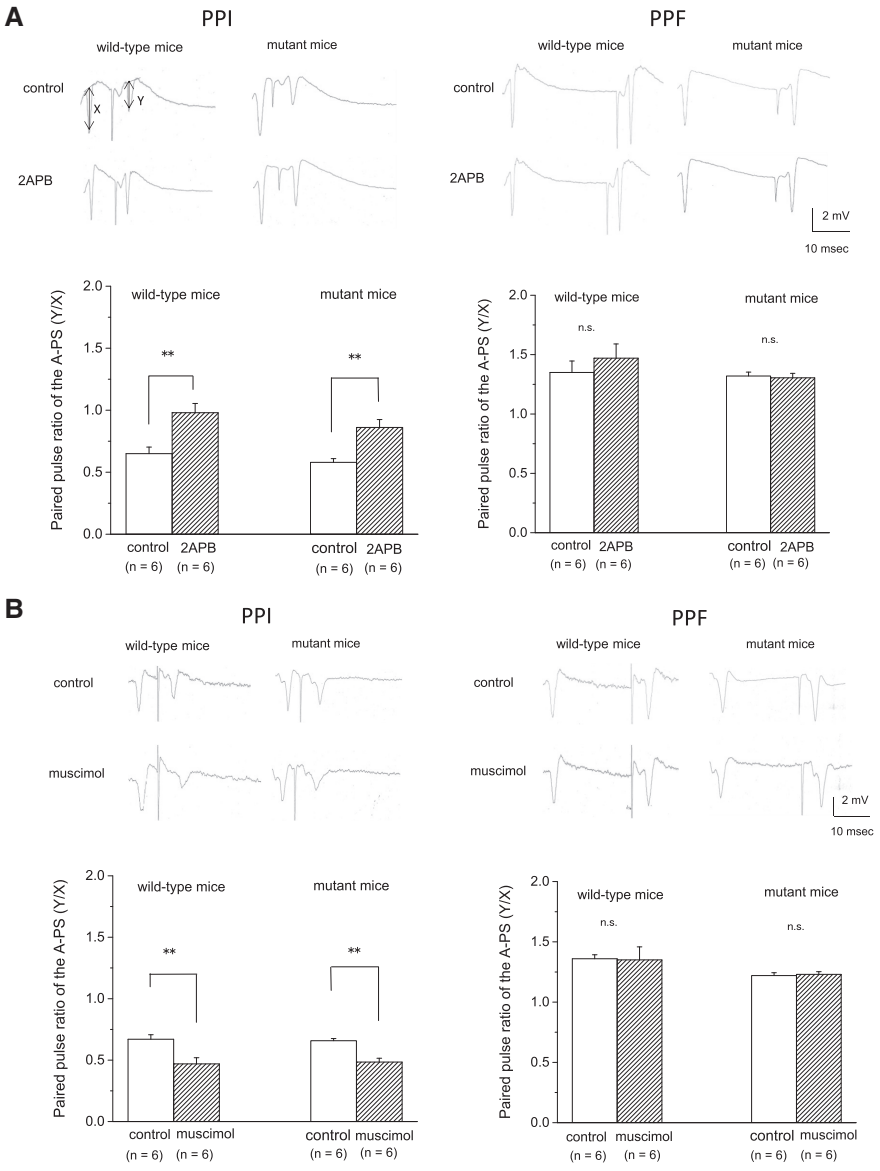

Figure 3. Effects of an IP_3R antagonist or a $GABA_A$ receptor agonist on paired-pulse inhibition (PPI) and paired-pulse facilitation (PPF) induced in postsynaptic CA1 neurons of mice lacking IRBIT. (*A*) The *top* panels show typical examples of PPI (*left*) or PPF (*right*) responses induced in postsynaptic CA1 neurons of a wild-type and a mutant mouse. Pairs of stimuli (paired-pulse stimulation [PPS]) delivered at an interval of 10 msec induced PPI of the responses, the second A-PS being smaller than the first, whereas those delivered at an interval of 30 msec induced PPF, the second A-PS being larger than the first. These pairs of stimuli were delivered to CA1 neurons in the standard solution (control) and in the presence of 10 μM 2-APB (PPSs in Fig. 2A). The *bottom* panels show summarized data for the PPI (*left*) and PPF (*right*) in wild-type (empty bars) and mutant (hatched bars) mice. The level of PPI and PPF are expressed as the paired-pulse ratio of the A-PSs (*Y*/*X*), the ratio of the second A-PS (*Y* in the *top* panel) divided by the first one (*X* in the same one). In both genotypes, the PPI induced the presence of 2-APB was significantly weaker ([**] $P < 0.01$) than that induced in the standard solution. However, the PPF induced in both genotypes was not significantly changed (n.s.) by the application of 2-APB. (*B*) The *top* panels show typical examples of PPI (*left*) and PPF (*right*) responses induced by pairs of stimuli in the standard solution (control) or in the presence of 1 μM muscimol (PPSs in Fig. 2B). The *bottom* panels show summarized data for the PPI (*left*) and PPF (*right*) in wild-type (empty bars) and mutant (hatched bars) mice. In both genotypes, the PPI induced in the presence of muscimol was significantly stronger ([**] $P < 0.01$) than that induced in the standard solution. However, the PPF induced in both genotypes was not significantly changed (n.s.) by the perfusion of muscimol.

160.2% ± 3.6%, 163.2% ± 2.9%, and 161.3% ± 5.7% of the pretetanic baseline level, respectively, whereas, in mutant mice, the corresponding values were 161.7% ± 6.5%, 151.4% ± 6.9%, and 150.0% ± 7.1%, with no significant differences between the groups. In the wild-type mice, the A-PS values were 160.9% ± 8.8%, 161.1% ± 8.9%, and 163.0% ± 9.6%, respectively, of the pretetanic baseline level, whereas, in mutant mice, the corresponding values were 182.8% ± 14.9%, 154.4% ± 9.4%, and 152.0% ± 5.7%, with no significant difference between the groups. These results show that the standard tetanus induced a similar degree of LTP in both genotypes, and that the LTP was maintained for at least 140 min.

In the hippocampal CA1 neurons of wild-type mice, LFS reverses homosynaptic LTP in the S-EPSPs and A-PSs (depotentiation of LTP [DP]) (Fujii et al. 1991). However, in slices from mutant mice, the standard LFS, applied 60 min after the standard tetanus, had a significantly smaller effect on established LTP (Fig. 4B). The S-EPSP values 60 min after tetanus were nearly identical in the two groups of mice, being 152.3% ± 10.9% and 153.6% ± 5.6% of the pretetanic baseline level in the wild-type and mutant mice, respectively. The A-PS values 60 min after tetanus were also nearly identical in the two groups, being 171.0% ± 8.8% and 171.5% ± 7.3% of the pretetanic control level in the wild-type and mutant mice, respectively. LFS delivered 60 min after tetanus significantly attenuated established LTP (DP) in wild-type mice, but had no effect in mutant mice (Fig. 4B).

In mutant mice, the S-EPSPs and A-PSs measured 60 min after the end of LFS were 151.0% ± 9.1% and 143.0% ± 3.9%, respectively, of the pretetanic control level, neither of which was different from the control LTP measured 140 min after tetanus (Fig. 4A). Thus, the mean percentage reduction in LTP in the S-EPSPs and A-PSs in mutant mice were only 9.1% ± 9.1% and 20.5% ± 4.9%, respectively.

In wild-type mice, however, the S-EPSPs and A-PSs measured 60 min after the end of LFS were 96.8% ± 6.6% and 126.9% ± 5.8%, respectively, of the pretetanic control level, both of which were significantly ($P < 0.01$) lower than the control LTP level measured 140 min after a standard tetanus (Fig. 4A). LTP levels measured as S-EPSPs and A-PSs 60 min after LFS were significantly ($P < 0.01$ and $P < 0.05$) different between wild-type and mutant mice, suggesting a failure of DP induction in CA1 neurons of mice lacking IRBIT. The mean percentage reduction in LTP in the S-EPSPs and A-PSs in wild-type mice were 110.0% ± 9.2% and 62.2% ± 7.8%, respectively, both significantly ($P < 0.01$) different from the corresponding values for DP in mutant mice. These results indicate no induction, or significant attenuation, of DP in the S-EPSPs and

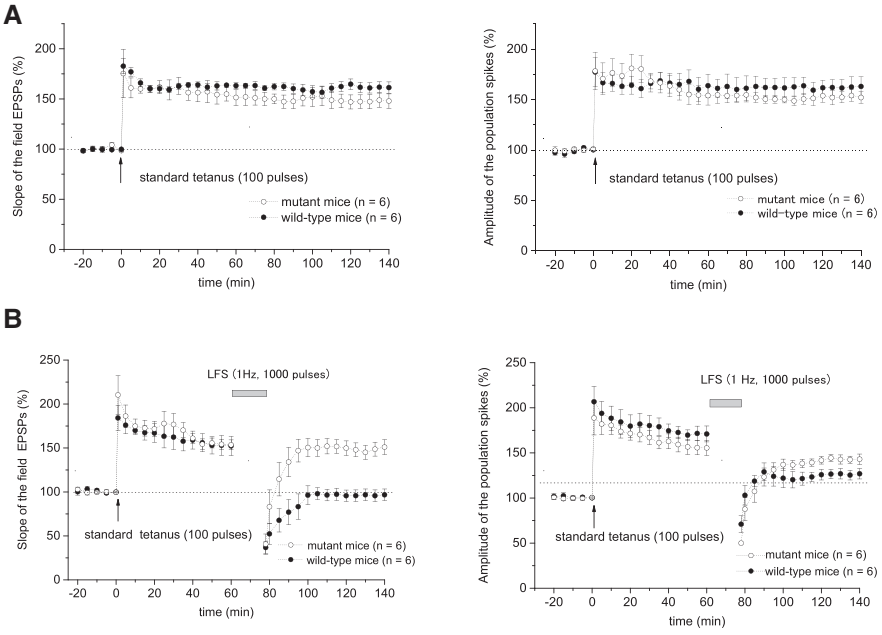

Figure 4. (*A*) The summarized time-course plots of LTP induced by the standard tetanus (100 pulses at 100 Hz) in hippocampal CA1 neurons of wild-type mice or IRBIT (−/−) mutant mice. Stable LTP was induced and maintained for 140 min after the tetanus (upward arrow) in the S-EPSPs (*left* panel) and A-PSs (*right* panel) in wild-type mice (filled circles) or mutant mice (empty circles). (*B*) Summary of time-course data for DP in S-EPSPs (*left* panel) and A-PSs (*right* panel) for wild-type ($n=6$, filled circles) or mutant ($n=6$, empty circles) mice. A train of low-frequency stimuli of 1000 pulses at 1 Hz (LFS, horizontal bar) given 60 min after the standard tetanus (upward arrow) reduced LTP close to the control level in wild-type mice, but did not reverse the established LTP in mutant mice.

A-PSs in mutant mice. Thus, we conclude that IRBIT is involved in the mechanism of LFS-induced DP in hippocampal CA1 neurons.

LTP suppression in hippocampal CA1 neurons of mice lacking IRBIT

In hippocampal CA1 neurons of wild-type mice, a train of LFS given 60 min prior to the standard tetanus suppressed subsequent LTP induction in the S-EPSPs (LTP suppression). Figure 5 shows summarized time-course plots of LTP suppression of the S-EPSPs (left panel) and A-PSs (right panel) in wild-type and mutant mice. The S-EPSPs (99.3% ± 2.1%) and A-PSs (99.0% ± 5.7%) 60 min after the end of LFS indicated that changes in these responses were scarcely induced by the standard LFS. Tetanus delivered 60 min after LFS produced STP, but induced significantly smaller LTP in either the S-EPSPs or A-PSs measured 60 min after tetanus. The S-EPSPs and A-PSs measured 60 min after tetanic stimulation were 112.1% ± 4.3% and 118.2% ± 5.5% of pretetanic levels, both significantly smaller ($P<0.01$) than those for the control of LTP in wild-type mice (Fig. 4A).

LTP suppression was inhibited in all slices of mutant mice tested. Figure 5 shows the summarized data of the time-course plots of S-EPSPs (left panel) and A-PSs (right panel). The S-EPSPs (90.1% ± 6.6%) and A-PSs (93.1% ± 9.7%) measured 60 min after the end of LFS in mutant mice were smaller than but not significantly different from those in wild-type mice. However, tetanus given 60 min after LFS resulted in robust LTP, the S-EPSPs (147.4% ± 11.1%) and A-PSs (150.8% ± 12.5%) measured 60 min after tetanic stimulation were both significantly greater ($P<0.01$, $P<0.05$, respectively) than those for the control suppression of LTP in wild-type mice. Because the percent changes in the S-EPSPs and A-PSs after tetanus are included in the levels of responses 60 min after LFS delivery, the magnitude of the LTP will be underestimated; when this was taken into consideration, the true magnitude of the LTP in S-EPSPs (163.3% ± 6.4%) and in A-PSs (165.5% ± 10.1%) were not significantly different from the values measured 60 min after the tetanus in the control LTP induced in naive slices from wild-type mice (Fig. 4A). These results indicate that LTP suppression is blocked at CA1 synapses of mice lacking IRBIT. Thus, we conclude that IRBIT is involved in the mechanism of LFS-induced LTP suppression in hippocampal CA1 neurons.

Discussion

In the present study, we found that LTP induction was not affected at CA1 synapses of mice lacking IRBIT, and that the mean magnitudes of these potentiated responses were not significantly different from those in wild-type mice (Figs. 1, 4A), indicating that

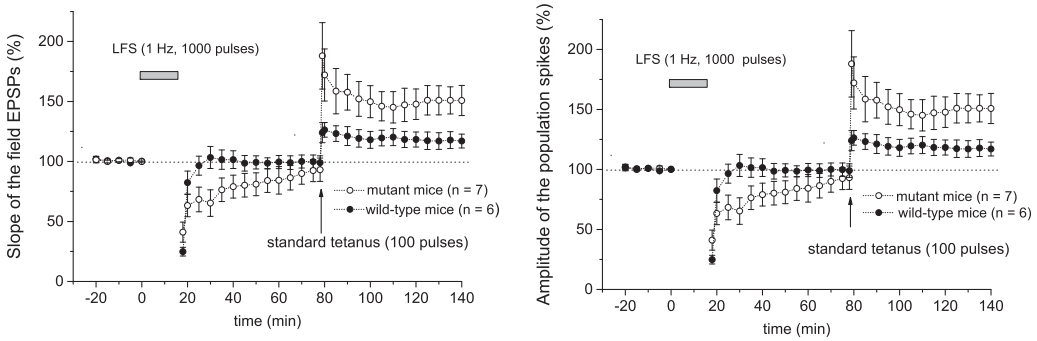

Figure 5. Summary of time-course data for LTP suppression in the S-EPSPs (*left* panel) or A-PSs (*right* panel) in slices from wild-type ($n=6$, filled circles) and mutant ($n=7$, empty circles) mice. A train of LFS (1000 pulses at 1 Hz) or HFS (100 pulses at 100 Hz) was applied where indicated by the horizontal bar or an upward arrow. In the mutant mice, LFS given 60 min prior to tetanus failed to suppress LTP induction.

IRBIT at CA1 synapses is not essential for LTP, while IP_3Rs themselves are involved in the mechanism of LTP induction in hippocampal CA1 neurons. However, we also showed that both DP and LTP suppression were markedly attenuated in hippocampal CA1 neurons of IRBIT (−/−) mutant mice (Figs. 4B, 5), and conclude that IRBIT plays an important role in blocking potentiation of synaptic transmission at CA1 synapses that is preconditioned by HFS or LFS.

It is widely believed that an increase in postsynaptic $[Ca^{2+}]_i$ due to Ca^{2+} influx through NMDA receptor/Ca^{2+} channels during or after tetanus is a necessary step in the induction of homosynaptic LTP in hippocampal CA1 neurons (Bliss and Collingridge 1993). We previously investigated the role of IP_3Rs in LTP in hippocampal CA1 neurons using mice lacking IP_3R1s (Fujii et al. 2000), and found that LTP induction was facilitated at CA1 synapses, and that the mean magnitude of the responses after delivery of a short HFS (10 pulses at 100 Hz) was significantly greater than in wild-type mice. On the other hand, two distinct calcium releasing sites on the surface of the internal stores of the endoplasmic reticulum have been identified: the ryanodine receptor and the IP_3Rs. The type 3 ryanodine receptor (RyR3) is enriched in hippocampal CA1 neurons (Furuichi et al. 1994). RyR3-deficient mice produced by a gene targeting method exhibited facilitated LTP induced by a short tetanus (10 pulses at 100 Hz) at hippocampal CA1 synapses, suggesting that CICR as a result of Ca^{2+} influx during or after tetanus contributes to attenuate LTP induction in hippocampal CA1 neurons (Futatsugi et al. 1999). We previously investigated the role of IP_3Rs in LTP in hippocampal CA1 neurons using mice lacking IP_3R1s (Fujii et al. 2000), and found that LTP induction was facilitated at CA1 synapses, and that the mean magnitude of the responses after delivery of a short HFS (10 pulses at 100 Hz) was significantly greater than in wild-type mice. Therefore, it is possible that Ca^{2+} released from the internal stores of the endoplasmic reticulum during or after tetanus generates calcium signaling for attenuation of LTP induction at hippocampal CA1 synapses of mice.

The present study demonstrated that mice lacking IRBIT did not show the facilitated LTP induced by a short tetanus (10 pulses at 100 Hz) at hippocampal CA1 synapses and that this was dependent on activation of IP_3-induced Ca^{2+} release through IP_3Rs and $GABA_ARs$ (Fig. 2A,B). This suggests that, in hippocampal CA1 neurons, LTP induction by a short tetanus is triggered by Ca^{2+} influx through NMDA receptor/Ca^{2+} channels whereas it is actively blocked by postsynaptic IP_3Rs and $GABA_ARs$. It is therefore possible that, in these cells, increases in the $[Ca^{2+}]_i$, mediated either by Ca^{2+} influx through NMDA receptors or by efflux from internal stores via IP_3Rs play opposite roles in LTP induction at CA1 synapses.

Group I mGluRs activate PLC, which hydrolyses phosphatidylinositol bisphosphate into IP_3 and DAG, the former opening IP_3R channels and the latter activating PKC. PKC is thought to be involved in the mechanism of LTP induction in hippocampal CA1 neurons (Wang and Feng 1992; Abeliovich et al. 1993; Ren et al. 2013). Bannai et al. (2015) reported that slow mGluR signaling activates both IP_3R-dependent calcium release and PKC to promote clustering of $GABA_ARs$ on the postsynaptic neuronal membrane. From the results shown in Figure 3A,B, we conclude that activation of postsynaptic IP_3Rs increases $GABA_AR$ activity and is likely to alter the activity of local circuit interneurons in the CA1 region. These findings suggest that glutamate released during or after HFS activates both NMDA receptors and IP_3Rs, which generates spatiotemporal patterns of calcium signaling for opposing control of GABAergic synapses in postsynaptic CA1 neurons, and affects LTP induction at CA1 synapses.

In a previous study (Fujii et al. 2000), we found that DP and LTP suppression at CA1 synapses were attenuated in mice lacking IP_3R1s, and that the mean magnitudes of the responses after delivery of LFS (1000 pulses at 1 Hz) or HFS (100 pulses at 100 Hz) were significantly greater than in wild-type mice. In hippocampal CA1 neurons, both DP and LTP suppression are synapse-specific effects and are induced optimally by 1-Hz LFS, suggesting that DP shares some common features with LTP suppression (Fujii et al. 1991, 1996). As DP and LTP suppression at CA1 synapses were attenuated in mice lacking IRBIT (Figs. 4, 5) and as IRBIT binds to IP_3Rs and suppresses IP_3R activity in the resting state (Ando et al. 2003, 2006), we suggest that the lack of IRBIT is essential for the attenuation of DP and LTP suppression in CA1 neurons seen in mice lacking IP_3Rs. In CA1 neurons of guinea pig hippocampal slices, DP and LTP suppression were blocked by antagonists of NMDA receptors, mGluRs, or IP_3Rs applied in the presence of test synaptic inputs for a 10-min period after preconditioning HFS or for a 20-min period after preconditioning LFS (Fujii et al. 2016, 2020). These results suggest that, in CA1 neurons, following these preconditioning stimulations, coactivation of NMDA receptors and group I mGluRs due to sustained synaptic activity leads to the activation of IP_3Rs and an increase in IRBIT in postsynaptic neurons, and results in DP or LTP suppression of the responses.

In previous studies (Fujii et al. 2016, 2020), we suggested it was possible that postsynaptic increases in both $[Ca^{2+}]_i$ and IP_3 levels due to coactivation of NMDA receptors and group I mGluRs, caused by test synaptic stimuli after priming HFS or LFS, might stimulate IP_3Rs to release both IRBIT and Ca^{2+} into the cytoplasm, leading to calcineurin activation in postsynaptic CA1 neurons. As calcineurin, a Ca^{2+}/calmodulin-dependent phosphatase, has a high affinity for the Ca^{2+}/calmodulin complex (Meyer et al. 1992; Ye et al. 2008), and as IRBIT binds to CaMKIIα and inhibits CaMKIIα kinase activity by blocking the binding of the Ca^{2+}/calmodulin complex to CaMKIIα (Kawaai et al. 2015), we suggest that further CaMKIIα activation might be inhibited after the priming HFS or LFS by the binding of free Ca^{2+}/calmodulin complex to calcineurin in the presence of IRBIT in the dendritic spines of CA1 synapses. It is possible that, after subsequent LFS or HFS, levels of free Ca^{2+}/calmodulin, which is 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 a reduction in synaptic potentiation in hippocampal CA1 neurons.

In conclusion, we demonstrated cellular events involved in the induction of LTP, DP, and LTP suppression in postsynaptic CA1 neurons that include some new findings as well as findings elucidated in our previous studies (Fujii et al. 2016, 2020). Briefly, we postulate the following: (1) In LTP induction, an increase in the $[Ca^{2+}]_i$ due to coactivation of NMDA receptors and IP_3Rs during or after HFS increases Ca^{2+}/calmodulin levels and activates CaMKIIα, while an increase in the $[Ca^{2+}]_i$ and IP_3 levels due to activation of group I mGluR during or after HFS activates PKC to promote clustering of $GABA_ARs$ on the postsynaptic neuronal membrane (Fig. 6). (2) In LFS-induced DP at CA1 synapses, coactivation of NMDA receptors and group I mGluRs caused by sustained synaptic activity after a priming HFS results in activation of IP_3Rs, which leads to reversal of LTP of the responses (Fujii et al. 2020). The mechanism of DP triggered by the priming HFS involves an activation of group I mGluRs and an increase in levels of IP_3 and IRBIT, which are sustained by the test synaptic stimuli after the priming HFS in the postsynaptic CA1 neurons (Fig. 6). Ca^{2+} influx through NMDA receptor/Ca^{2+} channels during the subsequent LFS induces Ca^{2+}-induced Ca^{2+} release from intracellular stores in the dendrites of postsynaptic CA1 neurons, while the activation of group I mGluRs by LFS contributes to increased levels of IP_3 and IRBIT in the cytoplasm of postsynaptic CA1 neurons. In the presence of IRBIT, which inhibits CaMKIIα kinase activity, LFS increases Ca^{2+}/calmodulin levels and activates calcineurin in the dendritic cytoplasm of the postsynaptic CA1 neurons, and the calcineurin activation that occurs in the postsynaptic CA1 neurons during LFS is maintained after LFS by the test synaptic inputs applied to

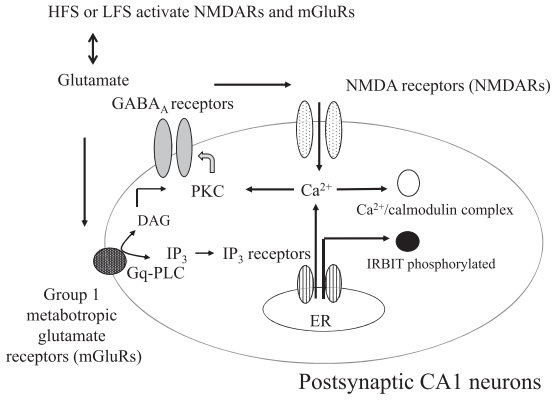

Figure 6. The proposed role of IRBIT in the mechanisms of LFS-induced DP and LTP suppression in hippocampal CA1 neurons. Synaptic inputs after HFS or LFS activate NMDA receptors and mGluRs on postsynaptic CA1 pyramidal neurons. The activation of IP_3Rs on the membrane of the endoplasmic reticulum (ER) occurs downstream from group I mGluRs and phospholipase C (PLC) in the signaling cascade. Group 1 mGluRs activate PLC, which hydrolyses phosphatidylinositol bisphosphate (PIP_2) into IP_3 and DAG, the former releasing Ca^{2+} and IRBIT from the IP_3Rs into the cytoplasm the latter activating PKC. IP_3R-dependent Ca^{2+} release and activated PKC promote clustering of $GABA_A$Rs on the postsynaptic neuronal membrane. The increase in $[Ca^{2+}]_i$ due to the coactivation of NMDA receptors 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. In the presence of IRBIT, which inhibits CaMKIIα kinase activity, the subsequent LFS or HFS increases Ca^{2+}/calmodulin levels and activates calcineurin in the dendritic cytoplasm of the postsynaptic CA1 neurons, leading to failure of the reversal LTP or the suppression of LTP induction in CA1 neurons.

CA1 synapses, leading to decreased potentiation of synaptic responses. (3) In LTP suppression at CA1 synapses, coactivation of NMDA receptorss and group I mGluRs caused by sustained synaptic activity after the priming LFS results in activation of IP_3Rs, which leads to failed induction of LTP of the responses (Fujii et al. 2016). The mechanism of LTP suppression triggered by the preconditioning LFS involves the sustained activation of group I mGluRs by the test synaptic input, which increases levels of both IP_3 and IRBIT in the cytoplasm of postsynaptic dendritic spines of CA1 neurons (Fig. 6). As Ca^{2+} influx through NMDA receptor/Ca^{2+} channels during the subsequent HFS induces Ca^{2+}-induced Ca^{2+} release from intracellular stores in the dendrites of postsynaptic CA1 neurons (Alford et al. 1993), the activation of NMDA receptors by HFS will contribute to an increase in dendritic $[Ca^{2+}]_i$ in postsynaptic CA1 neurons. In the presence of IRBIT, which inhibits CaMKIIα kinase activity, the HFS increases Ca^{2+}/calmodulin levels and activates calcineurin in the dendritic cytoplasm of the postsynaptic CA1 neurons, leading to failure of LTP induction. We therefore conclude that IRBIT is not essential for HFS-induced LTP, but is involved in the mechanisms of LFS-induced DP and LFS-induced LTP suppression in CA1 neurons.

Materials and Methods

Materials

All mice were maintained and handled following the guidelines of the Animal Care and Use Committee of the Yamagata University School of Medicine. The techniques used in these experiments were nearly identical to those described previously (Fujii et al. 2000). The procedures for IRBIT gene targeting and the production of homozygous mutant (−/−) mice on a C57BL/6J background were as previously described (Park et al. 2013). A neomycin-resistant J1 embryonic stem cell clone was identified as a homologous recombinant and used to generate homozygous mutant (−/−) mice, which grew normally and showed no obvious defects. Recordings were performed on hippocampal slices prepared from age-matched 1- to 3-mo-old homozygous mutant IRBIT knockout C57BL/6J mice and their wild-type littermates, and the results compared.

Slice preparation

Male mutant IRBIT knockout mice or wild-type littermates were decapitated and the hippocampi rapidly removed and cut into 500-µm-thick transverse slices using a rotary slicer (Dosaka DK-7700). The slices were preincubated for a minimum of 1 h at 30°C in a 95% O_2/5% CO_2 atmosphere in standard perfusion solution (124 mM NaCl, 5.0 mM KCl, 1.25 mM NaH_2PO_4, 2.0 mM $MgSO_4$, 2.5 mM $CaCl_2$, 22.0 mM $NaHCO_3$, 10.0 mM D-glucose) before being placed in a 1-mL recording chamber and completely submerged in standard solution and perfused continuously at a rate of 2–3 mL/min. The temperature in the recording chamber was maintained at 30°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 excitatory postsynaptic potentials (EPSPs) and population spikes (PSs), respectively, and a test stimulus was applied every 20 sec with a pulse duration of 0.1 msec. The slope of the field EPSPs (S-EPSPs) and the amplitude of PSs (A-PSs) were measured and plotted automatically. The experimental protocol used in this study was as follows: At the beginning of each experiment, the strength of the stimulus pulse was adjusted to elicit a population spike with an amplitude 40%–60% of maximal and was then fixed at this level. After checking the stability of the S-EPSPs and A-PSs for >15 min, a conditioning stimulus of tetanus or LFS was then delivered to induce synaptic plasticity at CA1 synapses of mutant or wild-type mouse slices.

After checking the stability of the responses to a test stimulus given at 20-sec intervals, either a short (10, 15, or 20 pulses at 100 Hz) or a standard (100 pulses at 100 Hz) tetanus was delivered to elicit LTP in CA1 neurons of mutant or wild-type mice. For DP, a LFS train (1000 pulses at 1 Hz) was delivered 60 min after the tetanus. In LTP suppression experiments, this LFS train was delivered 60 min before the standard tetanus used to elicit LTP. After delivery of the tetanus or LFS, the test stimulus was repeated every 20 sec and responses recorded for a minimum of 60 min.

To evaluate changes in the response, the mean value of the S-EPSPs or the A-PSs for the 10-min period before the delivery of the tetanus or LFS to naive slices was defined as the control (100%) level, and responses after tetanus or LFS were expressed as a percentage (mean ± SEM) of this control level. Changes in responses after tetanus or LFS were calculated as follows: (1) The percentage change in the responses after tetanus was calculated as $(Y/X) \times 100$, (2) the percentage change in the responses after LFS was calculated as $(Z/X) \times 100$, and (3) 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 tetanus or LFS, Y is the averaged value at 10–0 min immediately before LFS, and Z is the stable level at 50–60 min after the end of LFS. Using the equation given in (3), 100% or 0% indicate a complete reduction to the pretetanic control level or no induction of depotentiation, respectively (details are shown in our previous studies) (Fujii et al. 2020).

In addition, a short tetanus of 10 or 20 pulses was given in the presence of 10 µM 2-aminoethoxydiphenyl borate (2-APB; Sigma) or 1 µM muscimol (Sigma) and changes in the S-EPSPs or A-PSs were measured 60 min after the short tetanus. 2-APB and muscimol were applied 5 min before and during the short tetanus, then replaced by the standard solution just after the end of the short tetanus.

Before the delivery of the short tetanus to CA1 neurons, paired-pulse stimulation (PPS) at an interval of 10 or 30 msec was applied during the standard perfusate or in the presence of 10 µM 2-APB or 1 µM muscimol to the input pathway of the CA1 neurons of wild-type mice or mutant (Fig. 2A,B). PPS induces PPI or PPF of the A-PSs elicited by the second of the paired stimuli (Fig. 3A,B). In this study, PPI and PPF were expressed as the A-PS produced by the second pulse (Y) divided by that produced by the first pulse (X) (see the inset of a top panel in Fig. 3A).

All values are given as the mean ± SEM (percentage). The results were analyzed for statistical significance ($P < 0.05$ or $P < 0.01$) using two-tailed Student's t-test.

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