Brief Communications

Adaptive Regulation Maintains Posttetanic Potentiation at Cerebellar Granule Cell Synapses in the Absence of Calcium-Dependent PKC

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Posttetanic potentiation (PTP) is a transient, calcium-dependent increase in the efficacy of synaptic transmission following elevated presynaptic activity. The calcium-dependent protein kinase C (PKC_{Ca}) isoforms PKCα and PKCβ mediate PTP at the calyx of Held synapse, with PKCβ contributing significantly more than PKCα. It is not known whether PKC_{Ca} isoforms play a conserved role in PTP at other synapses. We examined this question at the parallel fiber → Purkinje cell (PF→PC) synapse, where PKC inhibitors suppress PTP. We found that PTP is preserved when single PKC_{Ca} isoforms are knocked out and in PKCα/β double knock-out (dko) mice, even though in the latter all PKC_{Ca} isoforms are eliminated from granule cells. However, in contrast to wild-type and single knock-out animals, PTP in PKCα/β dko animals is not suppressed by PKC inhibitors. These results indicate that PKC_{Ca} isoforms mediate PTP at the PF→PC synapse in wild-type and single knock-out animals. However, unlike the calyx of Held, at the PF→PC synapse either PKCα or PKCβ alone is sufficient to mediate PTP, and if both isoforms are eliminated a compensatory PKC-independent mechanism preserves the plasticity. These results suggest that a feedback mechanism allows granule cells to maintain the normal properties of short-term synaptic plasticity even when the mechanism that mediates PTP in wild-type mice is eliminated.

Introduction

Posttetanic potentiation (PTP) refers to the short-term increase in synaptic strength evoked at many synapses following a period of high-frequency (tetanic) stimulation (Magleby, 1987; Zucker and Regehr, 2002). As an important means of regulating synaptic efficacy, PTP contributes to working memory and information processing (Abbott and Regehr, 2004). PTP is thought to emerge as a result of accumulated residual calcium (Ca_{res}) in presynaptic boutons (Zucker and Regehr, 2002; Fioravante and Regehr, 2011). At some synapses, such as the crayfish neuromuscular junction (Delaney et al., 1989), the decay kinetics of Ca_{res} and synaptic enhancement are similar, whereas at other synapses, Ca_{res} decays more rapidly than PTP (Brager et al., 2003; Korogod et al., 2005; Beierlein et al., 2007; Fioravante et al., 2011), suggesting that Ca_{res} activates downstream biochemical cascades that determine the duration of PTP.

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The downstream signaling cascades that regulate PTP have been studied extensively. Several calcium-dependent targets have been implicated in PTP (Chapman et al., 1995; Wang and Maler, 1998; Fiumara et al., 2007; Lee et al., 2008; Khoutorsky and Spira, 2009; Rodríguez-Castañeda et al., 2010; Shin et al., 2010). The observations that PKC inhibitors eliminate PTP (Brager et al., 2003; Korogod et al., 2007) and that PKC activators occlude PTP (Korogod et al., 2007) have made PKC a leading candidate for mediating this plasticity. We recently tested this model at the calyx of Held and found that genetic deletion of both presynaptic calcium-dependent PKC (PKC_{Ca}) isoforms (PKCα and PKCβ) strongly attenuates PTP, thereby establishing the requirement for PKC_{Ca} in PTP (Fioravante et al., 2011). At the calyx of Held, PKCα and PKCβ both contribute to PTP, but PKCβ plays a particularly important role because its elimination prevents the bulk of this plasticity.

It is not known whether the PKC_{Ca} requirement for PTP extends to other synapses beyond the calyx of Held. PKC inhibitors disrupt PTP at hippocampal and cerebellar synapses (Brager et al., 2003; Beierlein et al., 2007), but the specificity of these inhibitors has been questioned (Lee et al., 2008). Additionally, due to their lipophilicity, PKC inhibitors have been used at high concentrations that do not discriminate between calcium-dependent and calcium-independent isoforms. We therefore used molecular genetics to examine PTP at the PF→PC synapse, where PKC has been implicated in PTP (Beierlein et al., 2007). Even though PKC inhibitors strongly attenuated PTP in wild-type mice, genetic deletion of PKCα and PKCβ, the only presynaptic PKC_{Ca} at this synapse, did not eliminate PTP. These apparently conflicting

results were explained by a PKC-independent compensatory process, which is revealed in the PKCα/β double knock-out (dko) animals and mediates PTP in the absence of PKC_{Ca} isoforms. In single knock-out animals, either PKCα or PKCβ alone could mediate PTP. These findings indicate that unlike the calyx of Held, there is a remarkable capacity for compensation and the preservation of PTP at the PF→PC synapse.

Materials and Methods

Tissue preparation. Mice of either sex [postnatal day (P) 12–14] were anesthetized with isoflurane and decapitated; transverse cerebellar slices (220 μm thick) were obtained. The PKCα and β knock-out mice were generated by M. Leitges (Leitges et al., 1996, 2002). PKCα/β dko and wild-type controls were obtained by crossing heterozygotes for both genes ($\alpha^{+/-}$; $\beta^{+/-}$). PKCγ animals were purchased from Jackson Laboratory. For genotyping, the following primers were used: PKCα: 5′-GAGCCCTTGGGTTTCAAGTATAGA-3′, 3′-CCTGGTGGCAATGGGTGATCTACAC-5′, 3′-GTCAGCGCAGGGGCGCCCGG-5′; PKCβ: 5′-CCTAGCCCCTCAGGTGTTACCAC-3′, 3′-CCTACCTTGACACTGGAATCCCTGC-‘5, 3′-CTGCCAGTTTGAGGGGACGACGA-5′; PKCγ: 5′-GCTCCGACGAACTCTATGCCA-3′, 3′-GTGGAGTGAAGCTGCGTGAGA-5′, 5′-CAGGTAGCCGGATCAAGGTATGC-3′, 3′-GAGCTCACCCTGGAAGCTCA-5′. All procedures involving animals were approved by the Harvard Medical Area Standing Committee on Animals. Slices were prepared as described previously (Beierlein et al., 2007). Electrophysiological and calcium imaging experiments were performed at 33 ± 1°C.

Immunohistochemistry. Cerebellar slices of P12–P14 animals (100 μm thick) were processed and imaged as described previously (Fioravante et al., 2011). The following antibodies were used: anti-PKCα rabbit monoclonal (Abcam), anti-PKCβ rabbit polyclonal (C-16; Santa Cruz Biotechnology), anti-PKCγ rabbit polyclonal (C-19; Santa Cruz Biotechnology), and goat anti-rabbit Alexa 488-conjugated secondary (Santa Cruz Biotechnology). In additional experiments, different anti-PKCα (C-20) and anti-PKCβ (C-18) antibodies (both from Santa Cruz Biotechnology) were used to confirm previous observations. Average fluorescence intensities were obtained from regions encompassing the entire molecular or granule cell layer using MetaMorph software (Molecular Devices).

Electrophysiology and calcium imaging. Whole-cell voltage-clamp recordings (holding potential −60 mV) from Purkinje cells (PC) were obtained using 1.0–1.7 MΩ pipettes. The internal solution contained the following (in mM): 35 CsF, 100 CsCl, 10 EGTA, 10 HEPES, 315 mOsm, pH 7.3. Recordings were performed in bicuculline (20 μM), CGP55845 or CGP54626 (2 μM), and AM251 (2 μM) to block $GABA_A$, $GABA_B$, and cannabinoid type I receptors. For pharmacological studies, slices were preincubated at room temperature for 1 h [GF109203X (2 μM), Gö6983 (3 μM), staurosporine (5 μM) or ML9 (10 μM)] or 15 min [KN62 (3 μM) or H89 (5 μM)] and inhibitors were also included in the superfusate. Chemicals were from Sigma, except for AM251, CGP55845, CGP54626, ML9, and H89 (Tocris Bioscience) and KN62 (Abcam). Calcium transients were measured from Magnesium Green AM-loaded parallel fibers (PF) as described previously (Myoga and Regehr, 2011). For excitation, a 470 nm LED (Thorlabs) was used and fluorescence was detected by a custom-built photodiode. Calcium transients were normalized to baseline fluorescence and expressed as $\Delta F/F$.

Statistical analysis. PTP was calculated as the ratio of EPSC amplitude 2.8 s after tetanization over average baseline. For calcium imaging experiments, the average response to 8 pretetanus stimuli (pre) was compared with the response 2.8 s posttetanization (post). Pairwise comparisons were performed using Student's *t* tests. Multiple group comparisons were performed using one- or two-way ANOVAs followed by Tukey *post hoc* tests. Significance level was set at $p < 0.05$ (two-tailed). Data are expressed as mean ± 1 SD in Fig. 2 and mean ± SEM elsewhere.

Results

PTP in PKC_{Ca} knock-outs

We examined PTP at the PF→PC synapse in slices from knock-out animals of PKC_{Ca} isoforms and wild-type littermates (Fig. 1).

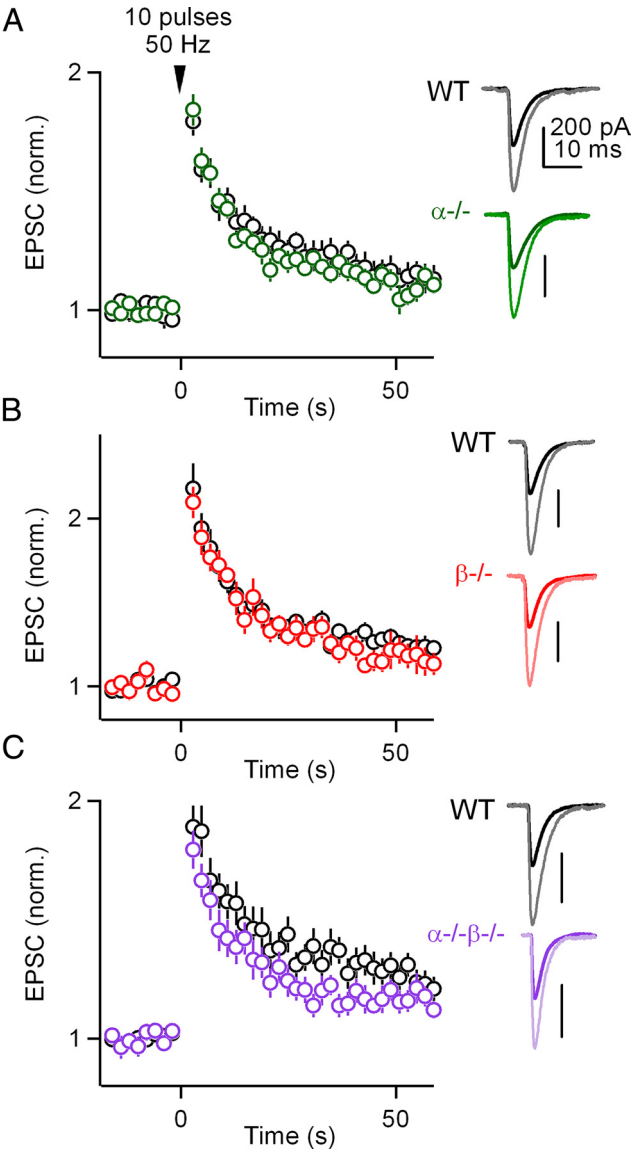

Figure 1. PTP is preserved in animals lacking calcium-dependent PKCs. Left, Plots of average normalized EPSC amplitudes as a function of time in slices from $\alpha^{-/-}$ (***A***), $\beta^{-/-}$ (***B***), and $\alpha^{-/-}\beta^{-/-}$ (***C***) animals and littermate controls. At $t = 0$, a brief tetanus (arrowhead) was delivered to induce PTP. Right, Representative traces of the first potentiated response (light traces) superimposed on the average baseline response (dark traces).

A tetanus (10 pulses, 50 Hz) was used to elicit PTP (Beierlein et al., 2007). Genetic deletion of PKCα (Fig. 1*A*), PKCβ (Fig. 1*B*), or both (Fig. 1*C*) did not affect PTP amplitude compared with wild-type ($\alpha^{+/+}$: 79 ± 6%, $n = 10$; $\alpha^{-/-}$: 84 ± 7%, $n = 11$, $p = 0.83$; $\beta^{+/+}$: 118 ± 15%, $n = 9$; $\beta^{-/-}$: 110 ± 9%, $n = 6$, $p = 0.87$; $\alpha^{+/+}\beta^{+/+}$: 89 ± 9%, $n = 13$; $\alpha^{-/-}\beta^{-/-}$: 80 ± 8%, $n = 10$, $p = 0.70$). Basal paired-pulse ratio (EPSC2/EPSC1) was also unaffected ($\alpha^{+/+}$: 1.84 ± 0.10, $n = 10$; $\alpha^{-/-}$: 1.94 ± 0.10, $n = 11$, $p = 0.49$; $\beta^{+/+}$: 2.18 ± 0.15, $n = 9$; $\beta^{-/-}$: 2.1 ± 0.09, $n = 6$, $p = 0.7$; $\alpha^{+/+}\beta^{+/+}$: 2.09 ± 0.12, $n = 13$; $\alpha^{-/-}\beta^{-/-}$: 1.98 ± 0.08, $n = 10$, $p = 0.78$; data not shown), suggesting that initial release probability was unaltered in knock-out mice. PTP appeared to decay more rapidly in PKCα/β dko mice, although the time course difference was not statistically significant [half decay time ($t_{1/2}$): $\alpha^{+/+}\beta^{+/+}$: 18 ± 3 s, $\alpha^{-/-}\beta^{-/-}$: 14 ± 3 s, $p = 0.27$].

These results were surprising because inhibiting PKC strongly reduces the magnitude of PTP at this synapse (Beierlein et al., 2007). The simplest explanation for the apparent discrepancy between genetic and pharmacological experiments is that PKC_{Ca} isoforms do not mediate PTP at this synapse, and that off-target effects account for the suppression of PTP by the PKC inhibitor. However, a number of alternative explanations must be tested. It is possible that all calcium-dependent isoforms are not eliminated from granule cells in PKCα/β dko mice. Alternatively, PKCα and PKCβ could mediate PTP in wild-type mice, but in PKCα/β dko mice a compensatory mechanism could mediate PTP. Such mechanisms could include a tetanus-induced increase in action-potential-evoked calcium entry, a calcium-insensitive PKC isoform, or activation of a PKC-independent biochemical cascade.

PKC_{Ca} expression in cerebellar granule cells

We performed immunohistochemistry to examine whether all PKC_{Ca} isoforms are eliminated from granule cells in PKCα and PKCβ knock-out animals. Although cerebellar granule cells are not thought to express the PKC_{Ca} isoform PKCγ (Barmack et al., 2000), PKCγ mRNA is transiently present during early postnatal development (Herms et al., 1993). We therefore tested the possibility that PKCγ expression might compensate for the loss of the other two PKC_{Ca} isoforms in PKCα/β dko mice. As expected, no PKCγ expression was detected in granule cells (Fig. 2*A1*,*A5*) in wild-type mice even though there was a strong signal from PCs (Fig. 2*A1*). No signal was detected in PKC$\gamma^{-/-}$ (Fig. 2*A2*,*A5*) or PKC$\alpha/\beta/\gamma$ triple knock-out (tko; Fig. 2*A4*,*A5*) mice, supporting the specificity of the antibody. No PKCγ signal was detected in granule cells from PKCα/β dko mice (Fig. 2*A3*,*A5*), indicating that there is no compensatory expression of PKCγ in granule cells from these mice.

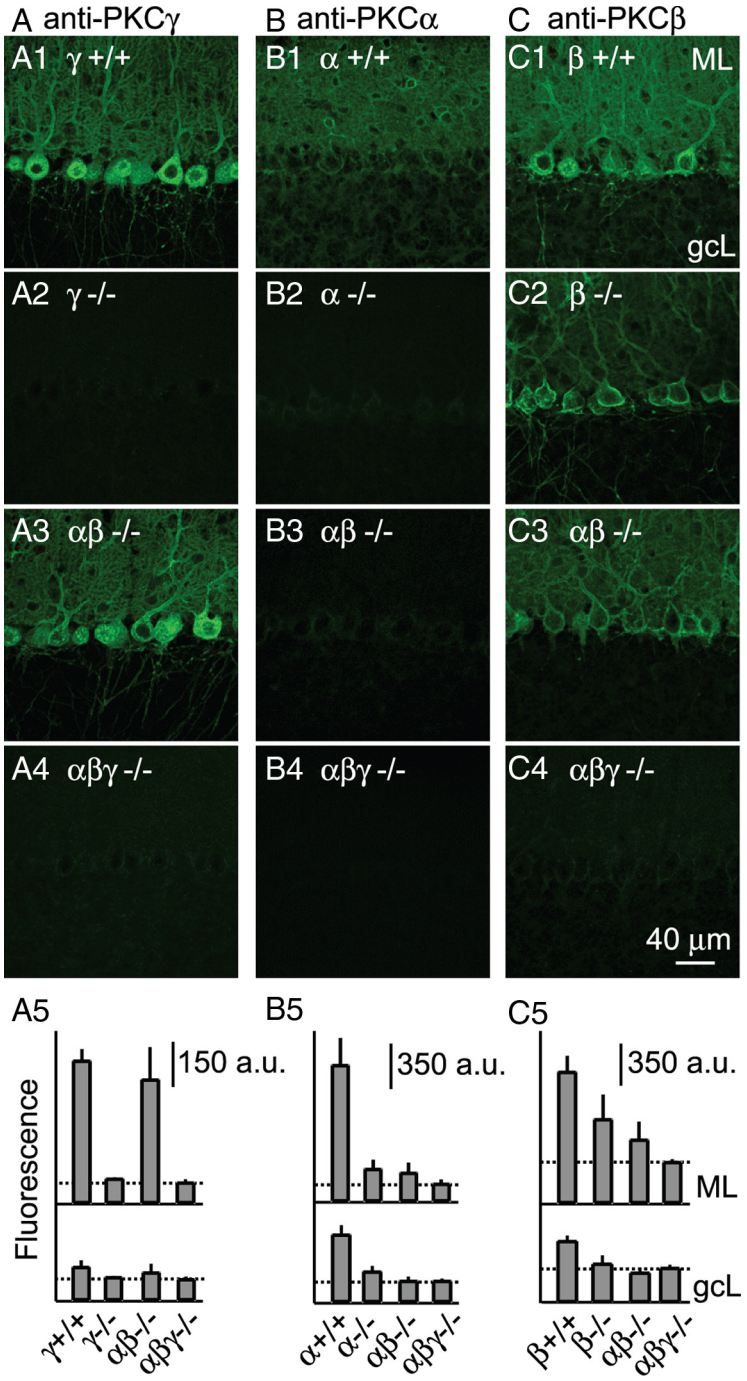

Figure 2. Expression of PKC_{Ca} isoforms in cerebellar granule cells in wild-type and PKC_{Ca} knock-out animals. Sagittal sections from PKCγ (***A1–A4***), PKCα (***B1–B4***), and PKCβ (***C1–C4***) wild-type, single knock-out, PKCα/β double knock-out and PKC$\alpha/\beta/\gamma$ triple knock-out animals, immunolabeled with antibodies against PKCγ (***A***), PKCα (***B***), and PKCβ (***C***). Graphs (***A5***, ***B5***, ***C5***) illustrate average fluorescence intensity ($\pm$1 SD) from molecular (ML) and granule cell (gcL) layers from three slices (each from a different animal) for each antibody.

We tested that no residual PKCα/β expression remains in the cerebellum of PKCα/β dko mice. PKCα signal was evident in granule cells and PFs from wild-type but not $\alpha^{-/-}$, α/β dko, or $\alpha/\beta/\gamma$ tko mice (Fig. 2*B1–B5*). Similarly, PKCβ labeling was observed in granule cells of wild-type mice but not $\beta^{-/-}$, α/β dko, or $\alpha/\beta/\gamma$ tko animals (Fig. 2*C1–C5*). The labeling of PCs with the anti-PKCα and anti-PKCβ antibodies (Fig. 2*B2*,*B3*,*C2*,*C3*) was predominantly due to cross-reactivity of the antibodies with PKCγ, because it was absent in slices from $\alpha/\beta/\gamma$ tko mice (Fig. 2*B4*,*C4*). These results indicate that residual presynaptic expression of PKC_{Ca} isoforms in PKCα/β dko animals cannot account for the PTP observed in these animals.

Presynaptic calcium signals

Tetanic simulation can enhance calcium entry evoked by subsequent action potentials, which can contribute to PTP (Habets and Borst, 2006; Korogod et al., 2007). Tetanic stimulation can also produce Ca_{res} increases that persist for tens of seconds after the end of the tetanus and can lead to synaptic enhancement

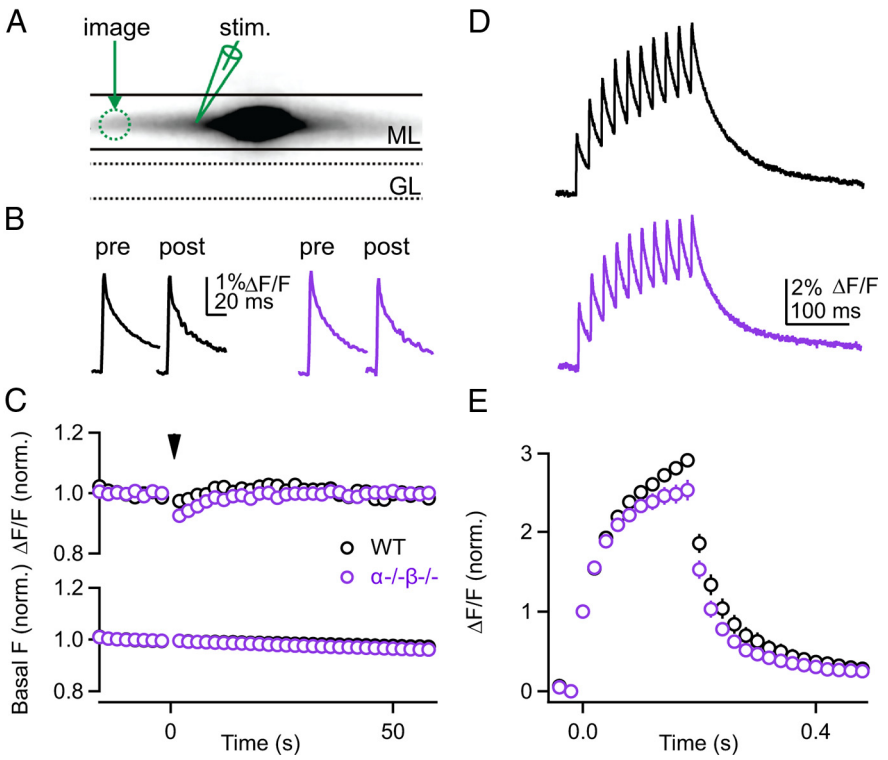

Figure 3. Presynaptic calcium entry and residual calcium signals in wild-type and PKCα/β dko animals. ***A***, Experimental setup of a transverse cerebellar slice with PFs loaded with indicator. ML, molecular layer; GL, granule cell layer; stim., stimulus. ***B***, Fluorescence calcium transients evoked by a single stimulus before (pre) and 2.8 s after (post) tetanization (the time point of peak PTP) in wild-type (black) and PKCα/β dko (purple) animals. ***C***, Plots of average normalized calcium influx (top) and residual calcium (bottom) as a function of time (arrowhead: PTP-inducing tetanus). ***D***, Fluorescence calcium transients evoked by tetanus (10 stimuli, 50 Hz). ***E***, Plot of average normalized fluorescence calcium signal (±SEM) shortly before (points 1–2), during (points 3–12), and immediately after (points 13–28) tetanus.

(Regehr et al., 1994; Habets and Borst, 2005; Korogod et al., 2007; Catterall and Few, 2008). We therefore examined the effect of tetanic stimulation on presynaptic calcium influx and Ca_{res} from PFs to determine whether increases in calcium influx and/or Ca_{res} accounted for PTP in PKCα/β dko mice (Fig. 3).

We introduced a membrane-permeable calcium indicator into granule cell PFs (Magnesium Green AM, $K_D = 6\ \mu M$) and measured stimulus-evoked presynaptic calcium transients (Fig. 3*A*). In wild-type mice, tetanic stimulation did not increase the amplitude of calcium transients ($\Delta F/F$, pre: 2.44 ± 0.25%, post: 2.39 ± 0.25%, $n = 8$, $p = 0.21$; Fig. 3*B*,*C*, top). This suggested that action potential-evoked increases in calcium entry do not contribute significantly to PTP at the PF→PC synapse. The Ca_{res} increase evoked by tetanic stimulation was surprisingly short-lived; by the time maximal PTP was observed (2.8 s posttetanus), Ca_{res} had already returned to basal levels (Fig. 3*C*, bottom, *D*,*E*).

We also examined calcium signaling in PKCα/β dko mice to determine whether PTP in these animals is mediated by a compensatory mechanism that involves alterations in presynaptic calcium signaling. As in wild-type mice, tetanic stimulation did not increase the amplitude of action potential-evoked calcium entry in PKCα/β dko mice; rather, it induced a modest (~6%) decrease ($\Delta F/F$ pre: 2.56 ± 0.08, post: 2.41 ± 0.07, $n = 8$, $p < 0.01$; Fig. 3*B*,*C*, top). There was no significant difference between wild-type and PKCα/β dko 2.8 s after tetanization [(normalized to pre) wild-type: 0.98 ± 0.02, PKCα/β dko: 0.94 ± 0.02, $p = 0.12$]. Moreover, increases in Ca_{res} evoked by the tetanus were slightly impaired in PKCα/β dko mice ($\Delta F/F$ wild-type: 2.9 ± 0.08, PKCα/β dko: 2.45 ± 0.11, $p < 0.01$; Fig. 3*E*). Nonetheless, they decayed at similar rates ($t_{1/2}$: wild-type: 37.5 ± 5.9 ms, PKCα/β dko: 32.0 ± 3.7 ms, $p = 0.48$) and by 2.8 s posttetanus, Ca_{res} was not different between PKCα/β dko and wild-type mice [(normalized to pre) wild-type: 0.99 ± 0.002, PKCα/β dko: 0.99 ± 0.001, $n = 8$, $p = 0.7$; Fig. 3*C*, bottom].

These results indicate that calcium influx and Ca_{res} are not increased upon deletion of all presynaptic PKC_{Ca} isoforms. Moreover, they establish that PTP in PKCα/β dko mice is not mediated by a compensatory mechanism that involves an alteration in presynaptic calcium signaling.

Compensation in PKC_{Ca} knock-outs

To further evaluate the possible explanations for the disparity between pharmacological and genetic studies, we examined the effects of PKC inhibitors on wild-type and knock-out mice. We found that the PKC inhibitor GF109203X (GF) significantly reduced the extent of PTP (control: 82 ± 8%, $n = 5$, GF: 5 ± 5%, $n = 9$, $p < 0.05$; Fig. 4*A*) without affecting initial release probability, as indicated by unaltered basal paired-pulse ratios (control: 2.30 ± 0.14, GF: 2.26 ± 0.14, $p = 0.86$; data not shown). These results indicate that PTP is sensitive to PKC inhibition in mice as in rats, and that the disparity between pharmacological and genetic results cannot be attributed to species-specific differences. We further examined the sensitivity of PTP to GF in PKCα/β knock-out animals. We reasoned that if GF had off-target effects, or if PTP were mediated by PKC isoforms other than PKCα and PKCβ, then GF should inhibit PTP in PKCα/β dko animals. In contrast, if a PKC-independent compensatory process sustained PTP in PKCα/β dko animals, then GF should not suppress PTP. We found that in PKCα and PKCβ single knock-outs, GF reduced the magnitude of PTP (in GF: $\alpha^{-/-}$: 20 ± 6%, $n = 8$, $p < 0.05$; $\beta^{-/-}$: 23 ± 5%, $n = 11$, $p < 0.05$; Fig. 4*B*,*C*). However, GF had no significant effect on PTP in PKCα/β dko mice (in GF: $\alpha^{-/-}\beta^{-/-}$: 61 ± 8%, $n = 9$, $p = 0.34$; Fig. 4*D*). We obtained similar results with a different PKC inhibitor: in Gö6983, PTP was reduced to 25 ± 4% ($n = 5$) in wild-type mice but not in PKCα/β dko mice (62 ± 9%, $n = 6$, $p < 0.01$; Fig. 4*E*). The ability of the inhibitors to impair PTP in wild-type but not PKCα/β dko mice suggests that they selectively target PKC in wild-type mice under our conditions. The inhibitors' inability to suppress PTP in PKCα/β dko mice indicates that there is indeed a PKC-independent compensatory mechanism that mediates PTP.

Several kinase inhibitors were used to provide insight into the mechanism of compensatory PTP (Fig. 4*E*). The broad spectrum inhibitor staurosporine strongly attenuated PTP in wild-type and α/β dko animals (in staurosporine: wt: 17 ± 6%, $n = 6$, dko: 6 ± 6%, $n = 6$, $p = 0.22$), indicating that compensatory PTP is mediated by a kinase. The myosin light chain kinase (MLCK) antagonist ML9 and the Ca/calmodulin-dependent kinase II (CaMKII) antagonist KN62 partially reduced the magnitude of

PTP to the same extent in wild-type and dko animals (in ML9: wt: 45 ± 6%, n = 11, dko: 33 ± 6%, n = 10, p = 0.17; in KN62: wt: 45 ± 8%, n = 8, dko: 46 ± 6%, n = 7, p = 0.9), excluding the preferential involvement of MLCK and CaMKII in compensatory PTP. However, H89 preferentially inhibited PTP in dko mice (in H89: wt: 78 ± 5%, n = 11, dko: 48 ± 8%, n = 12, $p < 0.01$), suggesting that the compensatory process is mediated by protein kinase A (PKA) or another kinase that is inhibited by H89 (Murray, 2008).

Discussion

Our major finding is that at the PF→PC synapse PKC_{Ca} isoforms mediate PTP in wild-type animals, but that in PKCα/β dko mice PTP is mediated by a PKC-independent compensatory mechanism. Even though PTP is mediated by PKCα and PKCβ at both the PF→PC and the calyx of Held synapses in wild-type mice, a compensatory mechanism only acts at the PF→PC synapse.

Presynaptic Ca signaling

Measurements of presynaptic calcium signals provided insight into the possible role of calcium entry and Ca_{res} in PTP. At the PF→PC synapse, tetanic stimulation does not alter presynaptic calcium influx (Fig. 3*C*). Regarding Ca_{res} following tetanic stimulation, there is a striking dissociation between the duration of Ca_{res} and PTP at the PF→PC synapse. At hippocampal and calyceal synapses, PTP also persists longer than the increases in presynaptic calcium following tetanization (Brager et al., 2003; Korogod et al., 2005; Fioravante et al., 2011). Here we find a particularly large disparity that highlights the significance of downstream signaling cascades in dictating the duration of PTP.

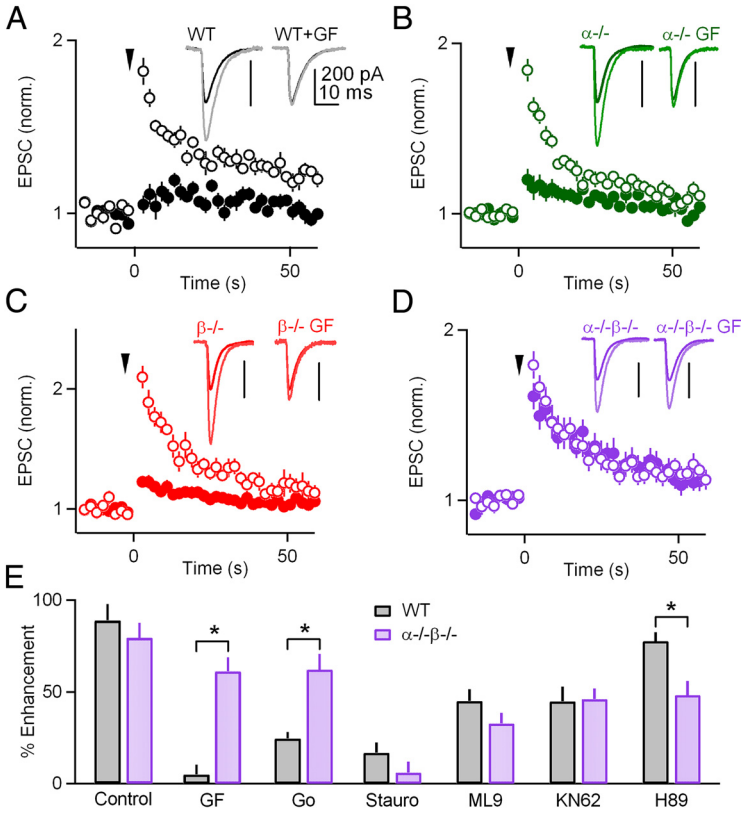

Figure 4. PTP is mediated by PKC in wild-type and single PKC knock-out animals but is PKC-independent in PKCα/β dko animals. ***A–D***, Plots of average normalized EPSCs from wild-type (***A***), $\alpha^{-/-}$ (***B***), $\beta^{-/-}$ (***C***), and $\alpha^{-/-}\beta^{-/-}$ (***D***) animals under control conditions (open circles) and in the presence of GF109203X (GF; filled circles), as a function of time. Insets show superimposed representative traces of the average baseline (dark traces) and the first potentiated response (light traces) in vehicle or GF for each genotype. ***E***, Average percent enhancement (±SEM) following tetanic stimulation in wild-type (gray) and PKCα/β dko (purple) animals in the presence of kinase inhibitors. *$p < 0.01$. Stauro, staurosporine; Go, Gö6983.

The roles of PKC isoforms in PTP

Even though previous studies had implicated PKC in PTP at several synapses (Brager et al., 2003; Beierlein et al., 2007; Korogod et al., 2007; Lee et al., 2007), the role of specific isoforms was examined only at the calyx of Held (Fioravante et al., 2011). Here, we investigated whether the contributions of PKCα and PKCβ to PTP observed at the calyx of Held could be extended to other synapses. Our findings suggest that even though PKCα and PKCβ are also important for PTP at the PF→PC synapse, their specific roles may differ. At the calyx of Held, elimination of PKCβ is sufficient to greatly attenuate PTP, whereas elimination of PKCα has only a small effect on this plasticity (Fioravante et al., 2011). In contrast, at the PF→PC synapse, the elimination of either isoform has no discernible effect. The observation that PKC inhibitors strongly attenuated PTP at the PF→PC synapse in single knock-out mice but not in PKCα/β dko mice suggests that either isoform can mediate PTP entirely on its own. This difference between the calyx of Held and the PF→PC synapse could reflect differences in the abundance or subcellular localization of the two isoforms. Our findings suggest that these isoforms can assume different roles at different synapses.

The compensatory mechanism of PTP

Our study revealed a novel compensatory process that rescued PTP in the absence of PKC_{Ca} isoforms (Figs. 1*C*, 4*D*). It was only through the combination of pharmacological and genetic approaches that compensatory PTP was uncovered. Alone, the pharmacological approach was difficult to interpret because of potential off-target effects. Moreover, genetic experiments alone could not distinguish between PTP being mediated by a PKCα/β-independent mechanism in both wild-type and PKCα/β dko mice, and, as we found to be the case, a PKCα/β-dependent mechanism in wild-type mice and a PKCα/β-independent mechanism in PKCα/β dko mice. Together, the two approaches validated the selectivity of the PKC inhibitors under our experimental conditions and established the existence of a compensatory mechanism.

The compensatory mechanism of PTP does not arise from changes in presynaptic calcium signaling (Fig. 3). Moreover, it requires removal of both presynaptic PKC_{Ca} isoforms (Fig. 4*B*,*C*). The insensitivity of the plasticity to PKC inhibitors indicates that compensatory PTP must involve proteins other than PKC, because the concentrations of the antagonists used (and required) to block PTP are sufficient to inhibit all isoforms of PKC (Toullec et al., 1991; Martiny-Baron et al., 1993; Gschwendt et al., 1996; Kewalramani et al., 2011). These proteins involve a kinase other than MLCK and CaMKII that is sensitive to H89

(Fig. 4*E*). A mechanism consistent with these observations is that elevated presynaptic calcium activates calcium-dependent adenylate cyclase leading to PKA activation and PTP. The existence of multiple mechanisms to sustain PTP at the same synapse highlights the importance of PTP at the PF→PC synapse.

The presence of a compensatory mechanism that preserves PTP is reminiscent of the compensation that occurs in cerebellar granule cells when α6-containing $GABA_A$ receptors are knocked out (Brickley et al., 2001). In wild-type mice, these receptors tonically inhibit granule cells, regulating their excitability. However, in α6 knock-out animals, there is no overt phenotype: granule cells remain hyperpolarized, but due to the compensatory upregulation of a potassium channel. For these functionally critical properties, neuronal excitability and PTP, granule cells display a remarkable capability to compensate to maintain normal neuronal behavior. Moreover, these findings suggest the presence of a mechanism that senses the desired state of granule cells and adjusts their properties to achieve that state, even in the absence of the mechanism that regulates that property in wild-type animals.

Many forms of homeostatic plasticity are known to regulate diverse neuronal properties (Turrigiano, 2008). The compensatory mechanism we reveal here maintains the magnitude and time course of PTP without altering initial release probability. This raises the possibility that a sophisticated feedback mechanism senses the short-term plasticity of the PF→PC synapse and regulates the properties of the synapse to achieve the desired short-term plasticity.

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