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Impaired hippocampal plasticity in mice lacking the $C\beta_1$ catalytic subunit of cAMP-dependent protein kinase

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ABSTRACT **Neural pathways within the hippocampus undergo use-dependent changes in synaptic efficacy, and these changes are mediated by a number of signaling mechanisms, including cAMP-dependent protein kinase (PKA). The PKA holoenzyme is composed of regulatory and catalytic (C) subunits, both of which exist as multiple isoforms. There are two C subunit genes in mice, Cα and Cβ, and the Cβ gene gives rise to several splice variants that are specifically expressed in discrete regions of the brain. We have used homologous recombination in embryonic stem cells to introduce an inactivating mutation into the mouse Cβ gene, specifically targeting the $C\beta_1$-subunit isoform. Homozygous mutants showed normal viability and no obvious pathological defects, despite a complete lack of $C\beta_1$. The mice were analyzed in electrophysiological paradigms to test the role of this isoform in long-term modulation of synaptic transmission in the Schaffer collateral–CA1 pathway of the hippocampus. A high-frequency stimulus produced potentiation in both wild-type and $C\beta_1^{-/-}$ mice, but the mutants were unable to maintain the potentiated response, resulting in a late phase of long-term potentiation that was only 30% of controls. Paired pulse facilitation was unaffected in the mutant mice. Low-frequency stimulation produced long-term depression and depotentiation in wild-type mice but failed to produce lasting synaptic depression in the $C\beta_1^{-/-}$ mutants. These data provide direct genetic evidence that PKA, and more specifically the $C\beta_1$ isoform, is required for long-term depression and depotentiation, as well as the late phase of long-term potentiation in the Schaffer collateral–CA1 pathway.**

A large body of evidence, both in humans and experimental animals, has revealed that the hippocampus is singularly important in the ability to use spatial, olfactory, auditory, and other contextual cues to learn new tasks and to commit those experiences to memory. Significantly, neurons of the hippocampus demonstrate the remarkable capacity to undergo persistent increases and decreases in synaptic transmission in response to electrical stimuli. Long-term potentiation (LTP) is an enhanced synaptic responsiveness that is elicited by high-frequency stimulation of the afferent neurons (1, 2). LTP can last for hours in an *in vitro* hippocampal slice preparation and for weeks when performed *in vivo*. Most neural pathways of the hippocampus also exhibit a second form of synaptic plasticity, long-term depression (LTD), in which low-frequency stimulation produces a decrease in synaptic responsiveness (3–5).

Both LTD and LTP in the CA1 region of the hippocampus are dependent upon *N*-methyl-D-aspartate (NMDA) receptor-mediated Ca^{2+} entry (4, 5). There is strong evidence for the involvement of calcium-regulated enzymes such as calmodulin kinase II and other protein kinases in the establishment of LTP and for phosphatases in LTD (1, 2, 6, 7). It is thought that these long-term changes in synaptic efficacy may be a mechanism by which enduring neural networks are established during memory acquisition and consolidation. In fact NMDA receptor antagonists have been shown to impair spatial and contextual learning in rats (8–10). Genetic studies also suggest that LTP in the CA1 hippocampus may be an important indicator of the potential for hippocampal synaptic modification during learning (11, 12). In contrast, no link between hippocampal LTD and learning has yet been demonstrated.

cAMP-dependent protein kinase (PKA) is involved in mediating long-term changes in synaptic strength, as first suggested by studies showing that β-adrenergic agonists and other activators of cAMP increased synaptic excitability and contributed to LTP in various hippocampal regions (13–17). Further studies showed a direct requirement for cAMP and PKA activity in mossy fiber LTP (18, 19) and in the late phase of LTP in CA1 neurons (20, 21). Because the late phase of LTP requires transcription (22), these data suggested that PKA mediates these transcriptional events by phosphorylating transcription factors such as CREB.

The ability to introduce targeted mutations into the mouse genome has made it possible to test the role of specific gene products in hippocampal function. Three mouse strains carrying gene disruptions in different components of the cAMP signaling pathway have recently been developed. The CREB mutants show a complete loss of the late phase of LTP in the Schaffer collateral–CA1 pathway, although the early phase of LTP is present (23). These mice also show a deficit in spatial learning, supporting the idea that phosphorylation of CREB by PKA is critical for the synaptic changes that underlie memory consolidation. A second mouse strain carrying a gene disruption in type I adenylyl cyclase has a defect in Schaffer collateral pathway LTP and shows a modest defect in performance on the Morris water task (24). Lastly, we recently reported that mice deficient in the RIβ regulatory (R) subunit of PKA lack multiple forms of hippocampal synaptic plasticity (3, 25). However, these mice have normal Schaffer collateral LTP and perform normally on spatial tasks.

Although evidence for the importance of PKA in hippocampal function has become ample in recent years, little is yet known about which PKA isoforms participate in the different forms of synaptic plasticity and which of many potential substrates are involved. All six of the mouse PKA catalytic (C) and regulatory (R) subunit genes, Cα, Cβ, RIα, RIβ, RIIα, and RIIβ, are expressed in brain. In addition, we have recently discovered (unpublished results) that the mouse Cβ gene gives rise to at least three splice variants with predicted unique amino termini and differential tissue distribution.

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Abbreviations: EPSP, excitatory postsynaptic potential; HFS, high-frequency stimulus; LFS, low-frequency stimulus; LTD, long-term depression; LTP, long-term potentiation; PKA, cAMP-dependent protein kinase; C, catalytic; R, regulatory; ES cell, embryonic stem cell.

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We have used gene targeting as a strategy to investigate the contribution of different PKA isoforms to hippocampal function. Our recent analysis of mice carrying a mutation in the RIβ subunit of PKA yielded the surprising observation that while Schaffer collateral LTP was normal, LTD and depotentiation were severely affected, providing direct evidence for involvement of the PKA signaling pathway in synaptic depression (3). The mutant mice also lacked mossy fiber-CA3 LTP, demonstrating an essential role for this PKA subunit isoform (25). However, these studies provided no information about which C subunit(s) of PKA mediates these functions. In the present study, we have created mutant mice carrying a gene disruption that specifically ablates the $C\beta_1$ isoform of the Cβ subunit. The electrophysiological consequences of this ablation further support the crucial role of PKA in multiple forms of synaptic modulation and suggest that both Cβ and RIβ are essential components of the signaling network.

MATERIALS AND METHODS

Construction of the Cβ Targeting Vector. The isolation of a Cβ clone from a BALB/c mouse genomic library was as described (26). An 8-kb genomic fragment containing exon 1 of the $C\beta_1$ splice variant was used to construct a targeting vector, Cβ-REC1. An approximately 200-bp segment of the gene, extending from the *Pvu* I site at amino acid 5 within the coding region of exon 1 to a *Sac* II site in the first intron, was replaced with the neomycin-resistance cassette from pKOneo; a thymidine kinase cassette was appended to facilitate negative selection (3).

Gene Targeting in Embryonic Stem (ES) Cells and Generation of Mutant Mice. Gene targeting in ES cells was performed essentially as described (27). Cβ-REC1 DNA was linearized with *Not* I and then electroporated at a concentration of 25 μg/ml into two ES cell lines—REK2 and REK3—at 1.2×10^7 cells per ml. G418 (GIBCO)- and ganciclovir (Syntex, Palo Alto, CA)-resistant colonies were screened by PCR. Four targeted REK2 ES-cell clones and three targeted REK3 ES-cell clones were identified, verified by genomic Southern blot analysis, and microinjected into C57BL/6 blastocysts to generate chimeric mice. Three REK2-derived cell lines and two REK3-derived cell lines contributed to the germ line.

Western Blot and Kinase Analyses. Tissue homogenates were prepared as described (28). For Western blot analysis, proteins were electrophoresed, transferred to nitrocellulose, and probed with antiserum 2328, a rabbit polyclonal antiserum raised against mouse Cβ amino acids 27–38 (29, 30). Signal detection was by the ECL method (Amersham). A homogenate of 3T3 cells that stably express mouse $C\beta_1$ was used for a $C\beta_1$ standard (31). Kinase assays were performed on brain homogenates as described (3, 28). Nonspecific kinase activity that persisted in the presence of the protein kinase inhibitor PKI peptide was subtracted.

Electrophysiology. Mice were studied on a C57BL/6 × 129 mixed strain background at 4–6 weeks of age. All experiments used age-matched controls and were performed blind to genotype. Hippocampal slices were kept in an interface chamber at 28°C, essentially as described (32). Stimulus intensity was adjusted to produce a response of approximately 1 mV amplitude, with an initial slope of approximately −0.5 mV/msec. CA1 LTP was induced by tetanic stimulation (100 Hz for 1 sec) delivered either once or four times with an intertrain interval of 5 min. Homosynaptic LTD was induced by a prolonged low-frequency stimulation (1 Hz for 15 min). For depotentiation experiments, the stimulus to produce LTP was 100 Hz for 1 sec, delivered twice with an interval of 20 sec, followed by a low-frequency stimulus (LFS) of 5 Hz for 3 min to produce depotentiation. The initial slope of field excitatory postsynaptic potentials (EPSPs) are reported, and error bars represent SEM.

RESULTS

PKA-$C\beta_1$ Null Mutant Mice Are Viable and Fertile. Mice that carry a targeted disruption in the Cβ subunit gene of PKA were created by homologous recombination in ES cells. The targeting construct, shown in Fig. 1*A*, encompasses 8 kb of genomic sequence spanning exon 1 of the Cβ gene. Approximately 200 bp of the Cβ gene extending from within exon 1 downstream into the first intron was deleted and replaced with the neomycin-resistance gene cassette, creating a disruption of the $C\beta_1$ splice variant at amino acid 5. Two other splice variants of the gene, $C\beta_2$ and $C\beta_3$, would not be affected by this strategy because they do not utilize this exon 1 (unpublished data). Electroporation of the targeting construct into ES cells yielded several homologous recombinant clones carrying the mutant allele. Five different recombinant clones, derived from two parental ES cell lines, gave rise to germ-line-competent male chimeric mice. The chimeras were bred with C57BL/6 females to generate offspring heterozygous for the mutation. Heterozygotes were bred, and the Southern blot shown in Fig. 1*B* demonstrates the wild-type 13-kb and mutant 4.4-kb Cβ alleles present in their wild-type, heterozygous, and homozygous offspring.

Western blot analysis was performed to demonstrate that the $C\beta_1$ isoform of PKA was indeed absent in the mutants and also to examine the remaining C subunit isoforms. In mouse, only one transcript has been characterized from the Cα gene, whereas we have identified three transcripts from the Cβ gene: $C\beta_1$ (30), and two splice variants, $C\beta_2$ and $C\beta_3$ (unpublished data). Fig. 1*C* shows a Western blot analysis with polyclonal antiserum directed against all known Cβ subunits. As shown, hippocampus and other brain regions from wild-type mice express both $C\beta_1$ and a smaller Cβ band (possibly a mixture of $C\beta_2$ and $C\beta_3$), whereas kidney expresses only $C\beta_1$. In the mutant mice, $C\beta_1$ is absent from all samples demonstrating the successful null mutation of the $C\beta_1$ isoform. The Cβ antiserum does not detect Cα, which comigrates with the $C\beta_1$ isoform and represents more than half of the C subunits present in both mutant and wild-type brain (data not shown).

Homozygous null mutant mice were obtained from heterozygous matings at the expected Mendelian frequency indicating no deleterious effect of the mutation on developmental viability. Similarly, no decrease in longevity and fertility and no overt behavioral, morphological, or histological defects were noted (data not shown). Kinase assays were performed with brain tissue homogenates in the presence and absence of cAMP to determine whether PKA activity was affected. As expected, no difference between mutant and wild-type mice was observed (Fig. 1*D*), reflecting the small fraction (≈10%) of total PKA activity in brain that is attributable to the $C\beta_1$ isoform. Compensatory changes in the levels of either R subunits or other C-subunit isoforms might have occurred to offset the loss of $C\beta_1$, but such small changes would be difficult to quantify by Western blot analysis.

$C\beta_1$ Mutants Have a Decrease in Late-Phase LTP in the Schaffer Collateral–CA1 Pathway. The role of the $C\beta_1$ isoform of PKA in LTP was investigated in the CA1 region of the hippocampus by using an *in vitro* slice preparation. A single high-frequency train produced significant enhancement of the field EPSPs in both wild-type and mutant mice (Fig. 2A). This stimulus paradigm, which elicits only the early phase of LTP, yielded no significant difference in synaptic response between the two mouse groups. After a series of four high-frequency trains of 100 Hz for 1 sec, wild-type slices showed a significant increase in subsequent field EPSPs, and this potentiation was maintained throughout the duration of a 3-hr experiment (Fig. 2*B*). In contrast, mutant mice showed a normal early phase, but the potentiation continuously decayed over time, resulting in a late phase of LTP that was significantly less than the wild-type mice (3 hr after stimulus, $P < 0.01$). This defect in

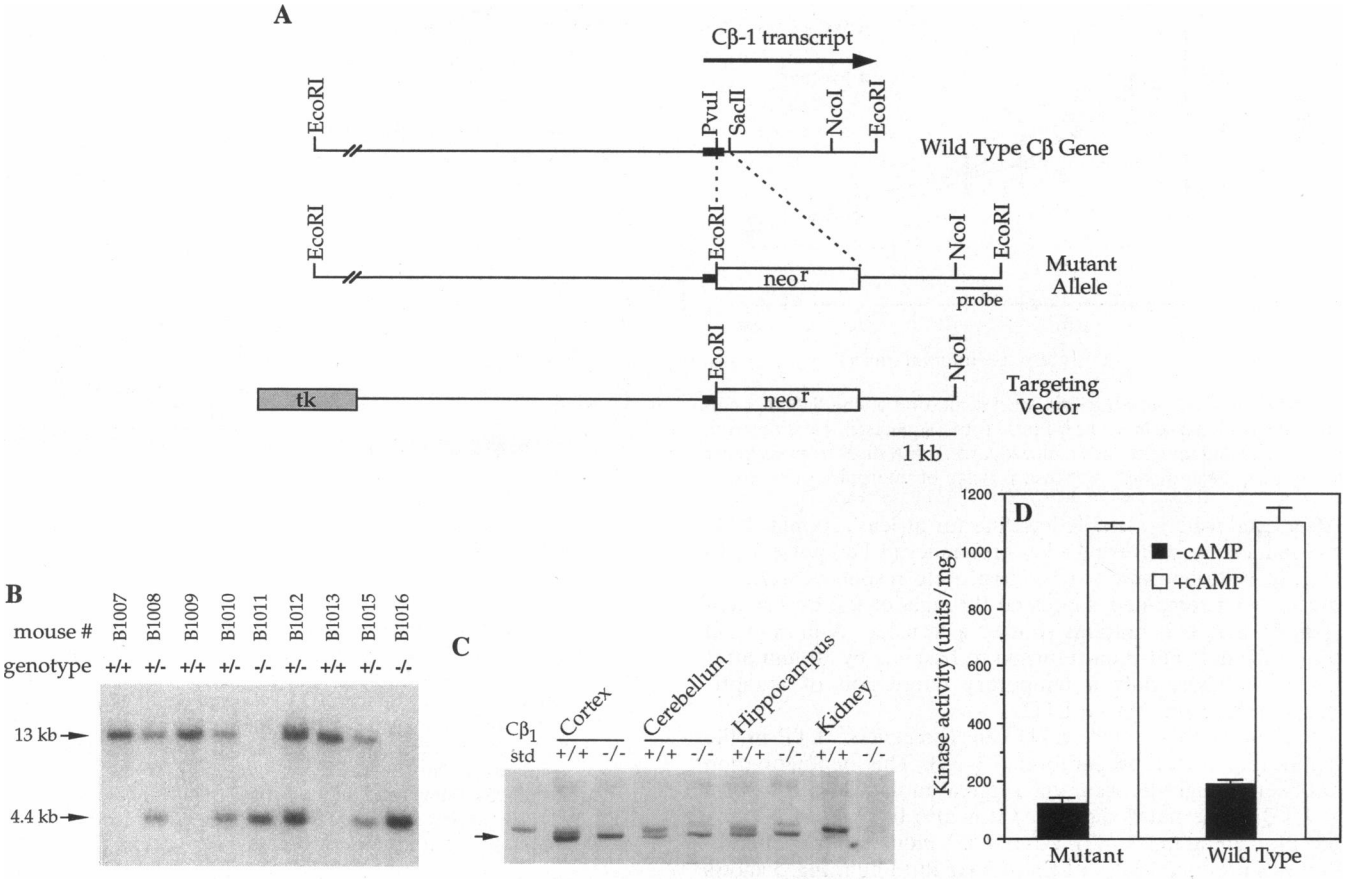

FIG. 1. Generation of $C\beta_1^{-/-}$ mice. (*A*) A restriction map of the wild-type Cβ genomic region (*Top*), the targeting vector (*Bottom*), and the mutant allele that results from homologous recombination of the targeting vector at the Cβ locus (*Middle*). The probe used for genomic Southern blots is indicated. (*B*) Genomic Southern blot analysis of offspring from heterozygote crosses. Total nucleic acid from tail biopsies was digested with *Eco*RI, yielding a wild-type 13-kb band and a mutant 4.4-kb band after hybridization. Heterozygous (+/−), homozygous (−/−), and wild-type (+/+) genotypes are indicated. (*C*) Western blot analysis of wild-type (+/+) and homozygous mutant (−/−) mice. Protein samples (40 μg) from the indicated brain region or kidney were loaded. For comparison, 15 μg of a homogenate prepared from a $C\beta_1$-overexpressing cell line was also loaded ($C\beta_1$ std). The blot was probed with a polyclonal anti-Cβ peptide antiserum. The position of the 40-kDa molecular mass standard is indicated (arrow). (*D*) PKA activity in homozygous mutant and wild-type mouse brain, in the absence or presence of 5 μM cAMP, using Kemptide as the substrate. PKI peptide-inhibitable activity is shown ± SEM.

the late phase was selective; both the early phase of LTP and paired-pulse facilitation (in which the second of a pair of stimuli evokes a greater response than the first due to an augmented accumulation of presynaptic Ca^{2+}) were normal. No difference in paired-pulse facilitation between wild-type and mutant mice was observed over the entire range of interpulse interval lengths (Fig. 3). $C\beta_1$, therefore, is not required for this presynaptic phenomenon. In contrast, $C\beta_1$ mutant mice showed a significant deficit in the ability to maintain a lasting increase in synaptic efficacy in response to tetanus.

LTD and Depotentiation Are Both Absent in the $C\beta_1$ Mutants. Homosynaptic LTD was examined at the same Schaffer collateral–CA1 pyramidal cell synapse studied above.

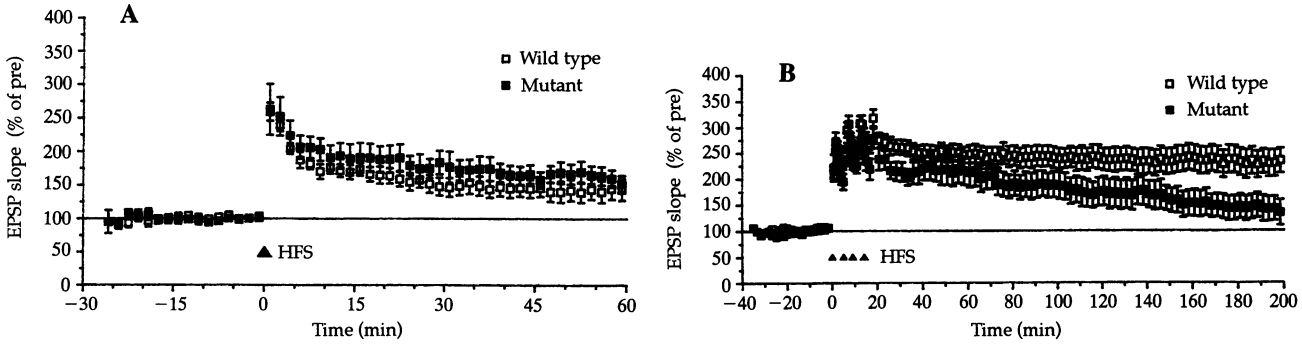

FIG. 2. LTP at the Schaffer collateral–CA1 synapse. (*A*) The early phase of LTP induced by a one-train tetanus (solid triangle) in $C\beta_1^{-/-}$ mutant mice (solid squares; $n = 7$ slices from seven mice) is similar to that in wild-type mice (open squares; $n = 9$ slices from eight mice). Mean field EPSP during the final 10 min was 143 ± 16% for wild-type mice, compared with 158 ± 12% for the mutant mice. (*B*) Field EPSPs after a high-frequency stimulus (HFS, four solid triangles) show potentiated responses throughout the time course in wild-type mice (open squares; $n = 8$ slices from eight mice). $C\beta_1^{-/-}$ mutant mice (solid squares) have normal potentiation at early times, which then decays significantly ($n = 9$ slices from nine mice). Mean field EPSP during the final 10 min was 232 ± 26% for wild-type mice, compared with 141 ± 23% for the mutant mice.

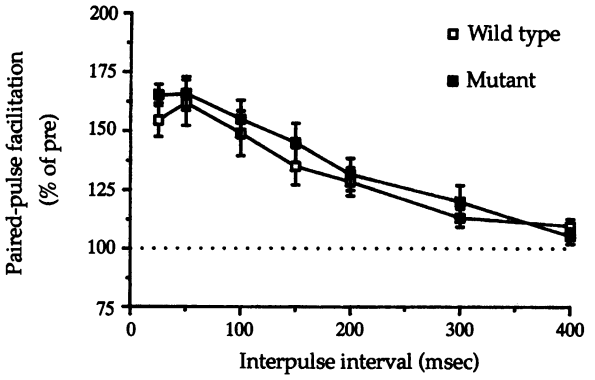

FIG. 3. Paired pulse facilitation. Facilitation at the Schaffer collateral–CA1 synapse in wild-type mice (open squares; $n = 8$ slices from eight mice) and mutant mice (solid squares; $n = 6$ slices from six mice) are not significantly different over a range of interpulse intervals.

After establishing a stable baseline for at least 20 min, LTD was induced by delivering a low-frequency (1 Hz) pulse for 15 min. In wild-type mice, subsequent field responses were depressed and remained depressed throughout the experiment (Fig. 4). The $C\beta_1$ mutants showed a transient diminution in responsiveness but then returned to baseline by 10 min after the LFS. Thus, only a temporary depression of synaptic strength occurred but no LTD.

Paradigms that induce LTD can also reverse LTP in the hippocampal slice preparation (33–35). This depotentiation has been thought to occur via a similar molecular mechanism as LTD in the naive slice since it is also blocked by NMDA receptor antagonists (35). After LTP induction, depotentiation was produced with a LFS of 5 Hz for 3 min. Fig. 5 shows field EPSPs in the mutant and wild-type mice after the LFS and, for comparison, the LTP produced in wild-type mice in the absence of the subsequent LFS. After LFS, responses in the wild-type mice were depressed to baseline as expected. In contrast, the mutant mice demonstrated only a brief depotentiation followed by a return toward the potentiated state. Again, the lack of $C\beta_1$ resulted in an inability to maintain synaptic depression. Thus, LTD and depotentiation are similarly affected in the $C\beta_1$ mutants. The coordinate loss of LTD and depotentiation further strengthens the idea that they share at least some common molecular underpinnings.

DISCUSSION

Multiple C-Subunit Isoforms Are Expressed in Brain. By using a genetic approach, we have completely eliminated

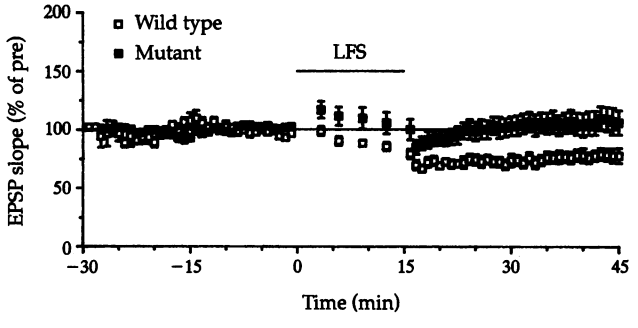

FIG. 4. LTD is absent in $C\beta_1^{-/-}$ mice. After a LFS, field EPSPs at the Schaffer collateral–CA1 synapse are significantly reduced in wild-type mice (open squares; $n = 14$ slices from nine mice). Mutant mice (solid squares; $n = 14$ slices from eight mice) show a transient depression and then return to near baseline within minutes. Average field EPSPs 25–30 min after LFS were 78 ± 6% for wild-type mice, compared with 108 ± 11% for the mutant mice, and these were significantly different (Student's t test, $P < 0.01$).

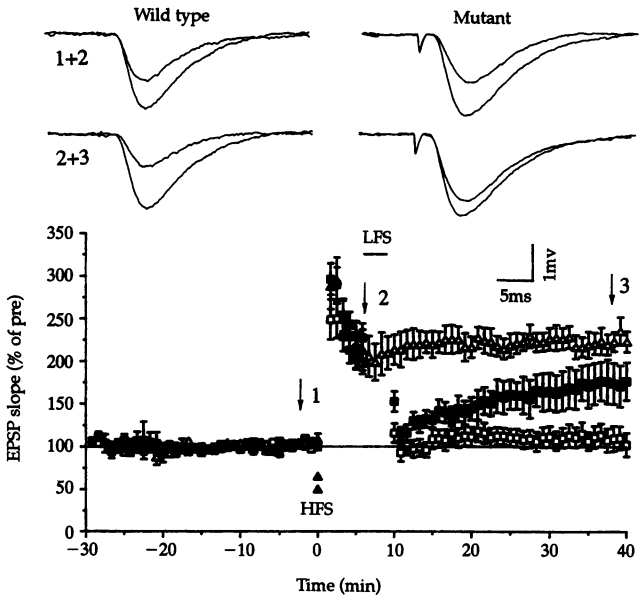

FIG. 5. Depotentiation is impaired in the $C\beta_1^{-/-}$ mice. Field EPSP was recorded at the Schaffer collateral–CA1 synapse in response to a HFS, which produced LTP in wild-type mice (open triangles; 217 ± 10%; $n = 6$ slices from six mice). HFS followed 6 min later by a LFS produced depotentiation in wild-type mice (open squares; $n = 11$ slices from nine mice) but failed to produce lasting depotentiation in the $C\beta_1^{-/-}$ mutants (solid squares; $n = 9$ slices from six mice). The average EPSP 25–30 min after LFS was 108 ± 13% for wild-type mice and 173 ± 24% for mutant mice. The magnitude of the depotentiation in the mutant mice was significantly smaller ($P < 0.01$).

expression of the $C\beta_1$ protein in mice. To understand the significance of this ablation in terms of total PKA activity, we must consider the repertoire of C subunits that are normally expressed in brain. We have identified three splice variants of the mouse $C\beta$ gene (ref. 30 and unpublished data), whereas the $C\alpha$ gene has only a single known transcript. $C\beta_1$ is the prototypic $C\beta$ subunit that has been cloned and characterized in several animal species. $C\alpha$ and $C\beta_1$ share 91% amino acid sequence identity (30), and both isoforms form functional holoenzymes with all of the various PKA R subunits. They show no unique substrate specificities, although they exhibit some preferences (36). The mouse $C\beta_2$ and $C\beta_3$ splice variants were only recently discovered and thus have not been extensively studied nor have their counterparts been identified in other species. A bovine splice variant of the $C\beta$ gene (that does not correspond to mouse $C\beta_2$ or $C\beta_3$) has also been described by cloning and RNA expression analysis (37).

In mouse, $C\alpha$ and $C\beta$ mRNAs are found in all tissues, including brain, at various levels (30). By *in situ* analysis, we have identified discrete regions of the brain that express $C\beta$ transcripts preferentially, although these studies were done with probes that would not distinguish between splice variants (38). By Western blot analysis, we estimate that $C\beta_1$ accounts for 7–10% of the total C subunits present in the hippocampus and other brain regions. Because $C\beta_2$ and $C\beta_3$ are not distinguishable on Western blots, no estimate of the relative levels of these two isoforms can yet be made.

How Might PKA Regulate Hippocampal LTP and LTD? Many cell signaling pathways have been implicated in LTP, including tyrosine kinases and a variety of calcium-dependent enzymes. Pharmacologic and genetic approaches have been used to demonstrate that the PKA system also plays a crucial role in synaptic potentiation. Studies on mice with targeted mutations in different components of the PKA signaling pathway—type I adenylyl cyclase and CREB—demonstrated that perturbation of this pathway severely impairs LTP (23,

24). We previously found that mice carrying a mutation in the RIβ-subunit isoform of PKA have a profound defect in CA3 LTP(25), although CA1 LTP is normal (3). In this report, the PKA-$C\beta_1$ mutant mice further demonstrate that PKA plays an integral and direct role in hippocampal synaptic modification. Future studies will likely reveal many layers of overlap and cross-talk between PKA and other signaling pathways.

Perhaps most interesting is our finding of a role for PKA in LTD. In 1989, Lisman (39) proposed a specific molecular model for hippocampal LTD (and LTP) that has helped to set the framework for subsequent studies. He proposed that modest Ca^{2+} influxes occur during LTD, activating a phosphatase cascade, whereas large influxes of Ca^{2+} occur during LTP, activating kinases. When this model was first developed, PKA was known to phosphorylate (and thus activate) the inhibitor 1 of phosphatase 1 and was predicted, therefore, to oppose LTD. Surprisingly, our subsequent pharmacologic and genetic studies revealed that PKA activity, and specifically the RIβ and Cβ isoforms, are required for both LTD and LTP. Although it is premature to attempt to incorporate PKA into a detailed molecular model of LTD, it is worth considering the known features of PKA and how the various isoforms might contribute to synaptic plasticity.

The remarkable similarity in phenotypes observed in the $C\beta_1$ and RIβ mutant mice suggests that these subunits perform similar or complementary functions in the relevant hippocampal cell types. Nevertheless, one cannot compensate for the other in its absence. An important characteristic of both of these isoforms is their sensitivity to cAMP. Holoenzymes containing $C\beta_1$ (in association with RIIα) are activated at a 6- to 10-fold lower cAMP concentration than holoenzymes containing Cα (E. Baude and M. Uhler, personal communication). Similarly, RIβ-containing holoenzymes are 3- to 7-fold more sensitive to cAMP than those containing RIα (in association with either Cα or Cβ) (40). One might speculate that the modest Ca^{2+} influx produced during the initiation of LTD activates a limited subset of adenylyl cyclase molecules producing a modest, perhaps localized, increase in cAMP. Only the more sensitive $C\beta_1$- or RIβ-containing PKA holoenzymes would respond to this small increase. The phenotypic similarity of the RIβ and Cβ mutant mice also raises the question of whether these two subunit isoforms might preferentially associate with each other to form holoenzymes in hippocampal neurons. We should note that an RIβ–Cβ holoenzyme does not confer additive sensitivity to cAMP (40). Although it is unknown whether RIβ and Cβ preferentially associate with each other, *in situ* hybridization studies indicated that in many cases their expression patterns are not coincident (38).

There is considerable evidence that subcellular compartmentalization of particular PKA isoforms occurs in neurons and other cell types (41, 42). For example, PKA is localized to postsynaptic densities via the interaction of its regulatory subunit (specifically RIIα) with the anchoring protein AKAP79. Colocalization of calcineurin with these complexes was also demonstrated in hippocampal neurons (43). This colocalization of signaling molecules invited speculation that it is functionally relevant, and evidence in support of this possibility is beginning to emerge. When anchoring was disrupted in hippocampal neurons by perfusion with blocking peptides, the PKA regulation of AMPA/kainate currents was lost (44). Similarly, these peptides prevented potentiation of voltage-dependent Ca^{2+} channels by PKA in skeletal muscle cells (45). These studies have not identified which C subunit of PKA is present within the compartmentalized holoenzymes. It is reasonable to expect that both Cβ and Cα are represented in these holoenzymes since there is no known discrimination among the C-subunit isoforms by R subunits *in vivo*. Furthermore, PKA might also be colocalized with other as yet unidentified receptors and signaling molecules. Thus, $C\beta_1$ may be present within PKA holoenzymes that are localized to specific subcellular regions, where they may be associated with critical signaling molecules, and there would confer greater cAMP sensitivity than that exhibited by Cα-containing holoenzymes. Perhaps both compartmentalization and cAMP sensitivity contribute to the requirement for $C\beta_1$ in synaptic function.

At present, one of the most important problems is the identification of PKA substrates that act to facilitate long-term changes in synaptic efficacy. Since many ion channels, neurotransmitter receptors, and transcription factors are phosphorylated *in vivo* by PKA, the possible candidates are numerous and it is likely that multiple substrates are involved. The recent literature in this area suggests two prime targets. LTP is associated with enhanced AMPA receptor-mediated synaptic transmission, and it is known that AMPA receptors are phosphorylated by PKA, resulting in enhanced activity (7). Additionally, there is clearly a requirement for gene transcription during the late phase of LTP, and the transcription factor CREB is directly activated by PKA phosphorylation in neurons. That CREB is a critical PKA substrate is supported by the finding that CREB knockout mice have a significant loss of late-phase LTP (23). It should be noted, however, that CREB is also a substrate for other neural kinases that may activate transcription without PKA involvement (46, 47).

Are CA1 LTP and LTD Required for Learning? LTP and LTD have frequently been discussed as electrophysiological indicators of synaptic plasticity and, therefore, as potential information storage mechanisms used by the hippocampus. In trying to understand the importance of these electrophysiological phenomena in hippocampal-mediated behaviors, we and others have used pharmacologic and genetic approaches to determine whether defects in hippocampal synaptic plasticity translate into defects in spatial learning and memory. For example, studies of the CREB knockout mice suggested that loss of the late, but not early, phase of CA1 LTP correlated with an inability to maintain long-term memory (23). However, the PKA-Cβ mutant mice described here, which also show a partial defect in the late phase of CA1 LTP, have shown no detectable deficit in spatial learning (25). Along with observations made on other mutant mice (24, 48–54), these data suggest that only severe LTP defects impair performance in typical spatial tasks.

The fact that mice with mutations in either the $C\beta_1$ or RIβ subunit of PKA lack LTD yet show no apparent behavioral deficits in paradigms that test spatial learning, including reversal (i.e., unlearning a solution to a maze and relearning a new solution), indicates that hippocampal LTD is also not essential for learning. This finding is particularly relevant for the interpretation of behavioral studies on other genetic mutants. In an analysis of the mGluR1 knockout mice, Aiba *et al.* (51) suggested that LTD, which was unaffected in the mutant mice, may account for the lack of a severe learning deficit despite a decrease in LTP. Similarly, Abeliovich *et al.* (53, 54) suggested that the protein kinase Cγ mutant mice, which show an impairment in LTP, exhibit only a mild learning deficit perhaps because of the unaffected LTD and, therefore, that both LTP and LTD might contribute to spatial learning. Our data on the $C\beta_1$ mutant and RIβ mutant mice suggest that the ability of hippocampal synapses to undergo LTD is not a critical factor in either spatial or contextual learning and thus does not support the idea that LTD is essential for such learning.

One of the more compelling attributes of the two PKA genetic mutant mouse lines that we have developed is that the electrophysiological deficits are very discrete, yet pronounced, without any appreciable developmental complications. Thus, it will be very informative to continue to explore other hippocampal-mediated behaviors in these mice to establish whether a link between hippocampal LTD and such behaviors can be found.

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