Adenylyl Cyclase Anchoring by a Kinase Anchor Protein AKAP5 (AKAP79/150) Is Important for Postsynaptic β-Adrenergic Signaling*

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Background: AKAP5 is emerging as an adenylyl cyclase (AC)-binding protein.
Results: Knockout of AKAP5 affects β-adrenergic postsynaptic signaling more than abrogating PKA targeting only in AKAP5 deletion mutants.
Conclusion: AC anchoring by AKAP5 is critical for postsynaptic signaling via cAMP and PKA.
Significance: β-adrenergic signaling, which depends on AKAP5-anchored AC, regulates synaptic transmission to augment alertness and memory.

Recent evidence indicates that the A kinase anchor protein AKAP5 (AKAP79/150) interacts not only with PKA but also with various adenylyl cyclase (AC) isoforms. However, the physiological relevance of AC-AKAP5 binding is largely unexplored. We now show that postsynaptic targeting of AC by AKAP5 is important for phosphorylation of the AMPA-type glutamate receptor subunit GluA1 on Ser-845 by PKA and for synaptic plasticity. Phosphorylation of GluA1 on Ser-845 is strongly reduced (by 70%) under basal conditions in AKAP5 KO mice but not at all in D36 mice, in which the PKA binding site of AKAP5 is mimics the endogenous long term potentiation induced by a 5-Hz/180-s tetanus, which severely impaired in AKAP5 KO than in D36 mice. In parallel, long term potentiation induced by a 5-Hz/180-s tetanus, which mimics the endogenous θ-rhythm and depends on β-adrenergic stimulation, is only modestly affected in acute forebrain slices from D36 mice but completely abrogated in AKAP5 KO mice. Accordingly, anchoring of not only PKA but also AC by AKAP5 is important for regulation of postsynaptic functions and specifically AMPA receptor activity.

AKAPs link PKA to several of its key substrates for fast, efficient, and selective phosphorylation of those targets (1, 2).

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1 The abbreviation used are: AKAP, A kinase anchor protein; AC, adenylyl cyclase; β- and β2-AR, β- and β2-adrenergic receptor, respectively; LTD, long term depression; LTP, long term potentiation; IP, immunoprecipitation; ACSF, artificial cerebrospinal fluid; EPSP, field excitatory postsynaptic potential; prolonged ISO, isoproterenol; ANOVA, analysis of variance; PTT-LTP, prolonged θ-tetanus-LTP; KI, knock-in.

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PKA phosphorylates GluA1 on serine 845 (17, 18), which is important for GluA1 surface expression (19–23), activity-induced postsynaptic accumulation (5, 24), and various forms of synaptic plasticity (25–29) (but see Ref. 30).

AKAP5 was named AKAP150 in rodents and AKAP79 in humans. AKAP150 is larger than AKAP79 due to an insert of 36 imperfect octapeptide repeats of unknown function (31). The C terminus of AKAP5 (amino acids 392–416 in AKAP79) anchors the regulatory RII subunits of PKA (32). The N terminus binds PKC, F-actin, cadherin, and phosphatidylinositol 4,5-bisphosphate and helps to target AKAP150 to dendritic spines (33–37). The central region of AKAP150 binds the Ca$^{2+}$ and calmodulin-activated phosphatase calcineurin (PP2B) (38–40). This interaction is important for long term depression (LTD) and for curbing long term potentiation (LTP) (41, 42).

AKAP5 is the main postsynaptic AKAP (43–47). Functionally, AKAP5 links PKA, PKC, and the antagonistic phosphatase PP2B via SAP97 and perhaps also PSD-95 to GluA1 for dynamic phosphorylation and dephosphorylation (17, 37, 48–50). On a molecular level, PKA is physically connected to GluA1 by AKAP5. AKAP5 can bind to the Src homology 3 and guanylate kinase domains of the postsynaptic scaffolding proteins PSD-95 and SAP97 (48, 51) (Fig. 1). PSD-95 interacts with its first and second PDZ domain with stargazin ($\gamma_2$) and its homologues $\gamma_3$, $\gamma_4$, and $\gamma_8$ (collectively called TARPs), which in turn associate with AMPA receptors for their postsynaptic targeting (52–55). SAP97 can directly bind with its first or second PDZ domain to AMPA receptors for their postsynaptic targeting (52–55).

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Because AKAP5 can recruit AC activity to GluA1 (10), we hypothesized that anchoring of AC by AKAP5 is important for postsynaptic $G_i$ protein-coupled receptor-$G_i$-AC-PKA-GluA1 signaling. We systematically compared $\beta$-adrenergic regulation of Ser-845 phosphorylation and of postsynaptic glutamate receptor responses in AKAP150 KO mice (59) and mice in which the last 36 residues of AKAP150 had been deleted (D36 mice) (43, 45–47) to test the functional roles of AKAP150 with respect to PKA versus AC targeting. We found that basal Ser-845 phosphorylation and its up-regulation by $\beta$-adrenergic stimulation is much more drastically impaired in AKAP5 KO than in D36 mice. The increase in basal glutamatergic synaptic transmission upon $\beta$-adrenergic stimulation is compromised in forebrain slices from AKAP5 D36 and KO mice. However, LTP induced by a 5-Hz/180-s tetanus, which requires $\beta$-adrenergic stimulation in addition to the electric stimulus train, is only modestly reduced in D36 but completely abrogated in KO mice. We conclude that anchoring of AC by AKAP5 is important for positive regulation of postsynaptic functions that include AMPA receptor activity by cAMP-PKA signaling.

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—(-)-Isoproterenol bitartrate salt, ICI118551, CGP20712, microcystin LR, and (+)-propanolol hydrochloride were from Sigma. IEM1460 was from Tocris. Antibodies against the $\beta_1$-AR (V-19; Lot K1209) and $\beta_2$-AR (H-20; Lot J0305) were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The antibodies against synaptophysin, PSD-95, GluA2, GluN1, GluN2B, and ACs (panspecific) were as given earlier (3, 5, 56, 60–62). The rabbit anti-AC5/6 antibody was from Santa Cruz Biotechnology, Inc. (C17; sc-590). The phosphospecific antibodies against Ser-831 and Ser-845 were produced against the synthetic peptides LIPQQpSINEA1K (GluA1 residues 826–836) and TLPpNpSGAG (GluA1 residues 840–850) (where pS represents phosphoserine) (see Ref. 18) and the anti-GluA1 antibody against the peptide MSHSSGMPLATGGL, which corresponds to the very C terminus of GluA1 (residues 894–907). All peptides had been coupled to bovine serum albumin for immunization of rabbits, as described earlier (63). Nonselective control antibodies were from Zymed Laboratories Inc.. HRP–coupled protein A was from Amersham Biosciences. ECL and ECL Plus reagents were from GE Healthcare. Other reagents were from the typical suppliers and of the usual quality.

Animal Use and Origin—All procedures followed National Institutes of Health guidelines and had been approved by the Institutional Animal Care and Use Committees at the University of Iowa and University of California, Davis. The production of AKAP150 KO mice (by insertion of a neomycin phospho-transferase cassette into the intronless AKAP150 gene) and of D36 mice (by creation of a premature stop codon) and their genotyping were described earlier (43, 46). Both KO and D36 mouse lines used in the current work had been back-crossed to C57Bl/6 (Taconic Farms) for at least 10 generations. All mice were between 8 and 16 weeks old except for postsynaptic density (PSD) preparations, for which up to 8-month-old mice were used.

Immunoprecipitation (IP) and Immunoblotting—Forebrain slices containing hippocampus (see below) were extracted with a 10-fold excess (v/w) of buffer A (150 mM NaCl, 10 mM EDTA, 10 mM EGTA, 10 mM Tris–HCl, pH 7.4, and protease inhibitors) containing 1% Triton X-100 with a glass–Teflon homogenizer. Samples were cleared from non-solubilized material by ultracentrifugation (250,000 × g for 30 min) before IP with anti-GluA1 (2 μg/ml of antiserum), H2O against the GluN1 (1 μg/ml), anti-GluN2B (1 μg/ml), or an equivalent amount of nonspecific rabbit IgG (typically 2–8 μg; overnight at 4 °C) and subsequent immunoblotting as described (60, 61). Proteins were separated by SDS-PAGE, transferred overnight onto polyvinylidene difluoride (PVDF) membranes, incubated with primary antibodies for 1 h, washed, incubated with HRP-protein A for 1 h, and washed for 4 h before detecting ECL or ECL Plus signals by film. Multiple exposures with increasing time periods were obtained to ensure that signals were in the linear range, as described (64, 65).

PSD Preparation—For each preparation, four forebrains per genotype were homogenized on ice with a relatively loosely fitting glass–Teflon homogenizer in 10 ml of freshly made ice-cold buffer B (0.32 mM sucrose, 1 mM Tris, pH 7.4, 1 mM MgCl$_2$) containing the protease inhibitors leupeptin (10 μg/ml), aprotonin (10 μg/ml), pepstatin A (1 μM), and phenylmethylsulfonyl fluoride (PMSF; 200 μM) and the phosphatase inhibitor microcystin-LR (2 μM). The lysates were centrifuged at 1,400 × g for 10 min at 4 °C. The pellets were rehomogenized in an equal volume of buffer B, followed by centrifugation (10 min, 710 × g, 4 °C). The combined supernatants were centrifuged first at 710 × g (10 min) and then at 13,800 × g (10 min) to obtain the P2 fraction enriched with synaptic membranes. P2 was resus-
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pended in 3 ml of buffer C (buffer B without MgCl₂) and layered on top of a 0.85/1/1.25 m sucrose gradient. After centrifugation at 82,500 × g (2 h), the synaptosome-enriched interface was collected between the 1 and 1.25 m sucrose layers and extracted with an equal volume of Triton X-100 buffer. The synaptosome-enriched fraction (PSD) was collected from the 1.5/2M sucrose gradient. After centrifugation at 225,000 × g for 30 min, 350-μm-thick forebrain slices containing hippocampus were prepared as described above for SDS-PAGE and analyzed by immunoblotting.

Preparation and Treatment of Brain Slices—Mice that were typically 8–16 weeks old were decapitated, and brains were placed into ice-cold artificial cerebrospinal fluid (ACSF), containing 127 mM NaCl, 26 mM NaHCO₃, 1.2 mM KH₂PO₄, 1.9 mM KCl, 2.2 mM CaCl₂, 1 mM MgSO₄, and 10 mM D-glucose, 290–300 mosM/kg. ACSF was saturated with 95% O₂ and 5% CO₂ (final pH 7.3). About one-third of the rostral and caudal ends of the brain were trimmed off. 350-μm-thick forebrain slices containing hippocampus were prepared with a vibratome (Leica VT 1000A). Slices were kept in oxygenated ACSF for 1 h at 30°C and for 1–5 h at 24°C before they were used for experiments.

For phosphoanalysis, slices were equilibrated at 32°C for 30 min and treated with vehicle, isoproterenol (ISO) (10 μM) for 30 min. Slices were extracted with 1% deoxycholate before IP with anti-GluA1 and anti-GluN2B and immunoblotting with phosphospecific antibodies against Ser-831 and Ser-845 on GluA1, Ser-897 on GluN1, and GluN2B and immunoblotting with phosphospecific antibodies against Ser-845 (17, 37, 42–48). In detail, a central not completely defined region of AKAP5 interacts with the Src homology 3 (sh3) and guanylate kinase (GK) domains of PSD-95 and SAP97 (blue, 48, 51). SAP97 binds with its first and second PDZ domains to the C terminus of GluA1 (56–58), and PSD-95 binds with its first and second PDZ domains to stargazin (γ2) and its homologues γ3, γ4, and γ8 (collectively depicted as γ), which in turn associate with AMPA receptors (52, 54, 55). The β₂ AR binds with its very C terminus to the third PDZ domain of PSD-95 (5, 6).

RESULTS

AKAP5 Is Necessary for AC-GluA1 Interaction—Because AKAP5 interacts with not only PKA but also with at least six AC isoforms (10–12), we hypothesized that the previously described association of GluA1-containing AMPA receptors with AC (5) is mediated by AKAP5, which in turn is linked to GluA1 via SAP97 and possibly PSD-95 (Fig. 1). In fact, the AC-GluA1 co-IP was fully abrogated in AKAP5 KO but not affected in D36 mice, as revealed by a panspecific AC antibody that recognizes all isoforms, which all migrate with an apparent molecular mass of about 150 kDa (Fig. 2, A and B). Given the complete loss of the co-IP of ACs with GluA1, we conclude that AKAP5 is the main and probably only adaptor protein that links ACs to GluA1. As expected, co-IP of the PKA regulatory RIIα subunit with GluA1 was strongly reduced in both genotypes (Fig. 2, A and B). The incomplete loss of PKA association with GluA1 in D36 mice could be due to additional AC interactions with other AKAPs within the overall complex. In agreement with this notion is the nearly complete loss of PKA in parallel with AC in the AKAP5 KO mice. Total amounts of AC or RIIα were unaltered in brain lysates (Fig. 2, C and D).

AC5 and AC6 interact with the second of the three polybasic regions in the N terminus of AKAP5 (10). We found that an AC5/6-selective antibody recognized the appropriate AC subunit with GluA1-containing AMPA receptors but not NMDA receptors.

Anchoring of both PKA and AC by AKAP5 Is Important for Ser-845 Phosphorylation—AKAP5 functionally and structurally links PKA to GluA1 for Ser-845 phosphorylation, an important regulatory mechanism for GluA1 (48, 49, 50). Given our finding that AKAP5 also recruits ACs to GluA1, we evaluated whether Ser-845 phosphorylation is more severely affected in AKAP5 KO than D36 mice as a result of the loss of AC anchor-
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FIGURE 2. AKAP5 links ACs to GluA1. Forebrains from WT, AKAP5 D36, and AKAP5 KO mice were extracted with Triton X-100 and cleared of non-solubilized material by ultracentrifugation. A, C, and E, lysate samples underwent IP with 1 μg of antibody against GluA1 or GluN1 or 1 μg of control rabbit IgG (A and E) or were directly applied (C) to immunoblotting with a pan-specific antibody against all ACs and with antibodies against GluA1, RIIα, or AC5/6 as indicated. B, D, and F, immunosignals were quantified by densitometry. Depicted are film optical density (OD) ratios for pan-AC versus GluA1 signals (B), OD values for pan-AC (D), and OD ratios for AC5/6 signals in GluN1 versus in GluA1 IPs (F). Co-IP of ACs with GluA1 is nearly completely absent in AKAP5 KO mice but not affected in D36 mice (**, p < 0.01, one-way ANOVA). Co-IP of AC5/6 with GluN1 is nearly undetectable compared with GluA1 IPs (**, p < 0.01, t test). Error bars, S.E.

ing in addition to the loss of PKA anchoring in KO mice. In fact, basal phosphorylation levels of Ser-845 were unaltered in whole forebrain slices of D36 mice but strongly reduced in KO (Fig. 3, A (top panels) and C (left graph)). At most 10% and perhaps even much fewer GluA1 subunits are phosphorylated on Ser-845 under basal conditions (22, 66). However, the β-adrenergic agonist ISO stimulates Ser-845 phosphorylation severalfold (5, 6, 29, 67). The ISO-stimulated increase in Ser-845 phosphorylation was more than 5-fold for WT slices but less than 3-fold for KO and D36 slices (Fig. 3, A (top panels) and D (left graph)). This reduction in efficacy in up-regulation of Ser-845 phosphorylation, paired with the much lower level of basal phosphorylation in KO mice, translates into a dramatically reduced total ISO-induced phosphorylation in KO mice compared with WT. Although the 5-fold increase in Ser-845 phosphorylation by ISO is also strongly impaired in D36 versus WT mice, given that D36 mice had nearly WT levels of basal Ser-845 phosphorylation, the loss in total ISO-stimulated phosphorylation is much more modest in D36 than in KO mice. Augmentation of Ser-845 phosphorylation by ISO was blocked by the general β-adrenergic antagonist propranolol, confirming that ISO-induced Ser-845 phosphorylation was mediated by β-adrenergic signaling.

Basal phosphorylation levels for Ser-831, a PKC and Ca2+/calmodulin-dependent kinase II site (17, 18), were not statistically different in D36 and KO versus WT slices, indicating that loss of PKC anchoring to GluA1 by AKAP5 in the KO mice had no significant effect on basal Ser-831 phosphorylation (Fig. 3, A (middle panels) and D (left graph)). Also, ISO did not significantly stimulate Ser-831 phosphorylation. This result was expected because PKC is typically activated by Gq and not Gs-coupled receptors, and there is no evidence that either PKC or Ca2+/calmodulin-dependent kinase II would be activated upon stimulation of the β2-AR–AC–cAMP–PKA cascade at postsynaptic sites. We also monitored phosphorylation of Ser-897 in the C terminus of the NMDA-type glutamate receptor GluN1 subunit, which is an established PKA site of unknown function (68). Neither basal Ser-897 phosphorylation levels nor ISO-induced Ser-897 phosphorylation were affected in AKAP5 D36 or KO mice (Fig. 3, B (top panels) and C and D (right graphs)). Accordingly, loss of AKAP5 function affects AMPA receptor but not NMDA receptor phosphorylation by PKA. The NMDA receptor can bind PKA and AC1, AC2, AC3, and AC9 via the AKAP yotiao (9, 69), which might thus functionally be the more important if not the only PKA and AC anchor for NMDA receptors. Our results indicate that the deficit in GluA1 Ser-845 phosphorylation in AKAP5 D36 and KO mice is not a universal deficit in PKA-mediated phosphorylation in dendritic spines.

AKAP5 Is Not Necessary for β2-AR–GluA1 Interaction—The β2-AR is linked to GluA1 via PSD-95 and stargazin and their homologues (5). The β2-AR also binds directly to AKAP5 (70, 71). More precisely, the β2-AR C terminus interacts with the N-terminal ~200 residues of AKAP5 (71). Because AKAP5 binds to PSD-95 and SAP-97 on one hand and the β2-AR on the other, it is conceivable that AKAP5 is required for or contributes to stabilization of the β2-AR–PSD-95–stargazin-GluA1 complex in addition to the direct interactions of the β2-AR with PSD-95 and SAP97. We thus tested whether impaired ISO-induced Ser-845 phosphorylation in AKAP5 mutant mice could be due to loss of β2-AR from the GluA1 complex in addition to loss of AC. However, co-IP of GluA1 as well as GluA2, the other main AMPA receptor subunit in the forebrain, with the β2-AR is comparable for WT, D36, and KO mice (Fig. 4, A and B). Accordingly, loss of AKAP5 does not affect the formation or stability of the β2-AR-GluA1 complex.

To ensure specificity of the β2-AR antibody, we immunoprecipitated both the β2-AR and the β2-AR from WT mouse brain. Subsequent immunoblotting with either antibody showed β2-AR immunoreactivity in the β2-AR but not β2-AR IP and β2-AR immunoreactivity in the β2-AR but not β2-AR IP at the correct Mₐ of ~60,000 in both cases (Fig. 4C). Accordingly, either antibody recognizes its cognate target protein without cross-reacting with the other isoform.

To scrutinize whether the co-IPs of GluA1 and AKAP5 (Fig. 4, A and B) and of AC5/6 (Fig. 2, E and F) with the β2-AR are truly due to IP of the latter and not an off target cross-reacting protein, we immunoprecipitated the β2-AR from WT and β2-AR KO mouse brains. AC5/6, AKAP5, and GluA1 co-immu-
noprecipitated only from WT and not from β2-AR KO mice (Fig. 4D). We conclude that these co-IPs are due to IP of the β2-AR, indicating association of these proteins with the latter.

AKAP5 Is Required for Postsynaptic Targeting of AC in General and Specifically of AC5/6—To test whether AKAP5 is important for postsynaptic localization of ACs, we isolated PSD fractions from brains of WT, AKAP5 KO, and D36 mice for immunoblot analysis. The purity of the final PSD fractions was reflected by the loss of synaptophysin signal and the strong enrichment of the PSD marker PSD-95 (Fig. 5, A and C). Immunoreactivity of our panspecific antibody was drastically reduced in AKAP5 KO but not D36 mice (Fig. 5, A–D).

Because AKAP5 specifically links AC5/6 to GluA1, we analyzed AC5/6 immunoreactivity in PSD preparations from
AKAP5 KO mice. As for pan-AC signals, the AC5/6 signals were dramatically decreased in the KO mice (Fig. 5, A–D). These findings indicate that AKAP5 is the most critical docking protein for postsynaptic targeting of ACs and especially AC5/6, which is analogous to its central role in postsynaptic localization of PKA (43).

AKAP5 Is Not Necessary for Postsynaptic Targeting of the β2-AR—AKAP5 could more generally link the β2-AR to the PSD. To control for this possibility, we analyzed the β2-AR content of the PSD fractions from WT, D36, and KO mice by immunoblotting. The β2-AR was enriched in parallel with PSD-95 and GluA1 (Fig. 5, A and C), illustrating for the first time by such subcellular fractionation that the β2-AR is a component of the PSD. There was no difference between WT, D36, and KO brains with respect to the β2-AR content of PSDs (Fig. 5, B and D), demonstrating that neither the D36 deletion nor the complete KO affects postsynaptic targeting of the β2-AR.

Collectively, these results indicate that deficits in ISO-induced Ser-845 phosphorylation are not due to mistargeting of the β2-AR away from the PSD in general and specifically from GluA1.

We also monitored subcellular distribution of the β1-AR. We observed β1-AR immunoreactivity only in pre-PSD fractions and not in the PSD fractions themselves (Fig. 5, A and C).
thermore, hippocampal samples did not show a decrease in basal Ser-845 phosphorylation, in contrast to the forebrain sections (compare Fig. 3C (left graph) with Fig. 6, A (top) and B), possibly because basal β-adrenergic tone might be lower at postsynaptic sites in the hippocampus than in the cortex. As in the forebrain samples, GluA1 Ser-831 phosphorylation was not affected in AKAP5 KO mice; nor did ISO increase it in the hippocampus (Fig. 6, A and C).

**Up-regulation of Postsynaptic AMPA Receptor Responses by β-Adrenergic Signaling Requires AKAP5—**LTP induced by prolonged θ-rhythm tetani (5 Hz for 180 s; prolonged θ-tetanus-LTP (PTT-LTP)) at Schaffer collateral-CA1 synapses is an important form of synaptic plasticity because the θ-rhythm (5–12 Hz) is a prominent activity pattern of the hippocampus (72, 73). The dependence of PTT-LTP on β-adrenergic stimulation of adenylyl cyclase and PKA (27, 29, 74–76) is fundamentally different from more standard LTP triggered by multiple tetani of 50–100 Hz or θ-burst stimulations, which does not require PKA at all or, if induced by a single tetanus, only requires basal PKA activity (43). PTT-LTP is impaired in phosphorylation-deficient GluA1 S831A/S845A double KO mice (27) and in GluA1 S845A single KO mice (29). It does not require β-adrenergic stimulation in phosphorylation-mimetic GluA1 S831D/S845D double KO mice (77). Accordingly, Ser-845 phosphorylation is not only necessary but also sufficient to gate induction of PTT-LTP that would otherwise require β-adrenergic stimulation for gating. Given the simultaneous loss of AC and PKA from GluA1 in AKAP5 KO mice versus the loss of only PKA in D36 mice, we hypothesized that up-regulation of postsynaptic responses and PTT-LTP are more severely affected in KO than in D36 mice.

Similar to our previous findings (5), in acute slices from WT mice, ISO by itself increased fEPSP initial slopes in only ~60% of the recordings with little to no effect in the remaining ~40% (Fig. 7, A and B). D36 and KO mice never showed any increase in the postsynaptic response upon perfusion with ISO, suggesting that the ISO effect observed under basal conditions specifically requires anchoring of PKA by AKAP5 (Fig. 7, C–E).

In WT slices, induction of PTT-LTP increased fEPSPs by 67% if ISO by itself initially had no effect (Fig. 7, A and E) and by 30% otherwise (Fig. 7, B and E). The reduced degree of potentiation in slices that showed an increase to the ISO perfusion suggests that this increase might occlude a portion of PTT-LTP. Accordingly, the two regulatory mechanisms may share molecular mechanisms. Importantly, induction of PTT-LTP increased the fEPSP response by only 33% in D36 mice (Fig. 7, C and E). This finding indicates that PTT-LTP is significantly lower in D36 slices when compared with responses in WT slices in which the basal response to ISO was absent, as was the case in D36 slices. Most relevant with respect to the role of AKAP5 in AC targeting is the observation that KO mice did not show any PTT-LTP at all (Fig. 7, D and E). These results indicate that AKAP5-mediated anchoring of not only PKA but also specifically ACs is critical for PTT-LTP.

**Up-regulation of Basal Synaptic Transmission Is Mediated by β2-AR—**We recently demonstrated that PTT-LTP requires the β2-AR but not the β1-AR (29). Given that some recordings showed an increase in basal transmission upon ISO application,

Accordingly, the β1-AR is not enriched and perhaps is even absent in PSDs, in agreement with our recent findings that the β1-AR contributes much less than the β2-AR to postsynaptic signaling if at all (29).

**AKAP5 Is Important for Ser-845 Phosphorylation in the Hippocampus—**For a physiological evaluation of the relevance of AC anchoring by AKAP5, we turned our attention to the hippocampus. ISO treatment increased GluA1 Ser-845 phosphorylation in acute hippocampal slices by 60% (Fig. 6, A (top) and B), which is a much smaller increase than in whole forebrain slices. This much more modest response is in agreement with our earlier observation that ISO increased Ser-845 phosphorylation in hippocampal cultures by 80% (5). It is also consistent with our previous observation that ISO affects basal synaptic transmission much more severely and robustly in the prefrontal cortex than in the hippocampal CA1 area (5). Further,
whereas others did not, we tested whether the difference could be due to differential $\beta_1$ versus $\beta_2$-AR contribution. Although the $\beta_1$-AR appears to be absent from the PSD (Fig. 5A) it could be present and regulate AMPA receptor availability in the perisynaptic space surrounding the PSD. However, the increase in basal response by ISO was completely blocked by a 40 nM concentration of the $\beta_2$-AR-specific antagonist ICI118551 (Fig. 8, A and C) but not by a 1 $\mu M$ concentration of the $\beta_1$-AR-specific antagonist CGP20712 (Fig. 8, B and C). Importantly, we recently confirmed that the CGP20127 batch we used in the current experiments is active (29). The findings that ICI118551 completely blocked the basal ISO effect, whereas CGP20712 had no effect collectively demonstrate that the basal ISO effect is mediated by $\beta_2$- but not $\beta_1$-AR.

**PPT-LTP Requires Activity of GluA2-lacking AMPA Receptors**—Despite the fact that Ser-845 phosphorylation was dramatically reduced in our KO versus WT mice, basal synaptic transmission was normal in 8–12-week-old KO and also D36

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**FIGURE 7.** ISO-induced increases in basal synaptic transmission and PTT-LTP by a 5-Hz/3-min tetanus in the presence of ISO are impaired in AKAP5 D36 and KO mice. A–D, time courses of fEPSPs before and after perfusion with ISO (1 $\mu M$; top gray bar) and delivery of the tetanus (bottom black bar) from recordings without (A) and with (B) an ISO baseline response in WT slice and from D36 (C) and KO (D) slices. Shown are averages of initial slopes of fEPSP starting after the baseline had stabilized. Insets at top, examples of fEPSPs before ISO application (dashed lines), after the start of ISO application (gray lines), and after PTT-LTP induction (solid lines). Graphed are averages of 10 consecutive fEPSPs recorded at the indicated times (arrows), E, summary data of PTT-LTP. The baseline (Bsl) is the average of the fEPSP initial slopes from each individual experiment during the 5 min immediately preceding the start of the ISO application and equaled 100% for each experiment. The 5-Hz/3-min tetanus-induced PTT-LTP in WT group 1 ($p = 0.0002$; t-test), WT group 2 ($p = 0.0464$), and D36 ($p < 0.0001$) but not AKAP5 KO ($p = 0.943$; not depicted in diagram for simplicity). Compared with the interleaved WT recordings, the direct ISO effects as well as PTT-LTP levels were significantly lower for D36 and KO mice. Two-way ANOVA showed a genotype effect ($p < 0.0001$) and treatment effect (PTT-LTP induction) ($p < 0.0001$). ISO and PTT-LTP effects between genotypes are indicated in bar graphs (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Accordingly, PTT-LTP is significantly reduced in D36 versus WT and significantly more reduced (in fact abolished) in KO. Error bars, S.E.
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FIGURE 8. ISO-induced increases in basal synaptic transmission depend on β2-AR but not β1-AR. A and B, time courses of fEPSPs before and after perfusion with ISO (1 μM; bottom gray bar) in the presence of 40 nM ICI118551 (A) or 1 μM CGP20712 (B). Shown are averages of initial slopes of fEPSP starting after the baseline had stabilized. Insets at bottom, example of fEPSPs before (black lines) and after the start of ISO application (gray lines). Graphed are averages of 10 consecutive fEPSPs recorded at the indicated times (arrows). C, summary data. The black line is the average of the fEPSP initial slopes from each individual experiment during the 5 min immediately preceding the start of the ISO application and equals 100% for each experiment. The ISO bars show the increase in fEPSP responses, which were obtained by averaging the initial slope values measured 10–15 min after the onset of ISO perfusion. ISO did not induce any increase in fEPSPs in any of the six slices tested in the presence of ICI118551 (p = 0.3179; t test) but increased fEPSPs in the three slices tested in the presence of CGP20712 (*, p = 0.0297; t test). Error bars, S.E.

Because GluA1 homomeric receptors have four Ser-845 residues for PKA phosphorylation sites rather than two as in GluA1/A2 heteromers, the main AMPA receptor species in CA1 (15), it is possible that Ser-845 phosphorylation has a stronger effect on the homomers than heteromers with respect to their activity-induced postsynaptic targeting. In this way, Ser-845 phosphorylation could help to drive those GluA2-lacking GluA1 homomers under certain forms of synaptic plasticity to postsynaptic sites. Given the congruence in dependence of PTT-LTP on Ser-845 phosphorylation (29) and on AC anchoring by AKAP5 (Fig. 7); given that PTT-LTP strictly depends on activation of AC (and PKA) by β-adrenergic receptors (74); and given that β-adrenergically induced Ser-845 phosphorylation is heavily blunted in AKAP5 KO mice (Figs. 3, A (top) and C and D (left graphs) and 6, A (top) and B), we hypothesized that PTT-LTP depends on GluA1 phosphorylation on Ser-845 because it requires at least temporarily GluA2-lacking AMPA receptors. Such a finding would not only provide further support for our hypothesis that AC anchoring by AKAP5 is important for Ser-845 phosphorylation at postsynaptic sites and thereby for PTT-LTP, but it would also expand our knowledge of molecular details underlying PTT-LTP. We inhibited Ca2+-permeable, GluA2-lacking AMPA receptors with IEM1460, which has higher selectivity for those receptors than alternative inhibitors, such as philanthotoxin-433 (85–87). IEM1460 completely blocked PTT-LTP (Fig. 9, A and D). Because IEM1460 can also inhibit at higher concentrations NMDA receptors and those receptors are thought to contribute to PTT-LTP (74), we tested in parallel whether IEM1460 affected LTP induced by two tetani of 100 Hz/1 s, which depends on NMDA receptors but not on GluA2-lacking AMPA receptor in 8–12-week-old mice (43, 86). IEM1460 had no effect at all on this 2 × 100-Hz/1-s LTP (Fig. 9, B–D), indicating that it did not impair NMDA receptor function in postsynaptic signaling and synaptic plasticity. Accordingly, IEM1460 prevented PTT-LTP by acting on GluA2-lacking AMPA receptors rather than NMDA receptors under our conditions.

DISCUSSION

Importance of AC Anchoring by AKAP5 in Postsynaptic Signaling—Interaction of various ACs with GluA1 has recently been observed (5, 10), but the functional relevance of this interaction has so far not been evaluated. Association with the AKAP5–PKA complex inhibits the activity of some (AC2, AC5, and AC6) but not other AC isoforms (AC1, AC8, and AC9) (10).
Inhibition of AC2, AC5, and AC6 is probably mediated at least in part by negative feedback by PKA on the activity of those ACs (7), yet localization of ACs in close vicinity to PKA-substrate assemblies should augment signaling from AC to PKA via cAMP (12). Whether the interaction of ACs with GluA1 via AKAP5 increases or decreases the AC-PKA signaling in this complex was thus not predictable, although conceptually the former appeared more likely in our view, at least during the initial phase of AC activation. By comparing the effects of AKAP5 D36 deletion with full AKAP5 KO, we find that the loss of PKA plus AC anchoring in the KO mice impairs phosphorylation of GluA1 on Ser-845 (88) and postsynaptic regulation of AMPA receptor activity more severely than loss of PKA anchoring alone in D36 mice. We conclude that AKAP5-mediated docking of both AC and PKA to GluA1 is important for optimal signaling from AC to PKA and ultimately GluA1. Regulation of NMDA receptor phosphorylation on GluN1 Ser-897 by PKA is not affected in either genotype, indicating the importance of highly localized targeting and thereby signaling of both AC and PKA within dendritic spines. Because yotiao has the potential to recruit PKA and AC to NMDA receptors, the nearly complete loss of pan-AC immunoreactivity in PSDs from AKAP5 KO mice is puzzling (Fig. 5, A and B). The same is true for the dramatic loss of PKA from PSDs in D36 mice (43) and might reflect that either yotiao along with the associated AC and PKA is more easily extracted from PSDs than AKAP5 or that the interactions of AC and PKA with yotiao are easier disrupted by Triton X-100, which is required to remove presynaptic elements from the PSDs.

**Differential Effect of AKAP5 D36 versus KO on 100-Hz LTP versus PTT-LTP**—In contrast to WT mice, LTP induced by a single 100-Hz/1-s tetanus is nearly absent in adult D36 mice, and LTD is impaired in 12–14-day-old D36 mice (43, 45, 46). Remarkably, adult AKAP5 KO mice had normal 100-Hz LTP, and postnatal day 12–14 KO mice had normal LTD (but see Ref. 44 for deficits in adult LTD (but not LTP) in another AKAP5 KO mouse) and thus a milder phenotype than the D36 mutation (46). One potential explanation for the fact that D36 but not KO mice have a severe 100-Hz LTP deficit is that in the KO but not D36 mice, another AKAP could fill in for AKAP5 in certain complexes, possibly one that recruits only PKA and not PP2B, causing a shift toward higher phosphorylation. Although there is currently no evidence for such compensation (46, 59), it is difficult to rule this possibility out. In fact, AKAP12 (gravin, AKAP250) has recently been shown to be important for PKA-dependent forms of LTP induced by 100-Hz tetani and thus is a potential candidate that could at least partially compensate for loss of AKAP5 from postsynaptic sites (88). Interestingly, this work shows that AKAP12 is also important for PTT-LTP, although loss of AKAP12 does not affect phosphorylation of GluA1 on Ser-845 (88). Rather the function of AKAP12 in synaptic plasticity (88) appears to be mainly to recruit PKA to the β2-AR to foster its phosphorylation by PKA on Ser-345 and Ser-346 (89–91), which switches the coupling of the β2-AR from Gs/cAMP/PKA to Gq/ERK (92, 93).

Another potential explanation for the finding that D36 but not AKAP5 KO mice are strongly impaired in 100-Hz LTP is that the full KO of AKAP5 eliminated not only PKA but also PP2B anchoring, thereby less severely shifting phosphorylation of certain targets toward dynamic dephosphorylation than the D36 mutation, which preserves PP2B anchoring (46). However, AKAP5 KO but not D36 mice show a strong impairment in Ser-845 phosphorylation. Accordingly, it appears that targets other than Ser-845 may be more strongly affected in D36 versus AKAP5 KO mice, at least under the basal conditions, and their phosphorylations must be important for 100-Hz LTP. Alternative targets that are present at postsynaptic sites or dendrites and require AKAP5-anchored PKA for their regulation are the L-type Ca2+ channel Ca1.2 (3, 40, 59, 94, 95) and the K+ channel K4.2 (96). Phosphorylation of K4.2 on Ser-552 by PKA fosters internalization of K4.2, which in turn increases neuronal excitability (96, 97), thereby potentially fostering 100-Hz LTD. If K4.2 phosphorylation is more substantially decreased in D36 than KO mice, it would augment surface expression of K4.2, thereby reducing excitability and making it more difficult to induce LTP. L-type Ca2+ channels contribute to LTD induced by 200-Hz tetani (98, 99). Ca1.2 channel activity is increased by PKA (71). The reduction in phosphorylation of Ca1.2 by PKA that is observed in D36 mice (59) could thus potentially affect LTP induction by a single 100-Hz tetanus.
although L-type channels are typically not required for LTP induced by several 100-Hz tetani.

The requirement of PTT-LTP for AKAP5 shown here and for Ser-845 phosphorylation described recently (29) clearly differs from the corresponding requirements of 100-Hz LTP. Adult S831/845AA double KI mice are deficient in LTP induced by bursts of 100 Hz (8-burst-LTP) (25), which is similar to regular 100-Hz LTP. However, neither S831A nor S845A single KI mice show this loss in 8-burst-LTP (30), which is analogous to the fact that single 100-Hz LTP is normal in AKAP5 KO mice (46). Accordingly, neither Ser-831 nor Ser-845 phosphorylation is strictly required for LTP induced by high frequency stimuli as long as one or the other site is available. In other words, the presence of Ser-831 as PKC and Ca2

+—calmodulin-dependent kinase II target site and Ser-845 as PKA target site safeguards against impaired phosphorylation of one of the two sites, allowing for 100-Hz LTP even if one of the two sites is unavailable, as in S831A and S845A single KI mice (30), or if PKAanchoring by AKAP5 for effective Ser-845 phosphorylation is abrogated (46). In contrast, PTT-LTP requires Ser-845 phosphorylation (29). Accordingly, Ser-831 phosphorylation cannot substitute for loss of Ser-845 phosphorylation in PTT-LTP, explaining the complete absence of PTT-LTP in AKAP5 KO mice in which Ser-845 phosphorylation is more severely affected than in D36 mice. This finding also indicates that Ser-831 and Ser-845 are not equivalent, although molecular differences in their mechanistic functions might be modest.

AKAP5 anchors not only PKA but also PKC. It is conceivable that loss of PKC anchoring in the KO contributes to the complete abrogation of PTT-LTP, whereas the D36 mutant still can provide PKC targeting, which might be sufficient for a partial PTT-LTP. However, basal Ser-831 phosphorylation was not altered in AKAP5 KO mice, β-adrenergic stimulation did not lead to a significant increase in this phosphorylation, and S845A KI mice show little if any PTT-LTP (29), making a major contribution of Ser-831 phosphorylation by AKAP5-anchored PKC to PTT-LTP unlikely. Nevertheless, these observations do not exclude the possibility that PKC targets other than Ser-831 are involved in PTT-LTP.

Potential Role of AC Anchoring by AKAP5 in Vivo—Norepinephrine fosters arousal and learning, especially under novel and emotionally charged situations via β-adrenergic signaling (27, 100–106). β-Adrenergic signaling facilitates several forms of LTP in the hippocampal dentate gyrus and CA1 region of the hippocampus (27, 29, 74–76, 107, 108). Thus, our findings demonstrating that anchoring of both PKA and AC by AKAP5 is important for β-adrenergic stimulation of Ser-845, a critical PKA site at postsynaptic sites of glutamatergic synapses, and for PTT-LTP implicate AC anchoring by AKAP5 as a relevant molecular component in the regulation of alertness by norepinephrine.

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