NEUROSCIENCE

How Ca^{2+}-permeable AMPA receptors, the kinase PKA, and the phosphatase PP2B are intertwined in synaptic LTP and LTD

Johannes W. Hell*

Both synaptic long-term potentiation (LTP) and long-term depression (LTD) are thought to be critical for memory formation. Dell'Acqua and co-workers now demonstrate that transient postsynaptic incorporation of Ca^{2+}-permeable (CP) α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) is required for LTD in the exemplary hippocampal CA1 region in 2-week-old mice. Mechanistically, LTD depends on AKAP150-anchored protein kinase A (PKA) to promote the initial functional recruitment of CP-AMPARs during LTD induction and on AKAP150-anchored protein phosphatase 2B (PP2B) to trigger their subsequent removal as part of the lasting depression of synaptic transmission.

α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) are ionotropic transmembrane receptors for glutamate that mediate fast synaptic transmission in the central nervous system. AMPARs are formed by four homologous subunits: GluA1 to GluA4. Under basal conditions in wild-type mice, GluA1/GluA2- and GluA2/GluA3-containing AMPARs mediate ~80 and 20%, respectively, of postsynaptic responses in the hippocampus (*1*). These AMPARs are Ca^{2+}-impermeable (CI-AMPARs) because of the presence of GluA2 and show little if any inward rectification.

Adenylyl cyclase and protein kinase A (PKA) are linked to the GluA1 subunit by a complex between synapse-associated protein 97 (SAP97) and the A-kinase–anchoring protein AKAP150 and to the β_2 adrenergic receptor (β_2 AR) by auxiliary γ subunits and postsynaptic density protein 95 (PSD-95) (Fig. 1) to promote efficient and localized up-regulation of AMPAR channel activity by phosphorylating GluA1 on Ser^{845} (*2*–*4*). Phosphorylation of Ser^{845} also augments postsynaptic accumulation of GluA1-containing AMPARs (*2*, *5*, *6*) by promoting their accumulation in the perisynaptic space (*7*) [see also (*8*)]. AMPARs residing there are more immediately available for postsynaptic insertion upon activation of Ca^{2+}-calmodulin–dependent kinase II (CaMKII), which is especially important for postsynaptic recruitment of homomeric GluA1 AMPARs during long-term potentiation (LTP) (*6*).

In their new work, Sanderson *et al*. first evaluated synaptic transmission in mice in which residues 409 to 418 near the C terminus of AKAP150 have been deleted to abrogate the docking of PKA to this site (*9*). Accordingly, these mice (termed ΔPKA mice) have normal excitatory and inhibitory synaptic transmission, including miniature excitatory postsynaptic current (mEPSC) amplitude and frequency (although with a clear tendency to decreased frequency and increased amplitude), and a slightly increased spine density. These findings are by themselves worth noting because similar mice in which the last 36 residues (410 to 445) of AKAP150 are deleted (termed D36 mice) have a substantial increase in both mEPSC and miniature inhibitory postsynaptic current (mIPSC) frequency and spine density during early development (at 2 to 4 weeks old), although these parameters become normal at 8 weeks of age (*10*). The last 18 residues of AKAP150 bind to the distal C terminus of the central, pore-forming $\alpha_1$1.2 subunit of the L-type Ca^{2+} channel Ca_v1.2 (*11*, *12*). Loss of this interaction could affect signaling by this channel, which controls gene transcription (*13*, *14*), neuronal excitability (*15*), certain forms of LTP (*16*, *17*), and metabotropic glutamate receptor (mGluR)– but not *N*-methyl-D-aspartate receptor (NMDAR)–dependent long-term depression (LTD) (*18*). However, AKAP150 also interacts with $\alpha_1$1.2 through two other binding sites (*11*), which appear to be sufficient for stable AKAP150-Ca_v1.2 interaction, although changes in the quaternary structure with some physiological consequences cannot be excluded when binding of AKAP150 to the C terminus of $\alpha_1$1.2 is abrogated. The more extensive phenotype in young D36 compared with ΔPKA mice could be because of such subtle changes or because the more extensive D36 deletion affects other, as of yet unknown, AKAP150 interactions.

Next, Sanderson *et al*. (*9*) refined earlier results (*19*) that in wild-type mice LTP induced by a single 1-s 100-Hz tetanus requires CP-AMPARs at 2 but not 3 weeks of age. Accordingly, the change occurs between P14 and P17. Apparently, even a few days in the development, perhaps even just 1 day, can fundamentally change signaling network properties to profoundly alter synaptic signaling. Thus, careful selection of animal age is clearly critical for defining synaptic signaling mechanisms.

Surprisingly, when testing their ΔPKA mice, the authors find that in these mice LTP is present at 2 weeks. That ΔPKA mice still have LTP at 2 weeks of age is in contrast to what occurs in D36 mice, which lack LTP induced by the same 100 Hz/s stimulus protocol (*19*). As discussed above in regard to the increase in spine density and mEPSC frequency in D36 but not ΔPKA mice, that LTP is absent in D36 mice but present in ΔPKA mice might also be explained by the subtle difference in AKAP150 manipulations. Again, the slightly more extensive deletion of the AKAP150 C terminus in D36 mice may be affecting regulation and functions of Ca_v1.2 or other regulatory mechanisms more than the shorter deletion in ΔPKA mice.

Importantly, the LTP observed in 2-week-old ΔPKA mice is independent of CP-AMPARs, whereas LTP requires CP-AMPARs in 2-week-old wild-type mice (*9*). Apparently, the ΔPKA mice develop compensatory mechanisms that enable them to circumvent the requirement for CP-AMPARs. The seemingly normal basal synaptic transmission but altered LTP mechanisms in ΔPKA mice support my long-standing view [see (*10*, *20*)] that manipulations such as protein knock-down, knock-out, and knock-in of point mutations can change whole signaling networks. Many manipulations that appear to have no effect on basal synaptic transmission can cause covert changes that fundamentally alter regulatory mechanisms like those mediating LTP. The extensive work by Sanderson *et al*. (*9*) is paradigmatic for the

Department of Pharmacology, University of California, Davis, CA 95616–8636, USA.
***Corresponding author: Email: jwhell@ucdavis.edu**

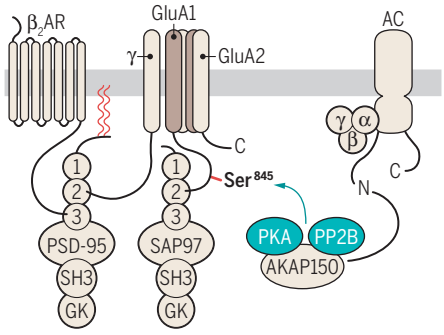

Fig. 1. The AMPAR–β_2 adrenergic receptor signaling complex. The AMPAR GluA1 subunit is phosphorylated on Ser^{845} in its cytosolic C terminus by PKA. For fast, effective, and selective signaling, PKA, its upstream regulator adenylyl cyclase (AC), and its antagonist PP2B are linked to GluA1 via AKAP150 and SAP97. The β_2 adrenergic receptor is connected to GluA1 via PSD-95 and the auxiliary AMPAR subunits known as TARP or γ subunits. How the trimeric G protein—consisting of an α, a β, and a γ subunit—preassociates with AMPARs is not yet known.

notion that even minimal manipulations of proteins can fundamentally modify signaling networks, even when most basal functions appear unchanged.

NMDAR-dependent LTD induced by 900 stimuli at 1 Hz was clearly impaired, although not absent, in 2-week-old ΔPKA mice (*9*). This finding is consistent with the earlier work with D36 mice (*20*). Accordingly, about half of LTD at this age depends on acute signaling by AKAP150-anchored PKA. Previous work indicates that AKAP150-anchored protein phosphatase 2B (PP2B) is important for LTD induced by a 1-Hz stimulus train and for removal of GluA1 from postsynaptic sites in cultured neurons upon chemical induction of LTD with PP2B acting by dephosphorylating GluA1 on Ser^{845} (*21*). In this earlier work, the reduction in electrically induced LTD in mice in which the PP2B binding site on AKAP150 had been eliminated (called ΔPIX mice) was rectified by blocking CP-AMPARs after LTD induction, whereas this treatment had no effect on LTD in wild-type mice (*21*). One possible explanation for all of these findings is that during LTD, CP-AMPARs are temporarily functionally recruited (likely by their transfer from perisynaptic to postsynaptic sites) in a manner that depends on AKAP150-anchored PKA. These CP-AMPARs are then removed within a few minutes by AKAP150-anchored PP2B; this step appears to be impaired in ΔPIX mice. AKAP150-anchored PKA, in turn, likely acts before and during the early phases of LTD induction by phosphorylating Ser^{845}, which promotes perisynaptic GluA1 accumulation for their facilitated postsynaptic targeting (*5*–*8*, *22*). Ser^{845} phosphorylation is likely especially effective in promoting perisynaptic accumulation of GluA1 homomeric AMPARs, which constitute most of the CP-AMPARs in the hippocampus (*23*), because those contain four Ser^{845} phosphorylation sites compared with only two in GluA1/GluA2 heteromeric AMPARs.

The degree of reduction in LTD in ΔPKA mice (*9*) and D36 mice (*20*) is comparable to the degree of reduction by the continued presence of 20 μM 1-naphthyl acetyl spermine (NASPM, a synthetic analog of Joro spider toxin) during and after LTD induction (*9*) and by the presence of PKA inhibitors (*20*). At 20 μM, NASPM inhibits CP-AMPARs but not NMDARs (*9*). Remarkably, wash-out of NASPM leads not only to a complete reversal of LTD but to a potentiation induced under these conditions by the prolonged 1-Hz stimulus train in wild-type mice (*9*). This finding suggests that blocking CP-AMPARs during LTD induction enables postsynaptic insertion of CP-AMPARs that are not removed during the early phases of LTD maintenance when Ca^{2+} influx through these very receptors is blocked. Such Ca^{2+} influx would otherwise activate PP2B via calmodulin to dephosphorylate Ser^{845}, which likely occurs within the nanodomain space surrounding the AMPAR-AKAP150-PKA/PP2B complex (*21*) (Fig. 1). Wash-out of NASPM after LTD induction in wild-type mice then reveals the continued presence of postsynaptic CP-AMPARs under these conditions (*9*). In ΔPKA mice, however, NASPM wash-out had no effect compared with LTD induced in the absence of any NASPM, suggesting that without AKAP150-anchored PKA, recruitment of CP-AMPARs to postsynaptic sites is negligible. The most parsimonious explanation is that in ΔPKA mice, CP-AMPARs do not effectively accumulate in the perisynaptic space. As a result, they cannot be readily recruited to postsynaptic sites during the 1-Hz stimulus train (Fig. 2A).

This molecular mechanism is strongly supported by the fact that in ΔPIX mice, blocking CP-AMPARs with IEM1640 after the 1-Hz stimulus train decreases postsynaptic responses, such that it nearly matches the LTD observed in wild-type mice, in which IEM1640 has no effect after LTD induction (*21*). Apparently, CP-AMPARs are recruited during the 1-Hz stimulus train to replace CI-AMPARs, which must simultaneously undergo removal from postsynaptic sites; otherwise, the overall postsynaptic response would increase. In turn, when NASPM is present during LTD induction in ΔPIX mice, which will inhibit newly recruited CP-AMPARs, NASPM wash-out after LTD induction results in a return of synaptic transmission to that seen in basal, pre-LTD conditions (*9*). This finding can again be explained by the notion that AKAP150-anchored PP2B is required for the removal of CP-AMPARs after LTD induction; wash-out of NASPM unmasks their continued presence in ΔPIX mice. Accordingly, all of these results are best explained by the hypothesis that in wild-type mice, temporary postsynaptic insertion of CP-AMPARs is reversed upon Ca^{2+} influx through the very same CP-AMPARs, which stimulates AKAP150-anchored PP2B to dephosphorylate Ser^{845}, which then promotes the removal of CP-AMPARs (Fig. 2B).

In fact, by using whole-cell voltage-clamp recordings, Sanderson *et al.* (*9*) found that when LTD induced by pairing 1-Hz presynaptic stimulation with postsynaptic depolarization from −65 to −30 mV for 6 min, rectification of AMPAR currents is substantially increased during and immediately after LTD induction. However, the rectification returns to original levels 5 to 10 min after the 1-Hz tetanus. This temporary change in rectification reflects the transient insertion of CP-AMPARs, which are inward-rectifying in contrast to CI-AMPARs. This temporary recruitment of CP-AMPARs in LTD is analogous to LTP in young mice (*24*), except that during LTD the rectification is reversed much faster than during LTP, where CP-AMPARs are present for ~15 min. In ΔPKA mice, the transient increase in rectification during this LTD is much smaller than in wild-type mice, indicating that AKAP150-anchored PKA is important for the temporary insertion of CP-AMPAR at postsynaptic sites. In ΔPIX mice, the rectification is irreversible, reflecting a lack of removal of CP-AMPARs in the later phases of LTD. This lack of reversal is consistent with the above data, indicating that AKAP150-anchored PP2B is required for the removal of CP-AMPARs (Fig. 2B).

These data suggest that PKA and PP2B, which are both linked to AMPARs via AKAP150, antagonize each other such that more prevalent PKA activity during LTP induction enables

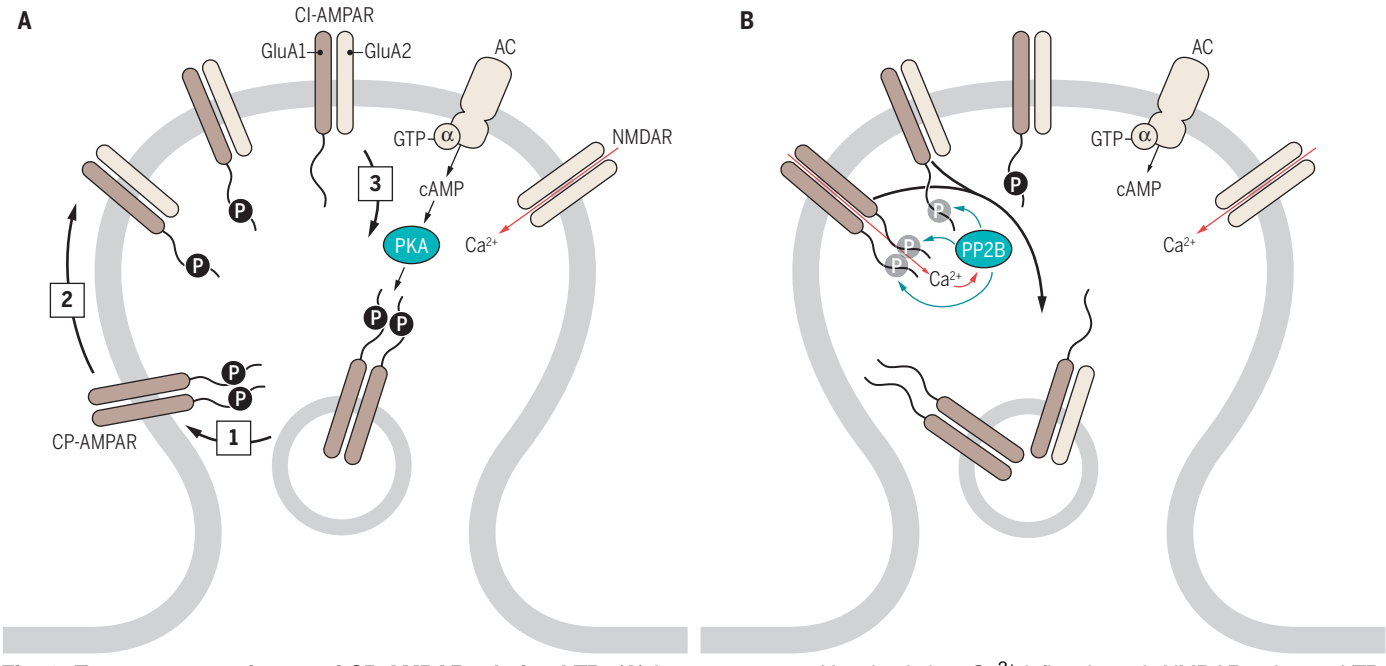

Fig. 2. Temporary recruitment of CP-AMPARs during LTD. (**A**) In response to 1-Hz stimulation, Ca^{2+} influx through NMDARs triggers LTD. Although how precisely Ca^{2+} induces LTD is unknown, given that ~50% of NMDAR-dependent LTD requires PKA, Ca^{2+} might act by activating the Ca^{2+}-sensitive adenylyl cyclases AC1 or AC8. Then, PKA linked to GluA1 via SAP97 and AKAP150 (Fig. 1) phosphorylates GluA1 on Ser^{845}, which promotes the accumulation of GluA1-containing AMPARs and especially of GluA1 homomers at perisynaptic sites (arrow 1). An additional signaling mechanism might involve activation of CaMKII, which then promotes accumulation of GluA1 homomers at postsynaptic sites (arrow 2), possibly via phosphorylation of the C termini of auxiliary γ subunits, which traps AMPARs that include GluA1 homomers at postsynaptic sites [CaMKII and phosphorylation of γ subunits are not depicted in the figure; for details see (*26*)]. In parallel, the 1-Hz stimulation triggers endocytosis of CI-AMPARs (mostly GluA1/GluA2 heteromeric AMPARs; arrow 3), presumably independently of PP2B. (**B**) As CP-AMPARs accumulate during LTD induction, they mediate Ca^{2+} influx, which stimulates PP2B. Like PKA, PP2B is also linked to GluA1 via SAP97 and AKAP150. It dephosphorylates the CP-AMPARs to promote their removal during the later phase of LTD.

potentiation, perhaps in part because CP-AMPARs are functionally available for longer time periods during LTP than they are during LTD induction when PP2B seems to outperform PKA and dephosphorylate Ser^{845} more effectively. As a result, CP-AMPARs are only available for <5 min during LTD induction (*9*), whereas they are present for ~15 min during LTP induction, as shown previously (*24*).

As detailed above, LTD and single-tetanus LTP depend on PKA. Why the two different conditions (long 1-Hz or short 100-Hz stimulus trains) have different outcomes (depression or potentiation) is not clear yet. Perhaps PKA activation might be less effective or shorter lasting or PP2B activation might be longer lasting during LTD than during LTP induction, such that PP2B outpaces Ser^{845} phosphorylation by PKA more effectively during LTD than LTP induction. In particular, one mechanism that may limit the duration of PKA signaling specifically during LTD induction is the PP2B-mediated translocation of the AKAP150-PKA complex from the plasma membrane and uncoupling from the PSD-95- or SAP97-GluA1 complex (*25*). Consequently, Ser^{845} phosphorylation would be shifted toward dephosphorylation during LTD as compared with LTP induction, with such swifter dephosphorylation of Ser^{845} promoting faster removal of CP-AMPARs than during LTP. As a result, CP-AMPARs are functionally available for longer time periods during LTP induction, which might promote LTP over LTD. Further defining the precise roles of PKA, PP2B, and CP-AMPARs in LTP and LTD is an important future quest.

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Johannes W. Hell

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