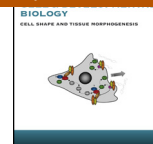




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Review

Subunit-specific trafficking mechanisms regulating the synaptic expression of Ca²⁺-permeable AMPA receptors

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ABSTRACT

AMPA receptors are the main excitatory neurotransmitter receptor in the brain, and hence regulating the number or properties of synaptic AMPA receptors brings about critical changes in synaptic transmission. Synaptic plasticity is thought to underlie learning and memory, and can be brought about by decreasing or increasing the number of AMPA receptors localised to synaptic sites by precisely regulating AMPA receptor trafficking. AMPA receptors are tetrameric assemblies of subunits GluA1–4, and the vast majority are GluA1/2 and GluA2/3 heteromers. The inclusion of GluA2 subunit is critical because it renders the AMPA receptor channel impermeable to Ca²⁺ ions. The vast majority of synaptic AMPA receptors in the brain contain GluA2, but relatively recent discoveries indicate that an increasing number of specific forms of synaptic plasticity involve not only an alteration of the number of synaptic AMPA receptors, but also changes to their GluA2 content. The resulting change in AMPA receptor Ca²⁺ permeability clearly has profound consequences for synaptic transmission and intracellular signalling events. The subunit-specific trafficking mechanisms that cause such changes represent an emerging field of research with implications for an increasing number of physiological or pathological situations, and are the topic of this review.

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Abbreviations: CP-AMPA, Ca²⁺ permeable AMPA receptor; CI-AMPA, Ca²⁺ impermeable AMPA receptor; LTD, long-term depression; LTP, long-term potentiation; VTA, ventral tegmental area; CARP, Ca²⁺-permeable AMPA receptor plasticity; TARP, transmembrane AMPAR regulatory protein; OGD, oxygen and glucose deprivation; NASPM, 1-naphthyl acetyl spermine; TBOA, threo-β-benzyloxyaspartic acid.

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1. Introduction

AMPA receptors mediate the majority of fast synaptic excitation in the brain. Therefore, the precise regulation of AMPA receptor (AMPA) trafficking is crucial to excitatory neurotransmission, synaptic plasticity and the consequent formation of appropriate neural circuits during learning and memory. The mechanisms that underlie AMPA trafficking under basal conditions and during certain forms of synaptic plasticity have been topics of intense research for two decades, and continue to be so [1–4]. In particular, long-term depression (LTD) and long-term potentiation (LTP) at the CA3–CA1 synapse in the hippocampus are the focus of fascinating research programmes from a large number of labs worldwide. LTP and LTD represent an increase or decrease in synaptic strength, respectively and are thought to be the molecular correlates of learning and memory. As well as electrophysiological studies, the quest for molecular cell biological mechanisms for these physiological processes has necessitated the use of dissociated cultures of hippocampal neurons that are amenable to certain imaging techniques that allow the localisation of specific proteins in real time at high resolution. Synaptic phenomena can be modelled in cultured neurons using chemically induced forms of plasticity that trigger receptor trafficking events via relevant signalling pathways. Using the combined approaches of electrophysiology in hippocampal slices and cell biology in dissociated cultures, an impressive amount of mechanistic information has been revealed about these forms of synaptic plasticity. For example, LTD involves dynamin- and clathrin-dependent endocytosis of AMPARs, which are subsequently sorted via specific membrane compartments in the endosomal system through the action of a number of accessory proteins, and may be recycled back to the plasma membrane according to the needs of the synapse in a complex process that we still do not fully understand [5–10]. LTP involves the plasma membrane insertion of additional AMPARs, which originate from the recycling endosomal system [11–13]. Using advanced imaging techniques it is thought that AMPARs are internalised from and inserted into plasma membrane regions away from the synapse, and are transported laterally in the plane of the plasma membrane [14–17]. Hence, as well as vesicle trafficking events, diffusional synaptic trapping represents a further level of regulation of AMPARs at the synapse during plasticity.

Synaptic plasticity has also been studied at a variety of other synapses in the brain, often uncovering striking mechanistic similarities to the hippocampus, but also showing important differences that presumably relate to the specific function of the associated neuronal circuit. General mechanisms of synaptic plasticity will not be described here, although some important examples of CP-AMPA trafficking, which occur at non-hippocampal synapses will be discussed in some detail in later sections.

AMPA receptors are tetrameric assemblies of subunits GluA1–4, and the vast majority of AMPARs in the adult brain contain GluA2, which renders AMPARs Ca^{2+} -impermeable [18–21]. This is essential to maintain an appropriately low cytoplasmic Ca^{2+} concentration under basal levels of stimulation. A small population of GluA2-lacking, Ca^{2+} -permeable (CP-)AMPARs exists, which are not

expressed at the majority of synapses under resting conditions [19,20]. A precise regulation of their synaptic expression can lead to physiologically important synaptic Ca^{2+} signalling events, usually for a restricted and regulated period of time [22,23]. Such events lead to the activation of Ca^{2+} sensitive signalling pathways that may be important for sustaining synaptic potentiation or for regulating the potential for subsequent plasticity. On the other hand, aberrant regulation of these mechanisms, leading to a prolonged synaptic incorporation of CP-AMPA receptors in cells that do not normally express synaptic CP-AMPA receptors, can result in excessive Ca^{2+} influx leading to synaptic dysfunction and cell death (excitotoxicity) in a number of diseases including brain ischaemia [24,25].

The focus of this review is to evaluate the current knowledge about the trafficking mechanisms and associated signalling pathways involved in regulating the synaptic expression of CP-AMPA receptors. To put these mechanisms in context, I will introduce some examples of physiological and pathological situations that all involve CP-AMPA trafficking, but may have subtle mechanistic differences. I will then discuss the source of CP-AMPA receptors, how they are trafficked to the synapse, the upstream signalling pathways and the subunit-specific interacting proteins that regulate these events. Finally, since Ca^{2+} influx must be tightly regulated, the duration of synaptic CP-AMPA expression and mechanisms that underlie the reversal of this process will also be considered.

2. Physiological paradigms involving the expression of CP-AMPA receptors at synapses

2.1. Hippocampal LTP

The CA3–CA1 synapse is the most-studied synapse in the mammalian brain, and it is widely accepted that a major component to the mechanism is the incorporation of additional AMPARs into the postsynaptic plasma membrane, which is largely driven by GluA1 subunit [11,26,27]. However, despite many years of extremely active research into LTP mechanisms, a role for CP-AMPA receptors was overlooked. In 2006, Isaac and colleagues reported the presence of CP-AMPA receptors at synapses within 3 min after a pairing induction protocol, which lasted for just 20 min [28]. Synaptic CP-AMPA receptors are needed for the initial expression, but not for the subsequent maintenance of LTP. Studies from other labs have not always reported similar findings [29], but more extensive investigations have revealed that developmental stage [30] and stimulation protocol [31] may be important factors, and another study suggested a shorter period (10 min) for the duration of CP-AMPA synaptic expression [32].

2.2. Fear conditioning

Potentiation at glutamatergic synapses in the lateral amygdala is central to the formation of fear memory, which is usually induced by pairing an auditory tone (conditioned stimulus) with a foot shock (unconditioned stimulus). Fear, manifested as a “freezing” response, can then be elicited in response to the conditioned stimulus alone [33,34]. As part of this potentiation, synaptic expression of

CP-AMPA, although undetectable at 2 h post-learning, gradually increases over 24 h, and then decreases at a similar rate [35]. The overall potentiation at the synapse and the fear memory remain. Hence, similarities exist with CA1 synapses, but the time course is dramatically extended. Indeed, the relatively slow time course does lead to the question of whether the regulation of trafficking fully accounts for this change. By repeated exposure of the conditioned stimulus without the foot shock, the fear response can be abolished in the process of fear extinction [34]. It has been suggested that fear extinction requires CP-AMPA expression, and hence that the presence of CP-AMPA at a synapse renders it labile [35].

2.3. Cocaine addiction

Drug addiction evidently involves long-lasting effects on brain circuitry [36,37]. A single cocaine injection can induce a potentiation of synaptic transmission at glutamatergic synapses on dopaminergic neurons in the ventral tegmental area (VTA) at approximately 3 h after drug injection, mediated by the incorporation of CP-AMPA [38–40]. The potentiation appears to be explained purely by the increased conductance of CP-AMPA, and not by an increase in AMPAR number [38], suggesting that CI-AMPA is replaced by CP-AMPA rather than an insertion of CP-AMPA on top of the existing receptor complement. Furthermore, this form of plasticity is very long lasting, persisting for around 1 week after drug injection [41], and it is thought to be mediated by the partial switch to CP-AMPA for this duration. The nucleus accumbens is another midbrain region involved in drug addiction, and CP-AMPA is expressed on GABAergic medium spiny neurons of this structure in response to extended access cocaine self-administration, but in this case taking around a month to develop [42].

2.4. Acute neuronal injury

Unregulated calcium influx through CP-AMPA is thought to underlie neuronal dysfunction and death in a number of diseases [24,25,43]. In some cases this is a chronic change involving altered gene expression, the detailed mechanisms of which are outside the scope of this review. Rapid changes in subunit composition of synaptic AMPA are also seen following acute neuronal insults such as oxygen and glucose deprivation (OGD), which is an *in vitro* model for ischaemia [44,45], and mechanical stretch, which is used to model traumatic brain injury [46,47]. The trafficking mechanisms that underlie these changes appear to differ according to neuronal type (our unpublished observations), but in hippocampal neurons, a large and sustained decrease in surface GluA2 is observed in response to both OGD and mechanical stretch, which ultimately results in neuronal death [44–47].

2.5. Cerebellar granule cell-stellate cell plasticity

The examples described above are all instances in which synapses express almost exclusively GluA2-containing CI-AMPA under basal conditions. The switch towards CP-AMPA occurs in response to a specific form of physiological or pathological stimulus. In contrast, cerebellar granule cell-stellate cell synapses express CP-AMPA under resting conditions, and high frequency stimulation of the granule cell parallel fibres induces a rapid switch towards CI-AMPA [48]. This process has also been coined Ca^{2+} -permeable AMPA receptor plasticity (CARP) [49].

3. Where do CP-AMPA come from, and how do they get to the synapse?

The disassembly of existing AMPAR tetramers followed by reassembly in different subunit combinations would be an energetically unfeasible event, and no mechanism to achieve this feat has been reported. A rapid switch in AMPAR subunit composition from GluA2-containing to GluA2-lacking AMPA therefore implies that CP-AMPA exist before the relevant stimulus, but are localised away from the synapse, either at perisynaptic sites, or at intracellular membrane compartments, most likely endosomes. Identifying AMPA complexes that are not expressed on the neuronal surface is challenging, because they are unavailable for electrophysiological detection. However, biochemical analysis of tissue extracts from CA1 and CA2 hippocampus estimated that around 8% of AMPA are GluA1 homomers, with a very small population of GluA1/GluA3 heteromers [19].

3.1. Perisynaptic regions

CP-AMPA have been detected at extrasynaptic sites following puff-application of AMPA to distal dendrites of hippocampal neurons under basal conditions [31]. Moreover, CP-AMPA have also been detected at perisynaptic sites, i.e. immediately adjacent to the PSD on the spine head [50]. Perisynaptic AMPA are not stimulated by presynaptic glutamate release under physiological conditions because glutamate transporters ensure efficient clearance of neurotransmitter from the synaptic cleft, but pharmacological blockade of transporters with TBOA allows glutamate spill over, and hence stimulation of perisynaptic receptors [50,51]. Using this approach, philanthotoxin-sensitive CP-AMPA currents have been detected at perisynaptic sites on hippocampal CA1 neurons prior to LTP induction [50]. Although lateral movement from perisynaptic to synaptic regions specifically of endogenous CP-AMPA has not been directly visualised, this would provide an efficient mechanism for the rapid addition of CP-AMPA to the synapse. Mechanisms for regulating such a lateral movement have not been elucidated, but PKC activity may be important in mediating the translocation of perisynaptic CP-AMPA to the synapse in the first few minutes after LTP induction [51]. The substrate for PKC in this process is unclear. It is possible that transmembrane AMPA regulatory proteins (TARPs) play a role in this process, since they have been shown to regulate AMPA lateral movement by trapping AMPA at postsynaptic sites via their interaction with PSD95 [52]. It has recently been suggested that specific TARP family members preferentially regulate CP-AMPA in cerebellar granule cells [53], although lateral diffusion has not been explored in this particular case.

3.2. Endosomes

AMPA are known to undergo constitutive cycling. That is, they are internalised from the plasma membrane by endocytosis and enter the endosomal system from where they are recycled back to the cell surface [1–3]. This multi-compartment system provides multiple points where trafficking can be regulated. For example, AMPA endocytosis is enhanced during chemically-induced (c)LTD [54], and receptors are targeted to late endosomes and then lysosomes instead of recycling endosomes [55]. Conversely, during LTP, recycling endosomes are physically manoeuvred into dendritic spines to enhance AMPA delivery to the synapse [56,57].

The specific membrane compartments involved in the surface delivery and synaptic incorporation of CP-AMPA have not been extensively studied. We recently reported mechanisms involved in endogenous subunit-specific AMPA trafficking during

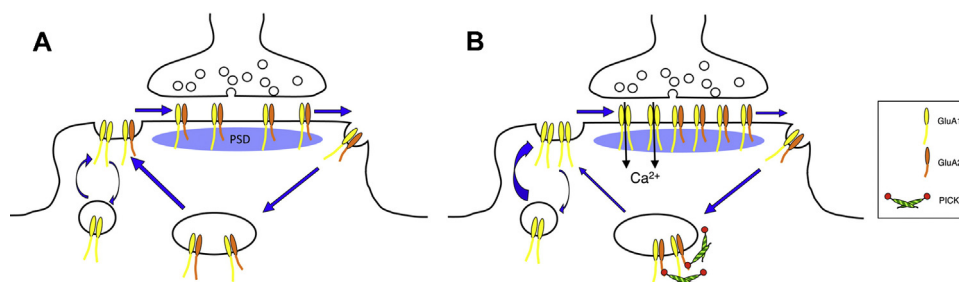


Fig. 1. Trafficking mechanism for the induction of CP-AMPA expression at synapses. This model is based primarily on data acquired for LTP in hippocampal neurons. Other examples such as cocaine exposure in the VTA or fear conditioning in the lateral amygdala may share similar mechanisms. (A) Under basal conditions, CP-AMPA receptors are not expressed at synapses, but are found in endosomal compartments and at perisynaptic sites. Both CP- and CI-AMPA receptors undergo constitutive endocytosis and recycling, but CP-AMPA receptors are restricted from entering the PSD. It is unknown whether CP- and CI-AMPA receptors traffic through identical or distinct endosomal compartments, but they are shown as distinct in this diagram, for clarity. (B) Following stimulation, PICK1 associates with CI-AMPA receptors on endosomes, restricting their recycling. A greater number of CP-AMPA receptors therefore traffic to the cell surface via the recycling pathway, and these receptors also move laterally from perisynaptic sites to the postsynaptic region.

glycine-induced chemical (c)LTP, suggesting that GluA2-containing AMPARs are held up in the recycling system in the first 10 min after stimulation, while the trafficking of GluA1 to the cell surface (in the absence of GluA2) is enhanced [58]. This mechanism exploits the constitutive recycling pathway, and implies that CP-AMPA receptors are available in the recycling compartment at the time of stimulation. Perhaps a small number of CP-AMPA receptors are trafficked through the recycling endosomal pathway alongside GluA2-containing receptors, but they lack a crucial signal to allow synaptic trapping, and hence are restricted to perisynaptic regions on the plasma membrane until the LTP stimulus. In this hypothetical model, LTP induction enhances the trafficking of CP-AMPA receptors to the surface while restricting CI-AMPA receptor traffic (see Section 5.1), and also enhances lateral movement of CP-AMPA receptors to the postsynaptic site (or enhances synaptic trapping). This would be in agreement with other studies demonstrating increased surface delivery of GluA1 via recycling endosomes during chemical LTP [12,57]. The reports from Ehlers and colleagues actually suggest an overall increase in endosomal recycling in response to chemical LTP induction, rather than a specific effect on GluA1 [12,57]. This further emphasizes the importance of restricted GluA2 trafficking as a key component causing an increase in surface expression of CP-AMPA receptors. This model is represented in Fig. 1.

The above represents a mechanism for synaptic potentiation, i.e. the total number of AMPARs at the synapse increases. In contrast, in response to OGD, hippocampal neurons exhibit a loss of surface GluA2, with no net change in GluA1 or GluA3 surface expression [45]. Since the only net change following OGD is reduced surface GluA2, there must be an overall reduction in surface AMPAR number. Functionally, this slight reduction in receptor number is largely counter-balanced by the higher conductance of CP-AMPA receptors compared to CI-AMPA receptors. It has been suggested that this subunit switch occurs via endocytosis of GluA1, GluA2 and GluA3, presumably as GluA1/2 and GluA2/3 heteromers, which is accompanied by the plasma membrane insertion of GluA1 and GluA3 [44]. An important unanswered question therefore is, what happens to GluA2-containing receptors that are internalised and not recycled? By analogy with the mechanism described above for cLTP, a likely explanation is that GluA2 recycling is restricted at endosomal compartments. The mechanism that underlies the increase in GluA1 insertion at the plasma membrane to balance the GluA1 internalised in complex with GluA2 is unknown, but might also have aspects in common with cLTP.

In other systems, similar mechanisms are likely to be involved. Mechanical stretch injury increases GluA2 internalisation in cortical neurons, leading to the synaptic expression of NASPM-sensitive CP-AMPA receptors [47]. The trafficking of other AMPAR subunits was not tested in this study. Suggesting a similar mechanism, cocaine

exposure in the VTA causes a redistribution of GluA2 from the synaptic plasma membrane to intracellular compartments [59]. In neither case have the endosomal sorting mechanisms been further investigated.

These observations point towards two categories of process for the synaptic expression of CP-AMPA receptors. One involves the addition of CP-AMPA receptors to the existing complement of CI-AMPA receptors at the synapse so that the total number of receptors is enhanced, ultimately leading to a stably potentiated synapse. Alternatively, CP-AMPA receptors replace CI-AMPA receptors, through the removal of GluA2-containing CI-AMPA receptors at the same time as the addition of CP-AMPA receptors. Although this results in no increase in total receptor number, it can still lead to synaptic potentiation, because of the higher conductance of CP-AMPA receptors.

4. Upstream signalling events leading to CP-AMPA expression

The signalling mechanisms that regulate GluA1 synaptic incorporation during LTP have been the subject of extensive research. A very large number of studies have unveiled a lot of information about these processes using tagged recombinant GluA1, which is thought to form homomeric receptor complexes when overexpressed in neurons. Since CP-AMPA receptors inserted immediately after LTP induction are likely to be predominantly GluA1 homomers [19,58], the mechanisms defined in these studies may apply to CP-AMPA receptor trafficking during LTP expression. However, only mechanisms that have specifically been shown to control endogenous CP-AMPA receptor expression will be discussed here.

4.1. GluA1 phosphorylation in hippocampal LTP

GluA1 is phosphorylated at two serine residues, S831 and S845, both of which are involved in AMPAR regulation during LTD/LTP. S831 is phosphorylated by CaMKII or protein kinase C (PKC), while S845 is phosphorylated by cAMP-dependent protein kinase (PKA) [60]. Increased phosphorylation at either of these sites potentiates AMPAR function, by regulating both trafficking and channel conductance. LTP is associated with an increase in GluA1 phosphorylation [61,62], and LTD with GluA1 dephosphorylation via the action of phosphatases [62,63].

CP-AMPA receptors are proposed to be inserted perisynaptically upon LTP induction, and subsequently move laterally to the synapse [51]. In neurons from mice with targeted mutations in the GluA1 S845 phosphorylation site (GluA1-S845A mutants), CP-AMPA receptors are absent from perisynaptic sites [50], suggesting that phosphorylation of GluA1 at S845 maintains the perisynaptic pool of CP-AMPA receptors. These non-phosphorylated receptors are targeted

for lysosomal degradation, indicating that an absence of S845 phosphorylation leads to endocytosis and subsequent endosomal sorting to lysosomes. AKAP150 is a postsynaptic scaffold protein that anchors PKA and calcineurin to enhance regulation of their synaptic substrates, for example regulating AMPAR activity and trafficking via phosphorylation [64]. A role for AKAP150-anchored PKA and calcineurin to regulate the phosphorylation of S845 has been shown to control CP-AMPA expression at synapses during LTP [65]. Sanderson et al. generated knock-in mice with an AKAP150 mutation that selectively abolishes regulation by the phosphatase calcineurin, and therefore increases S845 phosphorylation of GluA1. Neurons from these mice show an increase in synaptic expression of CP-AMPA and enhanced LTP [65]. Taken together, these observations suggest that S845 phosphorylation is predominantly involved in promoting the surface expression of CP-AMPA, which may initially be perisynaptic, hence making them available for lateral movement to the synapse.

4.2. *GluA1 phosphorylation in fear conditioning*

The importance of S845 is not restricted to hippocampal neurons. At glutamatergic synapses in the lateral amygdala, synaptic expression of CP-AMPA during fear conditioning is abolished in S845Ala mutant mice, but unaffected by an equivalent mutation at S831 [35]. This demonstrates specificity for the PKA site (S845), and also indicates that the PKC/CaMKII site (S831) is not important for CP-AMPA trafficking. Interestingly, although CP-AMPA expression is blocked in the lateral amygdala of S845A mice, the synapses are nevertheless potentiated by fear conditioning, indicating that CP-AMPA expression is not a prerequisite for maintenance of this form of plasticity [35].

4.3. *The role of CaM kinases*

CaMKII is well established as a kinase that is crucial for LTP expression by phosphorylating GluA1, leading to increased AMPAR channel conductance. CaMKII activity also enhances GluA1-dependent trafficking to the synapse [66]. CaMKI is a related protein that is much less studied in synaptic plasticity, however Guire et al. demonstrated that a theta burst protocol, but not a high frequency tetanus, leads to rapid and transient synaptic expression of CP-AMPA that is blocked by pharmacological inhibition of CaM kinase kinase (CaMKK), the upstream activator of CaMKI [31]. This suggests a role for CaMKI in the synaptic recruitment of CP-AMPA, which was further supported by the observation that infusion of active CaMKI into hippocampal neurons is sufficient to cause a potentiation of AMPAR EPSCs, which are sensitive to the CP-AMPA specific blocker IEM-1460 [31]. CaMKI has been shown to modulate intracellular signalling pathways involving the small GTPase Rac and the RacGEF β PIX in the regulation of actin polymerisation [67]. The increased synaptic expression of CP-AMPA induced by active CaMKI infusion is abolished by application of the actin polymerisation inhibitor latrunculin A, indicating that actin polymerisation is required for CP-AMPA expression at synapses following CaMKI activation [31]. Further details of how the actin cytoskeleton regulates CP-AMPA trafficking are still lacking, and it will be interesting to investigate how such mechanisms differ from those that regulate GluA2-containing AMPARs.

5. AMPAR accessory proteins that regulate CP-AMPA synaptic expression

Subunit-specific regulation of AMPAR trafficking requires a distinct set of processes to be directed at specific subunits. This could be via distinct post-translational modifications such as phosphorylation and/or through the specific binding of accessory proteins. For

example, a mechanism is required to restrict the traffic of GluA2, but not GluA1, through recycling compartments following LTP induction or exposure to OGD [44,58]. Investigations into mechanisms that are relevant specifically to the trafficking of CP-AMPA have therefore focussed mainly on GluA2-binding proteins.

5.1. *PICK1*

Protein Interacting with C-Kinase 1 (PICK1) is a Ca^{2+} sensing, PDZ and BAR domain protein that binds, via the PDZ domain, to GluA2 and GluA3 subunits, but not GluA1 [68]. An early study of PICK1 function in hippocampal neurons demonstrated that PICK1 overexpression causes the surface and synaptic expression of CP-AMPA by reducing surface levels of GluA2 [69]. It was also shown that PICK1 knockdown blocks CA3–CA1 LTP [70], and also that disrupting PICK1 PDZ domain interactions with interfering peptides prevents OGD-induced loss of surface GluA2 in hippocampal neurons and reduces subsequent cell death [45]. Whether PICK1 promotes GluA2 endocytosis or restricts its recycling (or both) is still not entirely resolved, although the majority of published evidence points towards a role in recycling [58,71,72]. In response to chemical LTP induction, PICK1 rapidly translocates to endosomal compartments, with colocalisation reaching a peak at 5 min after stimulus, followed by a gradual dissociation. Furthermore, the colocalisation of GluA2 with PICK1 on endosomes, and the biochemical association of PICK1 with GluA2 are transiently enhanced immediately after stimulus [58]. These observations demonstrate that PICK1 is well placed to restrict GluA2 trafficking through recycling endosomes during LTP (Fig. 1). PICK1 also plays an important role in the subsequent release of GluA2 to reset potentiated synapses to their normal subunit complement (see later section).

The precise function of PICK1 in the surface and synaptic expression of CP-AMPA during OGD is unclear, but since GluA1–3 are all internalised by endocytosis, and only GluA1 and GluA3 are recycled to the plasma membrane [44], it is likely that PICK1 plays a similar role in restricting GluA2 traffic at recycling endosomes as described above for cLTP [58]. Since OGD also causes AMPAR endocytosis, there remains the possibility that PICK1 is also involved in this trafficking event, however this has not been directly tested. Since PICK1 can physically bind both GluA2 and GluA3, it is unclear whether PICK1 discriminates between GluA2 and GluA3 in the subunit-specific trafficking processes described, and if so, how this might be achieved.

PICK1 has also been implicated in subunit-specific trafficking events in response to cocaine administration. TAT-tagged peptides that occupy the PICK1 PDZ domain block the rectification change associated with synaptic CP-AMPA expression in dopaminergic VTA neurons [38]. This observation is consistent with a mechanism involving PICK1-mediated restrictions on GluA2 recycling similar to that described above, but further work is needed to confirm this. Furthermore, disrupting PICK1 PDZ domain interactions with interfering peptides at synapses in the lateral amygdala causes a reduction in AMPAR rectification, and genetic deletion of PICK1 has the same effect [73]. At the time of writing, a role for PICK1 has not been directly tested in the fear conditioning model *in vivo*, but these results suggest that PICK1 could play a pivotal role in this process by restricting the traffic of GluA2 to the synapse and therefore establishing a synaptic population of CP-AMPA.

PICK1 has not only been implicated in the switch towards CP-AMPA expression at synapses as described above, but also in the reverse process, whereby CP-AMPA are replaced by CI-AMPA. The switch from CP- to CI-AMPA at cerebellar granule cell-stellate cell synapses is PICK1-dependent, with the forward traffic of CI-AMPA thought to be mediated by the interaction of PICK1 with GluA2 [49]. This proposed role in promoting the synaptic expression of CI-AMPA in stellate cells is in contrast to its role in

hippocampal neurons, where PICK1 is involved in restricting or removing GluA2 from the synapse [58]. Although the CP-AMPA receptors in stellate cells consist predominantly of homomeric GluA3 [74], which is also a PICK1 binding partner, the experimental evidence available suggest that PICK1 is not involved in regulating the trafficking of these receptors. In the absence of a representative cell culture model, imaging studies have not been carried out on this system, so the cell biology is not well characterised.

5.2. GRIP

Glutamate receptor interacting protein (GRIP) is the representative member of a family of multi-PDZ domain proteins encoded by two separate genes, with multiple splice forms [75,76]. It binds, via specific PDZ domains, to the same C-terminal site on GluA2 and GluA3 subunits as PICK1, hence GRIP and PICK1 compete for binding to AMPAR subunits. However, PICK1 also interacts directly with GRIP via non-PDZ regions [77]. A function for GRIP in regulating GluA2-specific trafficking is poorly defined compared to PICK1, although it has been implicated in the switch from CP- to CI-AMPA receptors in cerebellar stellate cells. Experiments using interfering peptides to differentially disrupt GRIP and PICK1 PDZ domains from their AMPAR subunit binding partners suggested that GRIP, and not PICK1, is responsible for maintaining the synaptic population of CP-AMPA receptors, which are mainly GluA3 homomers [78]. In the same study, it was suggested that dissociation of GRIP-AMPA interactions underlie the activity-dependent loss of CP-AMPA receptors from synapses during this form of plasticity. Since a proportion of AMPARs at stellate cell synapses contain GluA2/GluA3 heteromers [74], which are apparently unaffected by stimulation, this mechanism requires a differential regulation of GRIP-GluA2/3 and GRIP-GluA3 interactions. Either synaptic GluA2/3 heteromers remain bound to GRIP during stimulation, or they do not bind GRIP at all. Further work is needed to fully define this mechanism.

5.3. NSF

N-Ethylmaleimide sensitive fusion protein (NSF) is a hexameric ATPase classically involved in membrane fusion events by regulating SNARE complex disassembly. It has also been shown to promote the dissociation of PICK1 from GluA2 via a related mechanism [79], and it is involved in either stabilising GluA2-containing AMPARs at the cell surface, or enhancing the addition of GluA2 at the synapse in hippocampal neurons [80–83]. NSF has been implicated in the switch from CP- to CI-AMPA receptors in hippocampal CA1 neurons after LTP induction in a study that employed peptides to block the GluA2 binding site on NSF [51]. Infusion of the interfering peptide has no effect on LTP induction per se, but LTP induced under these conditions is largely mediated by CP-AMPA receptors even 1 h post-induction. The precise trafficking mechanism that underlies the role of NSF in this process is unknown. Since PICK1 restricts GluA2 from the synapse immediately after chemical LTP induction, and dissociation of GluA2 from PICK1 coincides with GluA2 synaptic delivery [58], these observations are consistent with a model in which NSF contributes to the release of GluA2 from PICK1 [79], allowing traffic of CI-AMPA receptors to the synaptic plasma membrane.

An *in vivo* behavioural study employed the same peptides to investigate a role for NSF–GluA2 interactions in fear conditioning. Long-term fear memory, but not short-term memory was significantly impaired, suggesting a role for NSF binding to GluA2 in the consolidation of fear memory [84]. Electrophysiological analyses were not performed in this study, so specific information about the synaptic expression of CP-AMPA receptors is not available. However, the results are consistent with the model put forward by Clem et al. in which GluA2-containing CI-AMPA receptors replace CP-AMPA receptors during memory consolidation [35]. NSF might therefore play a role

in allowing the forward traffic of CI-AMPA receptors to the synapse, or in subsequently maintaining their synaptic expression.

5.4. TARPs

TARPs are key determinants of AMPAR function, with effects on both localisation and channel characteristics. TARPs form very tight physical interactions with AMPARs, and it is thought that they associate with most, if not all, synaptic AMPARs. They are therefore often referred to as auxiliary AMPAR subunits [85,86]. A role for TARPs in the regulation of CP-AMPA receptor trafficking was first suggested following the observation that cerebellar stellate cell synapses of stargazer mice, which lack the prototypical TARP stargazin ($\gamma 2$) [87], express a greater proportion of CP-AMPA receptors compared to wild-type mice [88]. This suggested that CP- and CI-AMPA receptors could be differentially regulated by TARPs. TARP $\gamma 7$ was later found to selectively enhance the synaptic expression of CP-AMPA receptors, and also to reduce CI-AMPA receptor expression [53]. These results lead to the possibility that TARPs could play a role in stellate cell CP-AMPA receptor plasticity in these cells. The mode of regulation, for example vesicle traffic or lateral plasma membrane movement, and also whether specific TARPs regulate trafficking of CP- vs CI-AMPA receptors in other cell types is unknown.

5.5. GluA1 interacting proteins

Synaptic incorporation of GluA1-containing receptors is the predominant mechanism for CP-AMPA receptor expression in response to hippocampal LTP induction and fear conditioning plasticity in the lateral amygdala. A number of GluA1 accessory proteins have been reported, which regulate GluA1 trafficking to the surface or incorporation at synapses [57,89,90]. While these have not been implicated in CP-AMPA receptor trafficking per se, some have been shown to be involved in GluA1 trafficking events associated with LTP, and are therefore likely to regulate the synaptic expression of CP-AMPA receptors during this process. For example, the actin-based motor protein myosin Va also binds GluA1 directly, and is required for GluA1 synaptic delivery during LTP [89]. Further work is needed to determine whether such protein interactions are required for the synaptic expression of endogenous CP-AMPA receptors.

6. Reversal of CP-AMPA receptor synaptic expression

6.1. Hippocampal LTP

In some cases, CP-AMPA receptor expression is inherently transient, for example in hippocampal neurons during early LTP expression, they are expressed in detectable amounts only for 10–20 min [28,31,51,58]. Interestingly, the stimulus to reset the synapse back to its normal complement of GluA2-containing receptors has been suggested to come from the CP-AMPA receptors themselves. The increase in CP-AMPA receptor number during the first few minutes after induction leads to the dissociation of PICK1 from GluA2, presumably caused by the elevated Ca^{2+} influx through these newly incorporated receptors [58]. Hence, PICK1 is involved in both the retention of GluA2 away from the cell surface, and the subsequent release of GluA2 to the synaptic plasma membrane. These observations may correspond to the biphasic sensitivity of PICK1 to $[\text{Ca}^{2+}]$, whereby a moderate increase in $[\text{Ca}^{2+}]$ causes an increase in GluA2 binding, and a further increase in $[\text{Ca}^{2+}]$ weakens the interaction [91]. Whether the CI-AMPA receptors displace CP-AMPA receptors simply because they are in greater supply, or there is a specific process for the removal of CP-AMPA receptors is unknown. Importantly, if synaptic activity is blocked immediately after LTP induction while CP-AMPA receptors are functionally expressed at the potentiated synapse, the switch back to CI-AMPA receptors is abolished, supporting a role for CP-AMPA

activation in this process [51]. This provides a self-limiting mechanism, whereby the build-up of CP-AMPA stimulates their own removal, thus ensuring a restricted period of CP-AMPA expression at the synapse. Further work will be needed to investigate whether the duration of CP-AMPA expression can be modulated according to the specific requirements of the synapse.

6.2. Fear conditioning

CP-AMPA expression following fear conditioning at synapses in the lateral amygdala is also transient, but over a much longer time course. Although the mechanism behind this more protracted transience is unclear, signalling components have been identified that lead to a switch from synaptic CP-AMPA to CI-AMPA in a process that underlies fear extinction. Activation of metabotropic glutamate receptor mGluR1 is required for a form of LTD that specifically removes CP-AMPA from synapses, and in so doing, causes the erasure of the fear memory [35]. Fear extinction is therefore restricted to the time period during which CP-AMPA is expressed at synapses. Surprisingly, a peptide characterised as an inhibitor of GluA2 endocytosis blocks this plasticity, which presumably involves the internalisation of AMPARs that lack GluA2 [73]. Further work is therefore needed to clarify the mechanism involved.

6.3. Other processes

mGluR1 activation as a mechanism to remove CP-AMPA from synapses is common to other systems. For example, dopaminergic neurons of the VTA express an LTD of CP-AMPA that is induced by mGluR1 agonists *in vitro* or an mGluR1 positive allosteric modulator *in vivo* [38]. The same group later presented evidence to suggest that the GluA2 subunit that replaces the internalised CP-AMPA is rapidly synthesised in response to mGluR1 activation via the mTOR pathway [59]. Further details of the mechanism regulating GluA2 synthesis and subsequent synaptic incorporation are still lacking.

Another important feature of the VTA synapse is that cocaine-induced potentiation (and by inference, CP-AMPA expression) appears to be maintained for at least one week, rather than returning to baseline at some later time point [41]. It has not been directly demonstrated that this potentiation continues to be mediated by CP-AMPA, but since the total number of AMPARs is thought to be unchanged, then the persisting potentiation is presumably still mediated by the higher conductance of CP- compared to CI-AMPA [38]. In the case of OGD, cell viability is reduced 24 h after insult in cultured neurons [44,45], making it difficult to assess the surface or synaptic expression of CP-AMPA at later time points. In addition, GluA2 mRNA levels start to decrease 6 h after ischaemic insult *in vivo* [92], which contributes to CP-AMPA expression after this time. Therefore, although subunit-specific trafficking initiates the switch to CP-AMPA following OGD/ischaemia, the switch is largely maintained by changes in GluA2 gene expression. A similar mechanism could underlie the persistence of cocaine-induced plasticity in the VTA, although results have not supported this [93]. If the animal self-administers the drug, the potentiation persists for a month or even longer. Under these conditions, changes in GluA1 and GluA2 mRNA expression have been reported [94].

7. Conclusion

While CP-AMPA have been implicated in pathological processes such as ischaemia for many years [95], a role in “normal” physiological memory processes is only recently becoming accepted, and CP-AMPA are now emerging as an important additional feature of various forms of synaptic plasticity. This

has coincided with the observation that the subunit composition of synaptic AMPARs can change quite rapidly as a result of subunit-specific trafficking. Compared to the wealth of knowledge about AMPAR trafficking in general, little is known about the specific mechanisms that regulate the synaptic incorporation of CP-AMPA. As discussed above, GluA1-dependent mechanisms already defined as being central to LTP expression, but previously thought to apply predominantly to GluA1/GluA2 heteromers, may be synonymous with CP-AMPA trafficking immediately after LTP induction, and possibly additional forms of plasticity that involve CP-AMPA insertion as well. GluA2 internalisation that takes place as a component of CP-AMPA expression may share mechanistic details with LTD induction (which does not involve synaptic CP-AMPA expression). It will be important to see how signalling pathways upstream of AMPAR subunits and their accessory proteins are specific to CP-AMPA expression. Elucidating trafficking details relies on a valid cell culture system that can be used for high-resolution imaging in conjunction with acute genetic manipulations. While such a system is clearly available for hippocampal neurons, the study of some other neuronal types lag behind in this respect. However, it is already evident that many similarities exist between hippocampal neurons, VTA and lateral amygdala.

CP-AMPA trafficking has now been implicated in brain ischaemia, traumatic brain injury, drug addiction and fear memory and further examples will undoubtedly be revealed in the coming years. A full understanding of the mechanisms that underlie these changes at synapses is therefore crucial, and might uncover potential therapeutic targets.

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