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REVIEW

Probing the role of AMPAR endocytosis and long-term depression in behavioural sensitization: relevance to treatment of brain disorders, including drug addiction

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Modifying the function of postsynaptic α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid subtype glutamate receptors (AMPARs) is one of the most important mechanisms by which the efficacy of synaptic transmission at excitatory glutamatergic synapses in the mammalian brain is regulated. Traditionally these types of modifications have been thought to be achieved mainly by altering the channel gating properties or conductance of the receptors. A large body of evidence accumulated from recent studies strongly suggests that AMPARs, like most integral plasma membrane proteins, are continuously recycled between the plasma membrane and the intracellular compartments via vesicle-mediated plasma membrane insertion and clathrin-dependent endocytosis. Regulation of either receptor insertion or endocytosis results in a rapid change in the number of these receptors expressed on the plasma membrane surface and in the receptor-mediated responses, thereby playing an important role in mediating certain forms of synaptic plasticity, such as long-term potentiation (LTP) and depression (LTD). These studies have significantly advanced our understanding of the molecular mechanisms underlying LTP and LTD, and their potential contributions to learning and memory-related behaviours. Here I provide a brief summary of the current state of knowledge concerning clathrin-mediated AMPAR endocytosis and its relationship to the expression of certain forms of LTD in several brain areas. The potential impact of recent advancements on our efforts to probe the roles of synaptic plasticity in learning and memory-related behaviours, and their relevance to some brain disorders, particularly drug addiction, are also discussed.
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Abbreviations: AMPARs, AMPA receptors; LFS, low-frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; NAc, nucleus accumbens; VTA, ventral tegmental area

Introduction

Communication between neurons in the mammalian CNS is primarily accomplished through a process known as synaptic transmission. The ability of a synapse to change its strength (efficacy) of synaptic transmission, a process known as synaptic plasticity, is central to both brain function and dysfunction. Long-term potentiation (LTP) and depression (LTD) of synaptic transmission, particularly at hippocampal CA1 glutamatergic synapses, are two of the best characterized forms of synaptic plasticity in the mammalian brain and have been studied as cellular substrates of learning and memory (Bliss and Collingridge, 1993; Bear and Malenka, 1994; Collingridge *et al.*, 2004). Glutamate is the principal fast excitatory neurotransmitter in the brain and acts primarily via two classes of postsynaptic ionotropic glutamate receptors, the NMDA receptors and the AMPA receptors (AMPARs). NMDA receptors make little contribution to basal synaptic transmission due to voltage-dependent Mg^{2+} blockade but are essential for producing different forms of synaptic plasticity in AMPAR-mediated synaptic transmission, including LTP and LTD. AMPARs, on the other hand, are the primary receptors mediating basal synaptic transmission, but they also play an essential role in the expression of LTP and LTD, at least at the hippocampal CA1 synapse (Bliss

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and Collingridge, 1993; Bear and Malenka, 1994). It is now generally accepted that the induction of both LTP and LTD is postsynaptic and dependent upon Ca^{2+} influx through activated NMDA receptors. The mechanisms underlying the expression of LTP and LTD, while remaining hotly debated, likely involve both a presynaptic component via alteration of transmitter release and a postsynaptic one through the modification of AMPARs (Bliss and Collingridge, 1993; Bear and Malenka, 1994; Kullmann and Siegelbaum, 1995; Pittenger and Kandel, 2003). With regard to the possible mechanisms of the postsynaptic expression, a large body of evidence accumulated in recent years strongly supports a critical role for rapid changes in the number of postsynaptic AMPARs in the expression of LTP and LTD (Malinow and Malenka, 2002; Bredt and Nicoll, 2003; Collingridge *et al.*, 2004). These recent studies indicate that, as with most integral plasma membrane proteins, AMPARs undergo constitutive cycling between intracellular compartments and the plasma membrane via vesicle-mediated plasma membrane insertion (exocytosis) and internalization (endocytosis). Regulation of these processes can lead to rapid increases or decreases in the number of AMPARs expressed in the postsynaptic membrane, thereby providing a powerful means of altering synaptic efficacy. Thus, the expression of LTP involves a facilitated insertion (exocytosis) of AMPARs into the postsynaptic membrane, whereas LTD expression is absolutely dependent on clathrin-mediated AMPAR removal (endocytosis) from the synapse (Malinow and Malenka, 2002; Bredt and Nicoll, 2003; Collingridge *et al.*, 2004).

Although we have a much better understanding of the molecular mechanisms mediating both LTP and LTD than we did a decade ago, we still know very little about their exact roles in learning and memory as well as other experience-dependent biological processes. This is, in large part, due to the lack of specific inhibitors for either LTP or LTD. However, the recent advancements in our understanding of the detailed molecular mechanisms underlying LTP and LTD have lead to the development of some reagents that can interfere specifically with either the induction or expression of these forms of synaptic plasticity. Availability of these agents, particularly those that can be applied both systemically and locally within the involved brain structures in behavioural animals, has allowed investigators to begin to address the specific roles of LTP or LTD in some learning and memory-related behaviours (Brebner *et al.*, 2005; Rumpel *et al.*, 2005; Wong *et al.*, 2007). The present review focuses on recent advances in our understanding of the molecular mechanisms underlying the expression of LTD and their impact on efforts to discern the roles of LTD in some learning and memory-related behaviours. We begin by summarizing progress surrounding the critical roles of clathrin-mediated endocytosis of postsynaptic AMPARs in the expression of various forms of LTD in the mammalian brain and the development of several interference peptides that can specifically prevent facilitated AMPAR endocytosis and hence LTD expression. We will then discuss the potential of using these interference peptides as LTD-specific inhibitors to probe the roles of LTD in some learning and memory-related behaviours using an animal model of drug addiction as an example.

Early evidence for clathrin-mediated endocytosis of postsynaptic AMPARs in LTD

The hypothetical contribution of a change in the number of postsynaptic receptors to synaptic plasticity was put forward more than two decades ago (Lynch and Baudry 1984); however, providing direct evidence for this hypothesis has proved extremely difficult. Although some supporting electrophysiological discoveries such as 'silent synapses' emerged a decade ago (Kullmann, 1994; Isaac *et al.*, 1995; Liao *et al.*, 1995), direct evidence for the alteration of synaptic strength via a rapid alteration of the number of postsynaptic receptors lagged behind. The difficulty in detecting such a change in postsynaptic receptors may be, at least in part, attributed to the input-specific properties of certain forms of synaptic plasticity, a factor particularly true for hippocampal CA1 LTP and LTD. Because of this input specificity, only a small fraction of activated synapses undergoes plastic changes and these are easily masked by the overwhelming number of unactivated synapses. This makes it almost impossible to detect a change in the number of receptors at the small fraction of activated synapses using conventional morphological methods, such as electron microscopy, and biochemical techniques, such as plasma membrane fractionation and surface biotinylation.

The first evidence for a rapid change in the number of postsynaptic receptors as an efficient means of regulating the strength of synaptic transmission did not come from the glutamatergic synapse, but was rather described at GABAergic synapses in the hippocampal CA1 region. In an early study using hippocampal slices prepared from adult rats,Wan *et al.* (1997) reported that a brief (10 min) stimulation with insulin produced a long-lasting increase in synaptic efficacy of $GABA_A$ receptor-mediated synaptic transmission, manifested as an increase in both amplitude and frequency of $GABA_A$ receptor-mediated miniature inhibitory postsynaptic currents without altering their gating. Using quantitative immunogold electron microscopy in combination with electrophysiological recordings, the authors were able to demonstrate that the enhanced synaptic efficacy was primarily due to a rapid translocation of the receptors from an intracellular compartment to the postsynaptic plasma membrane (Wan *et al.*, 1997). This constituted the first direct evidence, suggesting that a rapid alteration of postsynaptic receptor number is an efficient means of regulating synaptic strength, and may therefore be an important mechanism underlying certain forms of long-term synaptic plasticity.

The advantage of insulin stimulation was quickly extended into studies of AMPAR trafficking and synaptic plasticity (Man *et al.*, 1998). In contrast to its effects on the miniature inhibitory postsynaptic currents, a brief application of insulin produced a LTD of AMPAR-mediated excitatory postsynaptic currents. Biochemical studies using membrane fractionation followed by western blotting further confirmed that such insulin-induced LTD is primarily caused by a rapid redistribution of the receptors from the plasma membrane fraction to the cytosolic compartment. Most plasma membrane proteins translocated to the intracellular compartment by clathrin-mediated constitutive and/or regulated endocytosis. Consistent with the involvement

of clathrin-mediated endocytosis, AMPARs are present in the AP2 complex (the adaptor protein complex associated with clathrin-coated pits) and insulin treatment increased the association of AMPARs with AP2. Moreover, administration of inhibitors for clathrin-mediated endocytosis into postsynaptic neurons prevented the insulin-induced LTD (Man *et al*., 1998). Together, these results demonstrate that insulin-induced decrease in the efficacy of synaptic transmission at the CA1 glutamatergic synapse is primarily mediated by a rapid reduction of postsynaptic AMPARs as a result of increased clathrin-mediated AMPAR endocytosis. The occlusion of insulin-induced LTD by low-frequency-stimulation (LFS)-induced LTD, the best-characterized form of hippocampal CA1 LTD, suggested that the two forms of LTD share a common pathway in their expression and that both might involve a rapid endocytic removal of postsynaptic AMPARs (Man *et al*., 2000b). Indeed, LFS-induced LTD is prevented by postsynaptic inhibition of clathrin-mediated endocytosis (Luscher *et al*., 1999; Man *et al*., 2000b). These studies established the critical role of clathrin-mediated AMPAR endocytosis in the expression of hippocampal CA1 LTD, representing the first set of evidence for a rapid alteration in postsynaptic AMPAR number in the expression of synaptic plasticity.

Forms of AMPAR endocytosis and their relation to synaptic plasticity

The discovery of insulin-induced rapid endocytosis of AMPARs not only facilitated our efforts in probing receptor changes in the synapses and their contributions to synaptic plasticity as briefly mentioned above, but also, through the reconstitution of regulated AMPAR endocytosis in a recombinant expression system, facilitated studies aimed at understanding the detailed mechanisms controlling AMPAR trafficking (Man *et al*., 2000b). Structurally, AMPARs are presumed to be heteromeric combinations of homologous subunits (GluR1–GluR4). Although the subunit combination of native AMPARs has not been fully characterized and likely varies according to the brain region and developmental stage, the most abundant native AMPARs are believed to be heteromeric complexes containing GluR1 and GluR2 subunits. Recombinant GluR1 or GluR2 homomeric receptors expressed in either HEK (human embryonic kidney) or CHO (Chinese hamster ovary) cells undergo internalization with a single exponential time constant of about 42 min at 37 °C, suggesting a rapid constitutive receptor internalization in a clathrin-dependent manner (Man *et al*., 2000b). Surprisingly, insulin stimulation has greatly different effects on the internalization of these homomeric receptors. Insulin produces a threefold increase in the rate of GluR2 internalization ($\tau = 15$ min), resulting in a rapid, long-lasting reduction of cell-surface GluR2 expression, but it has little effect on either internalization rate or cell-surface expression of GluR1 homomeric AMPARs. However, in cells co-expressing GluR1 and GluR2 subunits, insulin treatment decreased the surface expression of heteromeric receptors containing both subunits, suggesting the presence of GluR2 is sufficient for insulin facilitation of heteromeric AMPAR internalization. These data provided the first evidence that stimulated AMPAR endocytosis is GluR2 subunit specific (Man *et al*., 2000b). The current topology model suggests that each AMPAR subunit (GluR) consists of three transmembrane regions, MD1, MD3 and MD4 (MD; membrane domain), and a re-entrant membrane segment corresponding to MD2 (Figure 1). The major intracellular carboxyl-terminal (CT) region contains potential sites for protein phosphorylation and binding motifs for GluR-interacting proteins, including adaptor proteins involved in forming clathrin-coated

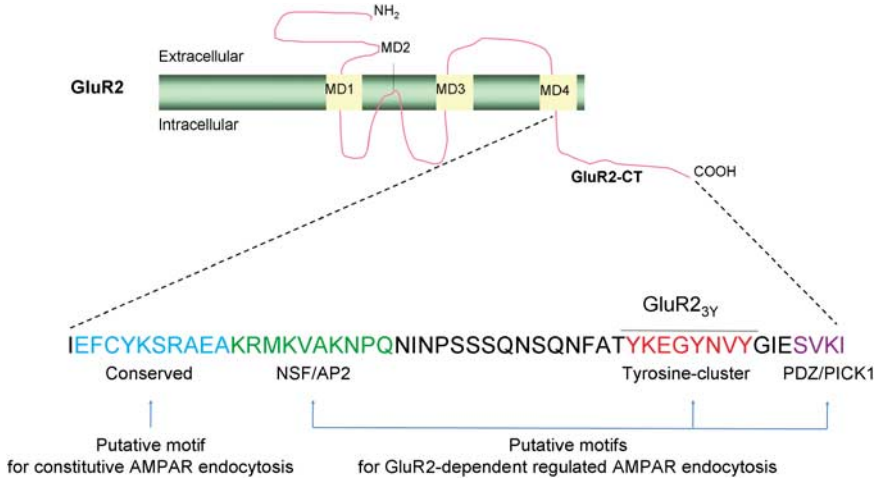

Figure 1 Schematic diagram of AMPAR GluR2 subunit showing the proposed membrane topology, along with detailed amino-acid sequences and proposed endocytic motifs of the carboxyl terminal region. MD represents the membrane domain. The putative motif for constitutive AMPAR endocytosis resides within a short stretch of amino acids, which are largely conserved (Conserved) among all four AMPAR subunits (Lin *et al*., 2000; Ahmadian *et al*., 2004). The putative motifs for GluR2-dependent regulated AMPAR endocytosis include the AP2-binding motif, which overlaps with the NSF-binding region (Lee *et al*., 2002), the tyrosine-containing motif (Tyrosine-cluster) (Ahmadian *et al*., 2004; Hayashi and Huganir, 2004) and PDZ/PICK1-binding motif (Kim *et al*., 2001; Chung *et al*., 2003). The sequence of the interference peptide used to probe the role of endocytosis and LTD in behavioural sensitization (Brebner *et al*., 2005) is also indicated (GluR2$_{3Y}$). LTD, long-term depression; NSF, *N*-ethylmaleimide-sensitive factor.

endocytic vesicles. Switching the CT regions between GluR1 and GluR2 subunits confirmed that the critical molecular determinants for the subunit-specific sensitivity of GluR2 to insulin reside within the GluR2 CT (Man *et al.*, 2000b).

Based on the mechanisms involved and functional consequences, clathrin-mediated AMPAR endocytosis may be classified into three distinct types: constitutive, regulated (stimulated) and ligand induced (Carroll *et al.*, 1999; Ehlers, 2000; Lin *et al.*, 2000; Man *et al.*, 2000b; Liang and Huganir, 2001). Constitutive endocytosis occurs across all AMPAR subunits and requires an amino acid stretch in the most juxtamembrane region of the CT that is 100% conserved among all AMPAR GluR subunits (Figure 1) (Lin *et al.*, 2000; Ahmadian *et al.*, 2004). Interfering with the constitutive pathway did not lead to detectable alterations in the number of AMPARs on the cell surface, likely due to compensation by a constitutive exocytosis pathway (Man *et al.*, 2000b; Liang and Huganir, 2001; Ahmadian *et al.*, 2004). The functional significance of such a feedback control mechanism would presumably be to ensure homoeostasis of receptor surface expression under most basal conditions.

Regulated endocytosis, on the other hand, is specifically associated with AMPARs containing the GluR2 subunit (Man *et al.*, 2000b; Wang and Linden, 2000; Ahmadian *et al.*, 2004). This specificity is mediated by several sequence motifs uniquely present within the GluR2-CT region, including a middle domain that binds to both *N*-ethylmaleimide-sensitive factor (NSF) and AP2 clathrin adaptor (Lee *et al.*, 2002) and a terminal postsynaptic density protein-95, disc-large tumor suppressor protein, zonula occludens-1 (PSD95/Dlg/ZO-1) PDZ-binding domain that interacts with a number of PDZ-containing proteins, such as GRIP and PICK1 (Kim *et al.*, 2001; Chung *et al.*, 2003) (Figure 1). In addition, through a systematic mutational analysis, a novel tyrosine phosphorylation-dependent endocytic motif ($_{869}$YKEGYNVYG$_{877}$) was identified to be absolutely required for both insulin-stimulated and LFS-induced AMPAR endocytosis (Ahmadian *et al.*, 2004). Although the detailed underlying mechanism remains unclear, it is relevant to mention that a recent study has reported that insulin stimulates β-adrenergic receptor endocytosis by phosphorylating one or more of three tyrosine residues in its CT region, thereby creating a binding site for the Grb2 adaptor protein (Karoor *et al.*, 1998). By analogy, it is reasonable to speculate that tyrosine phosphorylation of YKEGYNVYG may create a binding motif for a clathrin-coat adaptor protein, such as Grb2. The increased interaction between phosphorylated AMPAR and clathrin-adaptor recruits the receptors to clathrin-coated pits, allowing enhanced receptor endocytosis. Consistent with this hypothesis, a recent study has reported that the last tyrosine residue within this stretch can be phosphorylated and its phosphorylation is required for regulated AMPAR endocytosis in a culture model of LTD (Hayashi and Huganir, 2004).

Functionally, unlike constitutive endocytosis, regulated AMPAR endocytosis leads to a rapid and persistent reduction in the number of receptors expressed on the postsynaptic membrane surface, and hence plays an important role in controlling synaptic strength (Man *et al.*, 2000a, b). The ability to reduce cell-surface AMPARs makes regulated endocytosis an ideal candidate mechanism for the expression of certain forms of LTD. Indeed, several studies have shown that preventing the regulated endocytosis with peptides interfering with any of these GluR2-CT endocytic motifs (tyrosine containing (Ahmadian *et al.*, 2004; Fox *et al.*, 2007; Wong *et al.*, 2007), AP2 (Lee *et al.*, 2002) and PDZ/PICK1 (Kim *et al.*, 2001; Chung *et al.*, 2003)) can prevent the expression of hippocampal CA1 homosynaptic LTD in brain slices (Figure 1). Moreover, although the facilitated endocytosis of postsynaptic AMPARs is principally observed in conjunction with hippocampal CA1 LTD, it appears to be a common mechanism involved in the expression of certain forms of LTD in many other areas of the brain, including the cerebellum (Wang and Linden, 2000; Chung *et al.*, 2003), the nucleus accumbens (NAc) (Brebner *et al.*, 2005) and the visual cortex (Crozier *et al.*, 2007). It is important to note that in all of these studies, application of the interference peptides was found to block the expression of LTD without affecting basal synaptic transmission. These studies have firmly established a critical role for the regulated, but not the constitutive, AMPAR endocytosis in LTD expression. The data also suggest that these three GluR2-CT endocytic signals are indispensably involved in regulated AMPAR endocytosis and hence LTD expression, although the mechanisms by which these multiple internalization signals act in concert during various forms of synaptic plasticity remains to be illustrated. Importantly, as peptides interfering with any of these signals can specifically prevent the expression of LTD, without affecting either the basal synaptic transmission or the expression of LTP (Brebner *et al.*, 2005; Fox *et al.*, 2007), they may be used as specific LTD inhibitors to probe the functional roles of that phenomenon (Brebner *et al.*, 2005; Wong *et al.*, 2007).

In addition to GluR2-dependent regulated AMPAR endocytosis, mechanisms involving GluR1 dephosphorylation have also recently been implicated in LTD (Kameyama *et al.*, 1998) and may potentially be responsible for maintaining LTD in GluR2 (and GluR3) knockout animals (Meng *et al.*, 2003). It is also relevant to mention that several recent studies also implicate an important role of *Arc/Arg3.1*, an effector immediate-early gene, in mediating intracellular trafficking and plasma membrane expression of AMPARs via its interactions with some of the important clathrin-dependent endocytosis machineries (Tzingounis and Nicoll, 2006). Genetic manipulations of the *Arc/Arg3.1* expression result in alteration in both steady-state surface expression of AMPARs and NMDA-stimulated endocytosis of AMPARs (Chowdhury *et al.*, 2006; Rial Verde *et al.*, 2006), suggesting that *Arc/Arg3.1* may be involved in regulating both constitutive and regulated AMPAR endocytosis. As alteration of *Arc/Arg3.1* expression affects homoeostatic synaptic scaling (Shepherd *et al.*, 2006) and the expression of LTP and LTD (Plath *et al.*, 2006), memory retention deficits observed in the *Arc/Arg3.1* knockout mice cannot simply be attributed to its effects on alteration of either basal synaptic transmission or LTP or LTD. Thus, how alteration of AMPAR trafficking by *Arc/Arg3.1* contributes to synaptic plasticity and hence to learning and memory remains to be determined.

In addition to constitutive and regulated endocytosis, it has been reported that exposure of cultured hippocampal

neurons to the AMPAR agonists, glutamate or AMPA, can also trigger increased internalization of AMPARs within minutes, and this ligand-induced endocytosis seems also to involve a clathrin-mediated endocytic pathway (Beattie *et al*., 2000; Ehlers, 2000; Lin *et al*., 2000; Liang and Huganir, 2001). Functionally, this form of endocytosis may make important contributions to receptor desensitization, but its exact role in synaptic plasticity remains to be determined.

Evidence for the role of regulated AMPAR endocytosis and LTD in behavioural sensitization

Drug addiction is a complex neuropsychiatric disorder in which repeated self-administration of specific drugs, including amphetamines, cocaine, nicotine, heroin and alcohol, induces long-lasting changes in neural function and behaviour (Leshner, 1997; Hyman *et al*., 2006). Addictive behaviour is characterized by compulsion centred on the procurement and use of a drug of choice. Drug addiction is also defined as a chronic relapsing disorder, as individuals who have successfully abstained from drug use for extended periods are still susceptible to renewed episodes of drug seeking and abuse following a single exposure to environmental stimuli associated with prior drug use, or a small quantity of the drug itself. Brain-imaging studies with human drug addicts report that 'craving' induced by drug-associated stimuli is accompanied by activation of corticolimbic structures, including the prefrontal cortex, anterior cingulated cortex, amygdala, and ventral striatum (Breiter *et al*., 1997; Makris *et al*., 2004). These brain regions are innervated by the mesocorticolimbic dopamine system, which subserves the primary rewarding effects of many drugs of abuse, including psychostimulants and opiates (Hyman *et al*., 2006), highlighting the importance of dopamine in mediating some of the addiction-related behaviours. In addition, a growing body of evidence supports the hypothesis that addiction may involve mechanisms of neural plasticity implicated in learning and memory, in particular those associative mechanisms which link environmental stimuli with drug reward and strengthen instrumental responses required for drug self-administration (Wolf, 1998; Hyman *et al*., 2006).

Craving is a key feature of relapse to drug-seeking behaviour and is modelled in preclinical studies as increased motor activity (that is behavioural sensitization) induced by repeated intermittent administration of many drugs of abuse, including amphetamine, cocaine, heroin and nicotine. Behavioural sensitization, in turn, is linked to neural adaptations in the mesocorticolimbic regions of the brain (Wolf, 1998), including a terminal region in the NAc that receives dopaminergic projections from the ventral tegmental area (VTA) and excitatory glutamatergic inputs from the prefrontal cortex. Specifically, neural adaptations in the VTA play an essential role in the induction of behavioural sensitization, whereas synaptic modifications in the NAc are involved in its long-term maintenance (expression). Initial work on behavioural sensitization focused on pre- and postsynaptic changes in dopamine systems; however, evidence accumulated recently supports a critical role of synaptic plastic changes in glutamatergic transmission at both the VTA and NAc levels (Wolf, 1998). Consistent with differential roles of the VTA and NAc in mediating sensitization, repeated stimulation of glutamatergic cortical inputs to the VTA triggers sensitization, whereas the maintenance (expression) of this behavioural adaptation is blocked by inhibition of glutamatergic synapses in the NAc. Experiments characterizing the nature of synaptic plasticity in sensitized animals have observed both LTP (Yao *et al*., 2004) and LTD (Thomas *et al*., 2000, 2001) in the NAc. Several studies have provided correlative evidence for alterations in AMPAR trafficking and plasma membrane expression in the NAc in rats sensitized with drugs of abuse. In a recent study, by measuring the ratio of AMPA/NMDA excitatory postsynaptic currents in NAc neurons, Thomas *et al*. (2001) reported that the AMPAR-mediated component of excitatory postsynaptic currents was specifically reduced in sensitized rats. These results suggest that repetitive administrations of cocaine *in vivo* may elicit a LTD of excitatory synaptic transmission in the NAc as a result of enhanced endocytosis of postsynaptic AMPARs (Thomas *et al*., 2001). This initial electrophysiological evidence for a critical role of AMPAR trafficking in the NAc was further supported by several other studies exploring more direct measurements of neuronal surface AMPAR expression (Sutton *et al*., 2003; Boudreau and Wolf, 2005). However, it is particularly interesting to note that by comparing surface expression of AMPARs in the NAc in control and cocaine-sensitized rats, Boudreau and Wolf (2005) reported that sensitization is associated with a persistent increase rather than a reduction in the cell-surface expression of AMPARs in NAc neurons. These results appear to argue against an enhanced NAc LTD during behavioural sensitization as suggested by the initial electrophysiological study (Thomas *et al*., 2001).

In a more recent study, Brebner *et al*. (2005) investigated whether LTD is indeed produced in the NAc during behavioural sensitization and if so, whether it causatively contributes to the pathogenesis of the behavioural sensitization. This was primarily accomplished using $GluR2_{3Y}$, one of the recently developed GluR2-CT-derived interference peptides discussed above, that specifically blocks LTD expression by preventing GluR2-dependent AMPAR endocytosis (Ahmadian *et al*., 2004). The $GluR2_{3Y}$ interference peptide functions by targeting the tyrosine-containing motif in the GluR2-CT required for LFS-induced endocytosis of the receptor. As this motif appears to be unique to GluR2/3 receptors, off-target effects that could hypothetically be seen by targeting the AP2 or PDZ motif of GluR2 are avoided. Indeed, the $GluR2_{3Y}$ peptide has been shown to be able to specifically block the regulated AMPAR endocytosis and LTD while having little effect on other clathrin-mediated endocytic processes, including constitutive and ligand-induced AMPAR endocytosis (Ahmadian *et al*., 2004) and transferrin receptor endocytosis (Wang *et al*., 2004). Importantly, this peptide is the only one that has been shown *in vivo* to specifically prevent LTD without affecting either LTP or basal synaptic transmission (Fox *et al*., 2007). Using the $GluR2_{3Y}$ peptide along with other inhibitors of clathrin-mediated endocytosis, Brebner *et al*. (2005) first demonstrated that the expression of LFS-induced LTD in the NAc, like its

counterpart, the CA1 LTD in the hippocampus, is also mediated by the GluR2-dependent endocytosis of postsynaptic AMPARs. These results not only provided a mechanistic insight for the expression of NAc LTD, but also confirmed that the $GluR2_{3Y}$ peptide may serve as a research tool to probe the functional role of NAc LTD in behavioural sensitization.

In order to deliver peptide into neurons in the brain following systemic injections, the authors modified the wild-type $GluR2_{3Y}$ peptide, or its inactive control peptide in which the three tyrosines were replaced with alanines ($GluR2_{3A}$), by adding the cell-membrane transduction domain of the human immunodeficiency virus-type 1 Tat protein (YGRKKRRQRRR) (Schwarze *et al*., 1999). As expected, one-hour intravenous pretreatment with TAT-$GluR2_{3Y}$ peptide, but not the control TAT-$GluR2_{3A}$, abolished the expression of behavioural sensitization to a challenging dose of amphetamine, without any notable side effects in rats. The blockade of sensitization is due to specific action in the NAc, as the direct microinfusion of TAT-$GluR2_{3Y}$ into the NAc, but not the VTA, mimicked intravenous administration, preventing the expression of the behavioural sensitization. These data show that blocking the regulated endocytosis, and hence the expression of LTD in the NAc, prevents the expression of behavioural sensitization in the rat. This study also demonstrated the utility of peptides that disrupt AMPAR trafficking, the final step in the expression of synaptic plasticity, as tools to examine the critical role of LTD (or LTP) in specific aspects of learning and memory in conscious animals. Indeed, the strategy of interfering with AMPAR trafficking is being increasingly applied to probe the roles of LTP or LTD in learning and memory-related behaviours, including classical fear conditioning (Rumpel *et al*., 2005) and Morris water maze-based spatial learning (Wong *et al*., 2007).

Conclusion

To summarize, recent studies have made substantial advances to our understanding of the molecular and cellular mechanisms underlying the intracellular trafficking of postsynaptic ligand-gated neurotransmitter receptors, the AMPAR in particular, and their contributions to the expression of various forms of synaptic plasticity in different brain regions. Specifically, facilitation of GluR2-dependent endocytosis of postsynaptic AMPARs has been identified as a common final step in the expression of various forms of LTD observed in the mammalian brain. The advancement in our understanding of the detailed molecular steps underlying the GluR2-dependent AMPAR endocytosis has lead to the development of several GluR2-CT-derived interference peptides that can specifically prevent the facilitated AMPAR endocytosis, and hence the expression of certain forms of LTD. Accumulating evidence supports the hypothesis that these interference peptides can be used as LTD-specific inhibitors in probing the exact roles of LTD in some of the experience-dependent biological processes, including but not limited to, learning and memory and developmental maturation of neuronal circuits. Emerging evidence also suggests that similar interference peptides may be developed as specific inhibitors of LTP. It is clear that further characterization of the detailed molecular mechanisms of LTP and LTD and the availability of new and specific strategies for manipulating them will not only significantly advance our efforts in understanding the exact roles of synaptic plasticity in various brain functions and dysfunctions, but also lead to the development of novel therapeutics for some brain disorders, such as drug addiction. We can expect a rapid and exciting growth in this direction of neuroscience research in the next few years.

Conflict of interest

The author states no conflict of interest.

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