Molecular Neurobiology

ISSN0893-7648/96/12(1): 1–11/$7.75

Ionotropic Glutamate Receptors

Their Possible Role in the Expression of Hippocampal Synaptic Plasticity

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Abstract

In the brain, most fast excitatory synaptic transmission is mediated through L-glutamate acting on postsynaptic ionotropic glutamate receptors. These receptors are of two kinds—the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate (non-NMDA) and the *N*-methyl-D-aspartate (NMDA) receptors, which are thought to be colocalized onto the same postsynaptic elements. This excitatory transmission can be modulated both upward and downward, long-term potentiation (LTP) and long-term depression (LTD), respectively. Whether the expression of LTP/LTD is pre-or postsynaptically located (or both) remains an enigma. This article will focus on what postsynaptic modifications of the ionotropic glutamate receptors may possibly underly long-term potentiation/depression. It will discuss the character of LTP/LTD with respect to the temporal characteristics and to the type of changes that appears in the non-NMDA and NMDA receptor-mediated synaptic currents, and what constraints these findings put on the possible expression mechanism(s) for LTP/LTD. It will be submitted that if a modification of the glutamate receptors does underly LTP/LTD, an increase/decrease in the number of functional receptors is the most plausible alternative. This change in receptor number will have to include a coordinated change of both the non-NMDA and the NMDA receptors.

Index Entries: Glutamate receptor channels; NMDA; non-NMDA; synaptic plasticity; long-term potentiation; long-term depression; hippocampus.

Introduction

In the vertebrate central nervous system (CNS) most fast excitatory synaptic transmission is mediated by L-glutamate *(1)* acting on postsynaptic receptors. These glutamate receptors may play important roles in various physiological and neuropathological events in the brain, such as synaptogenesis *(2,3)*, synaptic plasticity in learning and memory *(4)*, development of chronic pain *(5)* and anxiety *(6)*, and excitotoxicity *(7,8)*. This article will address the possible role glutamate receptors may have in the expression of synaptic plasticity in the hippocampus.

The glutamate receptors constitute a family of receptors that can be categorized as iono-

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tropic (with an intrinsic ion channel) and metabotropic (acting through a G-protein) *(1,9)*. The ionotropic receptors are traditionally further subdivided, defined by specific agonists, into the *N*-methyl-D-aspartate (NMDA) receptor and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate (non-NMDA) receptor. Molecular cloning studies have identified several subunits of these ionotropic receptors. These subunits have been classified, based on sequence similarity and pharmacology, as AMPA-preferring (GluR1–4), kainate-preferring (GluR5–7, KA1,2), and NMDA-preferring (NMDAR1, 2A–D) *(10–12)*. The non-NMDA receptors in the hippocampus control a nonselective cationic channel permeable to sodium and potassium ions, whereas the NMDA receptors control a cationic channel permeable to these two ions and, in addition, to calcium. In the hippocampus, fast excitatory synaptic transmission is mediated by both the non-NMDA and NMDA receptor complexes, which are thought to be colocalized, at least at the majority of synapses *(13–15)*. During low frequency afferent activation, the postsynaptic current is mediated through the non-NMDA receptor channels *(16,17)*. This is because the NMDA receptor channels are normally blocked by magnesium ions, a block that can only be relieved by depolarization of the postsynaptic membrane *(18,19)*.

Modulation of Fast Excitatory Synaptic Transmission—Long-Term Potentiation and Long-Term Depression

The efficacy of the fast excitatory synaptic transmission in the hippocampus can be modified in an activity-dependent manner. Following brief (<1 s) high frequency afferent activation the efficacy is increased, a phenomenon commonly referred to as long-term potentiation (LTP). Although most extensively studied in the hippocampus, LTP has been described also in other regions of the cortex as well as in some subcortical ones *(4,20)*. Because of the associative requirement for its induction, as well as its capabiliy to be long-lasting (>days in chronic studies), LTP in the hippocampus has attracted much interest as a possible cellular correlate of certain forms of learning and memory. Long-term depression (LTD) has also been described in various brain regions. In the hippocampus, one form of LTD (homosynaptic LTD), described mainly in young animals, appears to serve as a functional reverse of LTP *(21,22)*. Thus, a prolonged (minutes) homosynaptic low frequency activation is believed to downregulate the same synaptic modification as that upregulated during LTP. As indicated above, LTP, as well as LTD, are used as broad terms for synaptic potentiation/depression processes in the brain and they may well correspond to different processes when comparing different brain regions, or even within a specific region *(22,23)*. This article will focus on the synapse-specific (restricted to activated synapses) NMDA receptor-dependent LTP and LTD in the hippocampus.

The role of the ionotropic glutamate receptors in the induction of NMDA receptor-dependent LTP and LTD in the hippocampus is well-established (*see* refs. *24–26*). The current notion is that activation of NMDA receptors, because of coincident pre-and postsynaptic activity, and the subsequent postsynaptic calcium influx, triggers processes leading to the expression of LTP and LTD. Coactivation of the non-NMDA receptors seems only needed to provide the depolarization necessary to open the NMDA receptor channels *(27–29)*. On the other hand, the role of the ionotropic glutamate receptors in the expression of LTP/LTD remains controversial. A number of findings have suggested that LTP/LTD is based on a presynaptic modification, i.e., on an increased/decreased release of transmitter *(30–34)*. If so, the ionotropic glutamate receptors do not play any direct role in LTP/LTD expression. On the other hand, other findings have suggested a direct involvement of the glutamate receptors *(35–40)*. These opposing views may depend on the fact that LTP/LTD can be based on both pre- and postsynaptic modifications, possibly dependent on specific experimental conditions *(41,42)*, or that forms of potentiation/depres-

sion other than the synapse-specific NMDA receptor-dependent ones sometimes have been studied (cf. refs. *43–45*). Alternatively, they may reflect that our present level of understanding of synaptic physiology is insufficient to make correct inferences from experimental data.

Our laboratory has lately focused on studies in which synapse-specific NMDA receptor-dependent LTP/LTD has been characterized with respect to temporal characteristics, and to the type of changes that appears in the non-NMDA and NMDA receptor-mediated synaptic currents. Data obtained from such studies must evidently put some constraints on what mechanisms are possible for LTP/LTD expression. In this article we will discuss such data and their implications for the understanding of the LTP/LTD process(es) and address the question: If LTP/LTD expression is based on a postsynaptic ionotropic glutamate receptor modification, what would this modification have to be?

The Temporal Characteristics of LTP

LTP has generally been defined as the rather stable potentiation that remains some 10–15 min and onward after a brief high-frequency activation when short-lasting alterations of synaptic efficacy *(46)* unrelated to LTP have subsided *(47,48)*. By using procedures that separate LTP from such short-lasting alterations it has been shown that LTP begins to appear already 2–3 s after the induction event, that it grows toward a peak value within 1 min, and that it thereafter can display a substantial decay before a subsequent stabilization *(49–51)*. By varying the induction strength, LTP can vary from a full decay within a few minutes to essentially no decay within its first hour *(50,52)*. This initial phase of the potentiation likely represents a dynamic feature of LTP itself *(53)*, rather than being a mechanistically distinct short-term potentiation (STP) process *(4)*. LTP expression must then, at least in its initial stages, be related to modifications that can be established quite rapidly (<1 min) and that can also be reversed. The above studies also seem to exclude the notion of temporally separate expression mechanisms for LTP within its first hour (e.g., suggested by refs. *36,54,55*).

Since chelation of postsynaptic calcium >2 s after the induction event has no effect on the subsequent LTP development *(56)* this development must be governed either by the temporal characteristics of biochemical systems activated by the calcium or by the properties of the LTP expression mechanism. The fast onset also implies that LTP has to rely on posttranslational changes, since *in situ* hybridization analyses indicate that the mRNA encoding for crucial molecules in the synapse (receptors, many messenger systems) are exclusively located in the cell body *(57)*. A signal transduction cascade from the synapse to the nucleus and back again *(58)* would take too long to account for the fast development of the expression mechanism.

How Is the Modified Synaptic Efficacy Seen with LTP/LTD Measured?

Measurements of synaptic transmission are accomplished either by intracellular recordings (excitatory postsynaptic potential/current, EPSP[C]) or by extracellular recordings (field excitatory postsynaptic potential, fEPSP). During low frequency afferent activation (<1 Hz) the postsynaptic current is mediated by the non-NMDA receptor and the increased/decreased synaptic transmission seen with LTP/LTD is mostly measured as changes in the initial slope of the non-NMDA receptor-mediated fEPSP (or EPSC). The rationale for using the initial slope, rather than the peak amplitude, is to avoid interference from disynaptic postsynaptic inhibition or somatic action potentials. A controversial issue has been whether the fEPSP (EPSC), evaluated when synaptic inhibition is pharmacologically blocked, is uniformly (symmetrically) increased/decreased with LTP/LTD. Abraham et al. *(59)* and Hess and Gustafsson *(60)* reported that LTP was not associated with any change in the shape of the fEPSP, and Isaacson and Nicoll *(61)* also found no changes in the EPSC time-course. These

results were challenged by Ambros-Ingerson et al. *(62)* who reported that LTP does induce changes in the fEPSP shape, suggesting a postsynaptic receptor modification. A subsequent analysis of such changes has, however, suggested them to be secondary to somatic spike activity *(63)*. NMDA receptor-dependent LTP thus differs from the prolonged potentiation induced by calcium influx through L-type voltage-sensitive calcium channels *(64–66)* that is mechanistically distinct from LTP and that is associated with a change in the time characteristics of the fEPSP *(44)*. Homosynaptic LTD has also been shown to be associated with a uniform decrease of the fEPSP *(67)*.

Is the NMDA Receptor-Mediated Current Modified in Association with LTP/LTD?

An early result that appeared to give strong support for a postsynaptic expression for LTP was that LTP seemed only associated with an increase in the non-NMDA receptor-mediated EPSP/fEPSP *(27,28,68)*. Procedures that enhanced transmitter release produced almost equal changes in the non-NMDA and NMDA receptor-mediated synaptic potentials *(27,38, 69,70)*. Later studies have, however, indicated a more complex situation. Bashir et al. *(71)*, and Xie et al. *(72)* reported substantial LTP of an isolated NMDA fEPSP/EPSP/EPSC. Asztély et al. *(69)*, measuring the non-NMDA and NMDA receptor-mediated fEPSPs in parallel, also demonstrated a substantial LTP of the NMDA component. However, in contrast with procedures known to enhance transmitter release, LTP was not found to be associated with equal changes of the two synaptic components, the relative increase of the NMDA fEPSP being only about one-third that of the non-NMDA one. Recent studies using whole-cell recording have also produced conflicting results. Whereas Perkel and Nicoll *(38)* found no potentiation of the NMDA EPSC, Clark and Collingridge *(73)* found equal changes of the non-NMDA and NMDA EPSCs. What different experimental conditions that may underlie this discrepancy in results is uncertain. With respect to homosynaptic LTD, experiments using parallel measurements of the two synaptic components demonstrated an equal decrease of the two fEPSP components, in cirumstances in which LTP was associated with unequal changes *(67,74)*.

Taken together, the above results demonstrate that the NMDA receptor-mediated synaptic component can exhibit LTP/LTD (NMDA LTP/LTD). The question is, then, whether this LTP/LTD is actually connected to that of the non-NMDA component. In other words, is the NMDA LTP/LTD a separate entity from the non-NMDA LTP/LTD, which in certain experimental situations appears together with the non-NMDA LTP/LTD (cf. ref. *75*), or are NMDA and non-NMDA LTP/LTD integral parts of the same process, with the lack of NMDA LTP sometimes observed caused by experimental factors specifically interfering with its expression? Which of these alternatives is correct can at present not be decided. Crépel et al. *(76)* demonstrated that a short period of hypoxia/hypoglycemia results in an LTP of the NMDA fEPSP, mediated by a redox modulatory site of the NMDA receptor *(77,78)*. Hammond et al. *(77)* have suggested this mechanism also to be operating in producing the LTP of the NMDA fEPSP observed after high-frequency afferent activation under the conditions of low extracellular magnesium used to record this potential. If so, non-NMDA and NMDA LTPs are likely separate entities. However, the NMDA LTP observed by Asztély et al. *(69)* could also be elicited in standard solution prior to the switch to low magnesium solution, and the temporal characteristics of the non-NMDA and NMDA LTP were found to be very similar. With respect to LTD, a very tight coupling between the depression of the non-NMDA and the NMDA fEPSPs, with respect to time-course and induction conditions, was also found *(67,74)*.

Are There Parallel Pre- and Postsynaptic Modifications?

Studies using quantal analysis have suggested that LTP expression may depend on the

state of the release probability of the synapses, the expression being mainly postsynaptic for synapses with a high probability of transmitter release *(77,78)*. This notion may seem supported by our result of a relatively larger non-NMDA than NMDA LTP *(69)*. In this scenario the "presynaptic" LTP would give an equal increase, and the "postsynaptic" LTP an isolated non-NMDA one. However, Asztély et al. *(51)* showed that the temporal characteristics of LTP are essentially the same whether elicited during conditions of high- or low-release probability, and the relative degree of NMDA LTP did not differ between these two conditions.

What Modifications of the Ionotropic Receptors Are Possible?

A direct postsynaptic modification of the ionotropic glutamate receptors in association with LTP/LTD can be either a change in the functional properties of existing receptors or a recruitment of new functional ones. Both the non-NMDA and the NMDA receptors have several consensus sequences for phosphorylation by various kinases *(10,79)* and phosphorylation/dephosphorylation of them can be a plausible mechanism for altering their functional state *(80,81)*. This notion of LTP/LTD being caused by ongoing postsynaptic kinase/phosphatase activity is supported by some studies *(82–84)*, although the results concerning LTP remain controversial *(53,85,86)*.

Modulation of Existing Receptors

A change in functional properties of the receptors could be an alteration of channel kinetics. This possibility has been discussed in relation to the findings that the effect of aniracetam, a drug that affects the kinetics of the non-NMDA receptor channels *(87)*, should interact with LTP *(88,89)*, and that LTP should induce changes in the time characteristics of the fEPSP *(60)*. Drugs that affect channel kinetics, such as aniracetam, also affect the fEPSP in the manner expected from its effect on single channels *(87)*. A change in the non-NMDA receptor channel-mediated currents, in a manner suggesting a change in channel kinetics, has also been reported following activation of cAMP-dependent protein kinase (PKA) *(90,91)* and calcium-and phospholipid-dependent protein kinase (PKC) *(92)*. However, Isaacson and Nicoll *(61)* and Asztély et al. *(93)* did not detect any interaction between the aniracetam-induced change of the EPSC/fEPSP and LTP. As noted above, the fEPSP shape modification described by Ambros-Ingersen et al. *(62)* can be explained by effects of spike activity rather than by changes in the fEPSP itself *(63)*. Thus, there is no clear evidence that LTP/LTD expression involves a change in channel kinetics. The functional properties of a receptor channel could also be altered via a change in its conductance. The non-NMDA receptor channels have been shown to exhibit multiple conductance levels *(94)*. However, as yet there is no clear experimental evidence that it is possible to modulate the conductance of postsynaptic glutamate receptor channels (cf. ref. *95*).

Recruitment/Derecruitment of Functional Receptors

The number of calculated functional postsynaptic glutamate receptors at a single site, <100 *(96–99)*, relative to the number of transmitter molecules in a vesicle (4000) *(100,101)*, suggests that the number of postsynaptic channels determines the size of a quantal event *(102–104)*. Whether the number of functional receptors is similar to the number of existing receptors is presently undecided. Morphological studies in the CA1 region have shown the existence of aggregates of particles on the postsynaptic membrane of excitatory synapses *(105)*; these particles are thought to represent postsynaptic receptors. The density of these particles in the postsynaptic density (PSD) was calculated to be 2800 μm^{-2}, which, assuming a total area of the PSD of 0.07 μm^2 in the CA1 region *(106)*, should give approx 200 particles (receptors) opposite to one release site. Thus, nonfunctional receptors may exist, and their conversion to functional ones may subserve

the fast (within seconds) 100–200% increase in synaptic efficacy that can be seen in association with LTP. Conversely, LTD could be related to processes converting functional receptors into nonfunctional ones. A similar scheme of recruitment of nonfunctional acetylcholine receptors into functional ones, without requiring *de novo* receptor synthesis, has been described in chicken ciliary ganglion cells *(107)*. LTP in the CA1 region has also been reported to be accompanied by an increase in non-NMDA receptor-binding (*108,109;* but *see* ref. *110*). Scatchard analysis revealed that this increase was caused by an increase in the number and not in the affinity of the non-NMDA receptors *(108)*. The finding by Asztély et al. *(69)* and Clark and Collingridge *(73)* of a parallel increase of the non-NMDA and NMDA receptor-mediated current in association with LTP indicates that an increase in the functional receptors should occur concurrently in both the non-NMDA and NMDA receptors and not be a selective "AMPAfication" *(111)*.

An expression mechanism based on an increased number of functional postsynaptic receptors is compatible with data suggesting a presynaptic expression for LTP, such as an increase in the frequency of spontaneous synaptic events *(33)* and a reduction in the rate of failure of miniature EPSCs *(34,112,113)*. This is because with quantal analysis of synaptic transmission, or in an analysis of spontaneous synaptic events, small quantal events might be buried in the background noise *(114)*. If this is the case, a recruitment of "clusters" of nonfunctional receptors into functional ones *(40,42)* leads to an increase in the amplitude of quantal events to above the detection level. Similarly, the increased number of failures observed after LTD induction *(34)* could be caused by loss of "clusters" of functional receptors.

Concluding Scenario for a Possible Postsynaptic Expression

As noted above, the aim of this article was not to cover all aspects of LTP/LTD expression or to state the nature of that expression. Rather, the aim was to describe some of our own findings regarding the characteristics of synapse-specific NMDA receptor-dependent LTP/LTD in the hippocampus in order to address the question: If LTP/LTD is expressed postsynaptically, what should the nature of that modification be?

Our results suggest that LTP, at least within its first few hours, is based on a single expression mechanism. The results do not favor the notion of either temporally separate pre- and postsynaptic mechanisms or temporally parallel ones. A postsynaptic expression mechanism then has to account for all of the observed characteristics of LTP (and LTD), including those pointing toward a presynaptic mechanism. As for a postsynaptic scenario, Lisman *(115)* and Lisman and Goldring *(116)* suggested that a bistable autophosphorylating calcium/calmodulin-dependent protein kinase (CaMK) in an unspecified manner could alter the efficacy of the ionotropic glutamate receptors, leading to LTP. Recently this hypothesis was elaborated such that a moderate increase in postsynaptic calcium (given by LTD-inducing stimulation) leads to activation of phosphatases that dephosphorylate the kinase *(117)*. In support of this notion, activation of CaMKII increases the non-NMDA receptor channel-mediated currents *(118)*, activation of postsynaptic CaMKII interacts with the expression of LTP *(119)*, and CaMKII activity is increased during LTP expression *(120)*. Furthermore, CaMKII is the major protein in the postsynaptic density (PSD) *(121,122)*, and it is locally synthesized in the dendritic tree *(57)*. The temporal characteristics of LTP described above would agree with this notion of a local posttranslational process. The dynamic character of LTP with a variable duration seems also compatible with the notion of two competing enzymatic systems (phosphorylation/dephosphorylation) controlling the expression.

Our results would suggest that this tentative phosphorylation/dephosphorylation of the receptor does not affect its channel kinetics, but rather alters the number of functional receptor channels. A scenario with a selective increase

in the number of non-NMDA receptors has recently been suggested–AMPAfication *(40, 111)*. However, if LTP expressiion is postsynaptic our results suggest that there should be a coordinated increase in the number of both non-NMDA and NMDA receptors in LTP, and a coordinated and equal drop-out of these receptors in LTD. Activation of CaMKII should then affect both receptor types, a result that remains to be demonstrated for the NMDA receptor.

Acknowledgments

This work was supported by the Swedish Medical Research Council (Project 05180); Göteborg Medical Society; and Svenska Sällskapet för Medicinsk Forskning.

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