Contribution of AMPA and NMDA receptors to early and late phases of LTP in hippocampal slices

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Abstract

Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptor mediated responses were investigated in rat hippocampal slices under 4 h of long-term potentiation (LTP) expression. A modified medium containing the NMDA receptor antagonist AP5 and low concentration of Mg²⁺ was used to monitor isolated AMPA responses. NMDA components were determined from composite excitatory postsynaptic potentials (EPSPs) under brief (15–20 min) wash-out of AP5. LTP was induced in a medium with low concentration of AP5, resulting in an about two-fold larger increase of the AMPA component than of the NMDA component at both 1 h and 4 h after induction. Similar results were obtained if LTP was induced in “normal Mg²⁺” and the NMDA components were assessed at the end of experiment, from either composite or isolated NMDA EPSPs, with or without blockade of GABAergic inhibition. It is generally believed that LTP undergoes biochemical and/or structural conversions during the first few hours. Our study, however, shows constant expression of LTP, at least in terms of AMPA versus NMDA components, during this time. The data support the notion that LTP initiates as a predominant amplification of AMPA receptors and remains so for at least 4 h.

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1. Introduction

Long-term potentiation (LTP) is an essential phenomenon believed to underlie learning and memory processes. Mechanisms of LTP induction include activation of N-methyl-D-aspartate (NMDA) type glutamate receptors and an increase in intracellular Ca²⁺ concentration. The relative contribution of different kinds of glutamate receptors after LTP induction is a key question, the resolution of which may help to trace basic mechanisms of synaptic strengthening (Sanes and Lichtman, 1999). Previous studies demonstrate selective alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor mediated potentiation (Kauer et al., 1988; Muller et al., 1992; Muller and Lynch, 1988; Perkel and Nicoll, 1993) as well as equal or variable involvement of AMPA and NMDA receptors (Clark and Collingridge, 1995; O’Connor et al., 1995; Watt et al., 2004; Xiao et al., 1995, 1996); still others claim a prevailing potentiation via NMDA, without firmly relating it to AMPA (Bashir et al., 1991; Berretta et al., 1991).

In analogy with memory, LTP consists of different temporal phases. Depending on induction parameters such as stimulus strength and temporal pattern, the duration of the induced potentiation may be short-term or long-term. Short-term potentiation (STP) usually declines within an hour whereas late phases of LTP last longer than 3 h. While early phases of LTP, possibly including STP, depend on activation of calcium/calmodulin-dependent protein kinase II (CaMKII), phases later than 1–2 h require activation of protein kinase C (PKC), protein kinase A (PKA), gene transcription and synthesis of new proteins (see Huang et al., 1996). Several studies emphasize a distinction between early, protein synthesis independent LTP, lasting up to 3 h, and late, protein synthesis dependent LTP (Frey et al., 1993; Nguyen and Kandel, 1996). Hence, LTP appears to undergo important transformations within the first few hours. A critical issue is to what extent this is reflected by changes in relative contributions of the main, ionotropic...
responses were assessed after 4 h of LTP expression by switching to ''low containing 4 pulses at 100 Hz with an interburst interval of 200 ms. NMDA solution by theta-burst stimulation (TBS). TBS consisted of 10 bursts, each magnesium concentrations being 2.5, 1.3 (''normal Mg\textsuperscript{2+}''') or 2, 0.1 (''low Mg\textsuperscript{2+}''').

Field excitatory postsynaptic potentials (EPSPs) were recorded from the CA1 apical dendritic layer by a glass micropipette filled with 3 M NaCl (3–10 M\textohm resistance). Stimulation was delivered as 0.1 ms negative constant current pulses via monopolar tungsten electrodes in the dendritic layer. Two stimulating electrodes were placed on either side of the recording electrode to provide for stimulation of two separate sets of afferents. The stimulating electrodes were activated alternately at a frequency of 0.05–0.1 Hz, usually 1 per 20 s with stimuli separated by 10 s between the two independent pathways. Stimulus strengths, usually in the range 20–40 \mu A, were adjusted to yield field EPSPs of nearly the same size for the two pathways. Stability of recordings was verified for at least 1 h before beginning the actual experiment.

The first group of experiments used \textasciitilde low Mg\textsuperscript{2+} solution to unblock NMDA receptors while low concentration of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 1 \mu M) was added to partially suppress AMPA receptors, producing a suitable balance of AMPA and NMDA components. To prevent decay of LTP due to weak NMDA receptor activation (Villarreal et al., 2002; Xiao et al., 1996), the antagonist 2-amino-5-phosphonopentanoic acid (AP5, 50 \mu M) was present in the bath except for a week after tetanization, such as for the NMDA component in some experiments (experiments in Figs. 3–5) are unlikely to affect the test/control ratio. The amount of LTP measured in this manner changed slightly when comparing values before, during and after the NMDA receptor activation period. Unless otherwise stated, measurements of the AMPA component were taken just before this period. The values are expressed as mean \pm S.E.M. Statistical comparisons were made using Student’s \textit{t}-test.

CNQX and AP5 were obtained from Tocris Cookson, UK; PTX was from Sigma Chemicals Co., MO, USA; prefabricated stimulating electrodes were obtained from World Precision Instruments, FL, USA, type TM33B.

3. Results

The first group of experiments was performed in the presence of 50 \mu M AP5 in ''low Mg\textsuperscript{2+}'' solution. AP5-free solution was transiently applied before induction, at 1 h after induction and at 4–5 h after induction of LTP, as illustrated in Fig. 1. The latter case will simply be referred to as 4 h. While LTP was associated with an increase of both AMPA and NMDA, there was a clear distinction between the levels of potentiation of the two components (164 \pm 9% versus 124 \pm 5% at 1 h and 156 \pm 8% versus 123 \pm 9% at 4 h, \textit{n} = 12). Notably, the potentiation of the AMPA component dominated over that of the NMDA component at both time points (see Fig. 2A). Paired-difference test revealed no significant distinction between values at 1 h and 4 h.

LTP of the AMPA component was estimated from isolated AMPA EPSPs recorded just before the NMDA receptor unblocking period. To examine whether the transient unblocking of NMDA receptors might interfere with LTP, values of AMPA LTP were compared before, under and after the unblocking period at 1 h after LTP induction. There was a slight decrease of the potentiation as defined by the ratio between test and control inputs (see Fig. 2B). The test/control ratio of AMPA components diminished, passing from 164 \pm 9% (before, see above) via 152 \pm 10% (under) to 155 \pm 10% (15 min after). These reductions of LTP, as compared to the values before unblocking, were significant by paired difference testing (\textit{p} < 0.05, \textit{n} = 12). Over a longer time scale (1 h after unblocking, or 2 h following LTP induction), the value of AMPA LTP still tended to be reduced (157 \pm 10%; \textminus 7 \pm 4%, \textit{p} > 0.05; not illustrated).

To exclude the possibility that the initial unblocking of NMDA receptors might prime LTP induction, possibly leading to secondary effects on its expression, experiments were carried out without the initial unblock yielding similar results (\textit{n} = 4, not illustrated). Actually, this unblock, performed for initial NMDA component matching, seems to be optional, because the initial equalization of AMPA components for the two pathways should to be sufficient (Dozmorov et al., 2004). This idea was verified by data from experiments in Fig. 2. After equalization of AMPA...
EPSPs the test/control ratio for the NMDA component during the initial unblock was near unity (104 ± 4%, n = 12).

LTP was also induced in “normal Mg2+” solution. Composite EPSPs were obtained after 4 h of LTP expression by switching to “low Mg2+” solution and NMDA components were measured. Similar to the above, there was a substantial AMPA-mediated LTP measured in “normal Mg2+” solution, and less potentiation of the NMDA component measured in “low Mg2+” solution (see Fig. 3). To counter any concerns about using composite EPSPs and assessing the NMDA component via late measurements, AMPA receptors were subsequently blocked by CNQX and isolated NMDA EPSPs were examined, using two different measurements, both an early and a late one (see Section 2). Additionally, the GABA_A-receptor antagonist PTX was added to further “purify” the NMDA response by blocking GABA_A-mediated inhibition. However, seizure-like waves tended to obscure the later part of the potentials, leading us to use an early time window to measure these “inhibition-free” NMDA EPSPs. Independent of details of NMDA response recording and/or measurement, all these experimental variants showed substantially less potentiation of NMDA than of AMPA components (see summary of data in Fig. 4). For instance, in the inhibition-free case we obtained 121 ± 7% for NMDA, compared to the AMPA value of 147 ± 7%.

Another way to induce LTP is to use theta-burst stimulation. Such activation resembles that in vivo and has been suggested to be a link between artificial and natural synaptic activity. LTP induced by such stimulation appears to be more robust and stable than LTP induced by conventional ways (Staubli and Lynch, 1987). Therefore some experiments were made with
TBS-induced LTP in “normal Mg^{2+}” solution (n = 12). The amount of LTP was significantly larger than in case of LTP induced by HFS in “normal Mg^{2+}” and “low Mg^{2+}” solutions (compare Figs. 2, 4 and 5A). After 4 h of LTP expression the solution was changed to “low Mg^{2+}” with 50 μM AP5. AP5 was then washed out, leading to appearance of an NMDA component that was estimated. The relation between AMPA and NMDA components was essentially the same as in previous cases, showing bigger potentiation of the AMPA component than of the NMDA component (179 ± 5% for AMPA and 138 ± 6% for NMDA, n = 12), see Fig. 5A. A scatter plot illustrating the relation between AMPA and NMDA components after 4 h of LTP expression is shown in Fig. 5B, displaying data from all three types of experiments (cf. Figs. 2, 4 and 5A). Linear regression of the pooled data revealed a correlation between the potentiation of the two components (r = 0.70, p < 0.05, n = 33). A diagonal line representing equal changes of AMPA and NMDA is drawn to emphasize that in almost all the cases the potentiation of AMPA component was larger than that of NMDA component.

4. Discussion

Despite extensive research efforts, the relative involvement of AMPA and NMDA receptors in LTP remains a contentious issue. Our present data show that LTP is associated with a major increase of the AMPA component, as measured both at 1 h and 4 h after LTP induction. Even so, the potentiation of the NMDA component was significant, amounting to about half of that of the AMPA component. We also found that the ratio between AMPA and NMDA components was the same at both time points, an issue somewhat unexpected in view of the wealth of data demonstrating a change from early, protein synthesis independent LTP into late, protein synthesis dependent LTP during this time (Frey et al., 1993; Nguyen and Kandel, 1996).

As both AMPA and NMDA receptors contribute to synaptic transmission, but with different voltage dependencies, our findings have implications for information transfer in neuronal circuits. Moreover, the balance between AMPA and NMDA receptors is a factor important in regulating the further induction of LTP or LTD.

Previous work has demonstrated that even small activation of NMDA receptors weakens LTP with time (Xiao et al., 1996). Consequently, our investigation of NMDA receptor involvement over an extended time period used a method with minimal exposure of synapses to NMDA current. Even so the brief periods of NMDA receptor unblocking slightly reduced the amount of LTP defined as test/control ratio (see Section 3). While the reduction of LTP observed during unblocking could be slightly contaminated by the NMDA response (Xiao et al., 1995), the reduction observed after unblocking should be reliable. It might then be that even such short NMDA receptor activation triggers processes leading to depotentiation or to depression that somehow interacts with LTP. Our impression is that the depotentiation was a lasting matter although the data did not reveal statistical significance. Future studies may help to further clarify this issue, which is complicated by the fact that LTP also has a decay of its own.

Our measurements using isolated NMDA EPSPs are consistent with those using composite EPSPs, arguing against an effect of AMPA receptor activation on NMDA responses (Bazhenov and Kleshchevnikov, 1999). Moreover, the results obtained under blockade of inhibition by PTX did not differ from those obtained without the drug. Accordingly, our method of assessing AMPA and NMDA components at different times after LTP induction seems appropriate.

The present data do not support some prior work (Xiao et al., 1995, 1996), as well as recent findings (Watt et al., 2004), which showed a substantial NMDA receptor mediated LTP at 1–2 h after induction, similar in size to that of AMPA. However, the divergence might be explained by differences in experimental
conditions. For instance, the two first-mentioned investigations monitored composite EPSPs continuously, implying a weak NMDA receptor activation throughout experiments. This might have “primed” slices with synaptic depression (Dozmorov et al., 2003, 2004), possibly altering LTP by promoting NMDA receptor mediated changes (Selig et al., 1995; Xiao et al., 1995). Other experimental conditions, with respect to recording type (field versus whole cell, slice versus culture) as well as induction parameters (e.g. strength, paring versus tetanization, chemically induced LTP), may also influence the degree of NMDA receptor contribution to LTP (Aniksztejn and Ben-Ari, 1995; Bayazitov and Kleschevnikov, 2000; Lisman, 2003; Watt et al., 2004; Xie et al., 1996). In our case, however, different induction protocols resulted in a similar degree of AMPA/NMDA potentiation. Thus, in terms of AMPA/NMDA receptor potentiation, LTP induced under different conditions may have similar expression mechanisms.

While our present data reveal constant AMPA/NMDA potentiation ratio in the 1–4 h time period, we cannot exclude that changes in this ratio may occur during the first hour of LTP as reported previously (Xiao et al., 1995, 1996; see also Watt et al., 2004). In fact, the experiment illustrated in Fig. 1 is compatible with such a change as no clear NMDA receptor potentiation is seen directly after tetanization. However, care is
needed when comparing full-size and partially blocked NMDA components, the latter often being quite small. Further systematic investigation of this issue is needed.

The simplest explanation of our data is that a major AMPA receptor enhancement persists for at least 4 h. As suggested by whole cell studies, using single or nearly single synapse resolution, the underlying process may be an “AMPAfication” of initially “silent synapses” (Durand et al., 1996; Lisman, 2003). However, limitations of this technique prevent direct studies of LTP over long time periods. It should also be noted that even though AMPA-mediated potentiation predominated, the potentiation of the NMDA component was not negligible but amounted to as much as half of that of AMPA. It is not clear to what extent this potentiation of NMDA is actually due to a postsynaptic modification at the receptor level or might reflect an enhancement of transmitter release (Errington et al., 2003). With respect to the postsynaptic alternative, it has been suggested that a “homeostatic” regulatory mechanism is at work to keep AMPA/NMDA ratio constant after an initial 1–2 h of varying ratio (Watt et al., 2004). A similar mechanism could also be operative in the present case except that the regulated ratio during LTP does not equal the pretetanus value.

The idea of a constant expression mechanism from 1 h to 4 h is at odds with the finding that new LTP could be induced after 4 h despite previous saturation, but not after 2 h (Frey et al., 1995). The latter result implies that LTP changes character at a time point somewhere between 2 h and 4 h. This is also the time that separates early and late phases of LTP, as defined by protein synthesis involvement (Frey et al., 1993; Nguyen and Kandel, 1996). Nevertheless, our present results suggest a constant AMPA/NMDA ratio in the 1–4 h period, and so, fitting this result with a variable expression mechanism appears problematic. However, a shift between a receptor modification and an increase in receptor number may still be compatible with the present results. Using chemically induced potentiation, claimed to be equivalent with the later parts of LTP, it was suggested that LTP eventually becomes associated with formation of new synapses (Bolshakov et al., 1997; Ma et al., 1999). Thus, an initial “AMPAfication” of the synapses might transfer later into

Fig. 4. Changes of AMPA and NMDA components during LTP in “normal Mg$^{2+}$” solution. Potentiation of AMPA and NMDA components at 4 h are shown, using four different estimates for NMDA: (1) late measurement of composite EPSP, (2) early measurement of isolated NMDA EPSP, (3) late measurement of isolated NMDA EPSP in PTX solution, avoiding the later, wavy part. All values are calculated as ratios relative to the naive pathway. Time windows for measurements were 0–1 ms for AMPA and 4–5 ms (early) or 35–45 ms (late) for NMDA. Bars indicate mean ± S.E.M.

Fig. 5. Changes of AMPA and NMDA components during LTP induced by theta-burst stimulation (TBS) in “normal Mg$^{2+}$” and a summary of AMPA and NMDA component changes under different conditions. (A) Potentiation of AMPA and NMDA components 4 h after induction of LTP by TBS (n = 12). Bars indicate mean ± S.E.M. (B) Potentiation of AMPA and NMDA components 4 h after LTP induction plotted as X–Y diagram. Open circles represent the values obtained with composite EPSPs after tetanization-induced LTP in “low Mg$^{2+}$” solution; data from Fig. 2, n = 12, marked “comp. (tet)”; triangles represent the values obtained with pure NMDA EPSPs without inhibition after tetanization-induced LTP in “normal Mg$^{2+}$” solution; data from Fig. 4(4), n = 9, marked “pNMDA (tet)”; filled circles represent the values obtained with composite EPSPs after TBS-induced LTP in “normal Mg$^{2+}$”; data from ‘A, n = 12, marked “comp. (TBS)”’. A regression line is shown for the pooled data. Comparison with diagonal dashed line implies a larger potentiation of AMPA component in almost all the cases.
structural changes. Our results may be difficult to reconcile with this scenario, unless the new synapses have an upregulated AMPA/NMDA receptor ratio. Still, we have only studied the first 4–5 h of LTP and changes in AMPA versus NMDA receptor expression towards a more equal relation may well occur at later stages. Thus, any homeostatic principle acting to preserve AMPA versus NMDA ratio before and after LTP (Watt et al., 2004) might work, in our system at least, on a longer time scale than 4–5 h. Further studies, perhaps in cultured slices, are needed to resolve the issue of AMPA/NMDA ratio in a very long-term perspective.

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