Lack of AMPA Receptor Desensitization During Basal Synaptic Transmission in the Hippocampal Slice

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INTRODUCTION

In area CA1 of the hippocampus, it is now generally thought that the decay of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated excitatory postsynaptic currents (EPSCs) is due to receptor deactivation, that is, the closing of the receptor and subsequent unbinding of the ligand, as opposed to receptor desensitization (i.e., the ligand remains bound to the receptor in a long-lasting nonconducting state) (Colquhoun et al. 1992). Despite this, outside-out patches pulled from CA1 cells clearly show desensitization in response to brief pulses of glutamate. In other words, when two pulses are given in rapid succession, the response to the second pulse is smaller than the first (Arai and Lynch 1996; Colquhoun et al. 1992).

Surprisingly, recent experiments with paired pulses and minimal stimulation protocols in the hippocampal slice have not seen evidence for desensitization (Hjelmstad et al. 1997; Stevens and Wang 1995). On the other hand, protocols using drugs that block receptor desensitization have led to mixed conclusions (Arai and Lynch 1998; Debanne et al. 1996; Kullmann 1994; Pananceau et al. 1998; Wang and Kelly 1996). To resolve this discrepancy, we have taken a number of approaches to specifically address whether AMPA receptors desensitize in response to synaptically released glutamate in the hippocampal slice.

METHODS

Methods for slice preparation, whole cell experiments, and minimal stimulation criteria are the same as previously described (Hjelmstad et al. 1997; Isaac et al. 1996) using 2- to 3-wk-old Sprague-Dawley rats. For the paired-pulse recordings in Fig. 2, single and paired pulses were interleaved, and the tail from the first pulse was subtracted before calculating the ratio.

Exogenous AMPA was applied by ionophoresis from a glass microelectrode containing 10 mM AMPA placed in stratum radiatum. Current pulses (200–500 nA, 20–60 ms) were controlled with a WPI constant-current generator. A small holding current was used to prevent leak from the electrode. AMPA was used instead of glutamate to negate the effects of reuptake through glutamate transporters.

The expected effects of desensitization (Fig. 2E) were based on values from outside-out patch experiments from the CA1 region of hippocampus where the recovery from desensitization was calculated as

\[
\text{Desensitization}_{\text{patch}} = 53 \times e^{-t/53}
\]

(Colquhoun et al. 1992). In this equation, \( t \) is the paired-pulse interval in ms; 53 is the percent of receptors in the patch desensitized after a 1-ms pulse of glutamate, and 58 is the time constant of recovery from desensitization. If the application of glutamate to outside-out patches were to mimic release at individual synapses, the amount of measured desensitization for a population of synapses should be

\[
\text{Desensitization}_{\text{population}} = p_r \times 53 \times e^{-t/53}
\]

where \( p_r \) is the mean probability of release. For Fig. 2E, \( t \) was set at 40 ms.

RESULTS

We initially reanalyzed paired-pulse data from a number of previous minimal stimulation experiments (Hjelmstad et al. 1997; Isaac et al. 1996) to specifically address whether desensitization occurs in response to synaptic stimulation. Only using cells that met criteria for single-site recordings (Isaac et al. 1996), we segregated responses on whether the response to the first of two stimuli was a success or a failure. If desensitization occurs, responses after a success should show desensitization and have smaller amplitudes than responses after a failure. To assay this, we compared the potency (Stevens and Wang 1994), or the mean amplitude of successes only, for the second response (Fig. 1A). The average ratio of the potency after a success to that after a failure for 10 cells was 1.01 ± 0.04 (Fig. 1B), suggesting that no desensitization occurred. Furthermore, there was no correlation between the paired-pulse interval and the potency ratio (interval range 30–70 ms). These data suggest that desensitization is not occurring in response to synaptic stimulation.
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FIG. 1. Single-site recordings do not show evidence for desensitization. \( A_1 \): average of all traces (\( n = 20 \)) from a single cell where there was a success in response to both the first and the second stimulus. \( A_2 \): average of all traces (\( n = 22 \)) where there was a failure in response to the first stimulus and a success in response to the second stimulus. \( A_3 \): overlaying responses to second stimulus after success or failure to the first stimulus shows no evidence for desensitization. \( B \): average potency ratio (after a success vs. after a failure) for 10 cells.

An assumption of this experiment is that we are recording from a single release site. If we were recording from multiple synapses, we would expect to see less evidence for desensitization because two subsequent EPSCs may not be from the same release site. Nevertheless, we would still expect to see some evidence for desensitization. For the example in Fig. 1, based on desensitization rates from outside-out patches (see METHODS), a single release site should show a potency ratio of 0.73. A simple binomial model of release with two release sites predicts a potency ratio of 0.85 and with three sites predicts a ratio of 0.89.

Because of the provocative nature of these results, we sought to obtain additional evidence by addressing this issue in other ways. If desensitization is occurring in response to synaptic stimulation, then drugs that block AMPA receptor desensitization, such as cyclothiazide (CTZ), should alter the magnitude of paired-pulse facilitation (PPF). Previous attempts to address this issue in the hippocampus (Arai and Lynch 1998; Debanne et al. 1996; Kullmann 1994; Pananceau et al. 1998; Wang and Kelly 1996) have provided inconsistent results. One possible explanation for this is that these previous experiments were all conducted under normal conditions in which the basal probability of release (\( p_r \)) is low. This will tend to underestimate the importance of desensitization because a given synapse will only occasionally respond to both stimuli, as illustrated in Fig. 2E. Therefore, to maximize the potential effects of desensitization on the paired-pulse ratio, we increased \( p_r \) by raising Ca\(^{2+}\) to 5 mM and by adding 50 \( \mu M \) 4AP, a protocol that more than doubles \( p_r \) (Hjelmstad et al. 1997; Hsia et al. 1998).

Additionally, this protocol should occlude any presynaptic effects of CTZ on \( p_r \) (Diamond and Jahr 1995). Under these recording conditions, EPSCs in response to paired stimuli (40 ms) showed no facilitation, and in some cases we observed paired-pulse depression (Fig. 2A). Addition of 100 \( \mu M \) CTZ had no effect on the paired-pulse ratio (1.01 ± 0.14 Control; 1.02 ± 0.12 CTZ, \( n = 6 \); Fig. 2B). The drug did slow the decay of the EPSCs (Fig. 2B) consistent with the effects of CTZ on receptor deactivation (Patneau et al. 1993). Analogous experiments with aniracetam, which also blocks AMPA receptor desensitization, likewise failed to show an increase in the paired-pulse ratio (Fig. 2D; 92.7 ± 4.3%, \( n = 3 \)).

An alternate method that has been used to show evidence for desensitization in the chick nucleus magnocellularis (Otis et al. 1996a) makes use of the voltage dependence of desensitization (Thio et al. 1991), the magnitude of which decreases with depolarization. Again, under conditions of increased \( p_r \), the paired-pulse ratio was measured at both hyperpolarized (–70 mv) and depolarized (+60) conditions (Fig. 2C). In the presence of 100 \( \mu M \) d-APV to isolate the AMPA receptor-mediated current. Scaling the two sets of synaptic responses to

![Diagram](http://jn.physiology.org/)

**FIG. 2.** Manipulations that alter desensitization do not affect paired-pulse ratios. A, left: paired pulses under conditions of high \( p_r \) do not show PPF. A, middle: addition of 100 \( \mu M \) cyclothiazide (CTZ) slows the decay but as shown in the scaled responses (right) does not alter the paired-pulse ratio. The second excitatory postsynaptic currents (EPSCs) were scaled to the amplitude of the first EPSCs. B: average of 6 cells shows that CTZ has no effect on the paired-pulse ratio but does prolong the decay of the EPSC. C: \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor EPSC paired-pulse ratio is not altered by the holding potential of the cell. C1: averages of 10 consecutive EPSCs recorded at –70 and +60 mv with either single or paired stimuli. C2: isolated second EPSC at 2 holding potentials superimposed after scaling to the amplitude of the first EPSC. D: average change in the paired-pulse ratio for CTZ (\( n = 6 \)), aniracetam (\( n = 3 \)), or depolarization (\( n = 7 \)). E: expected change in the paired-pulse ratio (40-ms interval) caused by desensitization based on outside-out patch experiments is dependent on the probability of release.
the amplitude of the first EPSC showed that there was no change in the paired-pulse ratio (−4.5 ± 4.7%, n = 7; Fig. 2, C and D).

The absence of desensitization in these assays raises the question whether AMPA receptors in the intact hippocampal slice are capable of desensitization at all. To address this, we measured the currents elicited by the ionophoretic application of AMPA to the dendrites of a CA1 pyramidal cell. After the bath application of 100 μM CTZ, the amplitude of the ionophoresis response increased dramatically (Fig. 3). Because CTZ affects deactivation in addition to desensitization, we also monitored miniature EPSCs from the same cells because it is thought that the time course of individual mEPSCs are not influenced by desensitization (Hestrin 1992). Consistent with data from hippocampal cultures (Diamond and Jahr 1995), CTZ caused an increase in both the mEPSC charge transfer (Fig. 3B) and the mEPSC frequency (not shown). More importantly, the increase in the charge transfer of the evoked responses was significantly larger than that of the minis (Fig. 3, B and C), suggesting that desensitization was occurring in response to exogenous application of AMPA.

In a final set of experiments, we bath applied a low concentration of AMPA (50 nM) and monitored the holding current of a voltage-clamped pyramidal cell. The application of AMPA had a negligible effect on the holding current, but after application of CTZ the holding current increased dramatically (Fig. 3D). Therefore under conditions of prolonged agonist application AMPA receptors in the intact slice exhibit marked desensitization.

**DISCUSSION**

We have shown that, although AMPA receptors in the intact hippocampal slice are capable of desensitization, there is no detectable desensitization in response to the synaptic release of glutamate. These results stand in contrast to data from the chick nucleus magnocellularis, where there is strong evidence showing AMPA receptor desensitization in response to synaptically released glutamate (Otis et al. 1996a,b).

There are a number of possible explanations for why desensitization was not detected in response to synaptic stimulation as opposed to the desensitization that occurs with glutamate application to outside-out patches. First, the magnitude of desensitization will depend on the degree of receptor occupancy at the synapse. If, for instance, the synapse is far from saturated by a single vesicle of glutamate, then a large pool of available receptors will obscure any desensitization caused by previous activity. Although a 1-ms pulse of 1 mM glutamate has been argued to mimic the time course of glutamate in the synaptic cleft, recent estimates indicate that the time course is dramatically faster (Clements 1996). Thus the disparity between the results from the patch and the synapse may be due to the slower clearance of glutamate from the outside-out patch.

It is also possible that the dynamics of desensitization are different between receptors in outside-out patches and at intact synapses. There are two possible scenarios by which this might occur. First, synaptic receptors may exhibit different properties than extrasynaptic receptors, perhaps because of some post-translational modification. Alternatively, a consequence of pulling outside-out patches may be to change some aspects of receptor function, as is the case for the NMDA receptor (Sather et al. 1992).

Our results differ from those of some previous studies (Arai and Lynch 1998; Wang and Kelly 1996), which reported a change in PPF after application of CTZ. Although the reason underlying these differences is not clear, the previous experiments measured either extracellular field responses or EPSCs with intracellular sharp electrode recordings. The synaptic responses that are recorded with these methods may be subject to nonlinear summation.

In summary, in this study we have presented data suggesting that a single EPSC does not cause significant AMPA receptor desensitization. It is possible, however, that during repetitive synaptic activity the accumulation of desensitized receptors may have significant influences on excitatory synaptic responses.

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