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Research Report

Post-tetanic potentiation of GABAergic IPSCs in cultured hippocampal neurons is exclusively time-dependent

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ABSTRACT

We have previously shown that post-tetanic potentiation (PTP) of GABAergic IPSCs in cultured hippocampal neurons involves activation of L-type Ca^{2+} channels. Although there is little Ca^{2+} entry by this route, it is possible that L-type Ca^{2+} channels mediate an increase in probability of release (Pr) by a mechanism that remains dormant in the absence of stimulation. We have tested this hypothesis in the present study using dual whole-cell patch clamp recordings. IPSCs were evoked by low-frequency stimulation (LFS; 0.2 Hz) of presynaptic GABAergic neurons. Run-down was corrected by linear regression. Following tetanic stimulation (80 pulses at 40 Hz), the presence of PTP was probed by resuming LFS after various post-tetanic intervals (PTI). To control for possible effects associated with LFS, the train and PTI were replaced by corresponding pauses. Following pauses ≥16 s, the first IPSC was significantly increased by 20–25% ($P<0.01$, paired *t*-test). These post-pause responses were subtracted from IPSCs following tetanic stimulation. Following correction, PTP was greatest (~50%) after the shortest PTI (4 s) and IPSC amplitudes declined back to the baseline value over 1–2 min. With a PTI of 16 s, the first IPSC was potentiated to the same level as that to which PTP with a PTI of 4 s had decayed with continued LFS. There was no significant PTP with PTIs of 64 and 128 s. Since PTP decays entirely in the absence of stimulation, it is concluded that the process(es) mediating the increase in vesicular Pr appear to be time-dependent, but not use-dependent.

1. Introduction

Central synapses typically display various forms of plasticity following periods of stimulation. Plasticity is categorized according to whether transmission is potentiated or depressed and its relative persistence (Thomson, 2000; Zucker and Regehr, 2002). Post-tetanic potentiation (PTP) is an increase in synaptic strength that can be observed following tetanic stimulation. Its duration (30 s to several minutes; Zucker and Regehr, 2002) distinguishes it from other types of short-term potentiation, such as facilitation and augmentation. PTP is related to the rise in the Ca^{2+} concentration in the bouton that accompanies tetanic stimulation and is therefore generally believed to be a presynaptic phenomenon (Zucker and Regehr, 2002). This increase in $[Ca^{2+}]_i$ is thought to represent the initial intracellular signal for induction of molecular cascades that culminate in various alterations of the synaptic release machinery (Zucker and Regehr, 2002). The biochemical mechanisms underlying most forms of plasticity, including PTP, have not yet been fully elucidated. However,

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Abbreviations: FCS, fetal calf serum; HS, horse serum; LFD, low-frequency depression; LFS, low-frequency stimulation; RRP, readily releasable pool; Pr, probability of release; PTD, post-tetanic depression; PTI, post-tetanic intervals; PTP, post-tetanic potentiation

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experiments suggest that potentiation processes are actually related to changes in both the number of docked and primed vesicles (Dobrunz, 2002; Habets and Borst, 2005) and their individual probabilities of release (Pr) (Stevens and Wesseling, 1999; Kalkstein and Magleby, 2004; Habets and Borst, 2005).

We have previously performed extensive studies of PTP at hippocampal GABAergic synapses, both in cultured neurons (Jensen et al., 1999a), and acutely isolated slices (Jensen and Mody, 2001). Intriguingly, L-type Ca^{2+} channels were shown to be crucial for the expression of PTP at these synapses (Jensen et al., 1999b; Jensen and Mody, 2001), which has also been subsequently confirmed by other studies on cultured GABAergic neurons (Ivanova et al., 2004). L-type Ca^{2+} channels make only minimal contribution to the overall increase in intrabouton "residual" $[Ca^{2+}]_i$ (Lambert et al., 2002) (Holmgaard, Jensen and Lambert, in preparation) and further the time course of PTP decay is not temporally correlated to the wave of residual calcium (Jensen et al., 2000a). Operation of L-type Ca^{2+} channels may, therefore, serve as a molecular trigger for subsequent events leading to the increase in Pr that underlies PTP (Jensen et al., 1999a).

Studies on PTP are usually performed with quite rigid stimulus protocols. Typically, a low baseline stimulation frequency is chosen on which is inserted a period of tetanic stimulation. Baseline stimulation is then resumed to chart the progress of the alteration in the synaptic responses. The choice of protocol may influence the results obtained. On the one hand, the very act of testing the response may result in its decline. Such use dependency has been dramatically illustrated by Volianskis and Jensen (2003) in their studies of short-term potentiation in hippocampal neurons. On the other hand, the molecular rearrangements during and following the stimulation may simply revert with the passage of time (time dependency). Hitherto, the physiological characteristics underlying the decaying phase of PTP in GABAergic neurons have not been investigated. This motivated the present descriptive investigation of PTP, in which we have tested whether PTP in GABAergic synapses is stored (i.e., whether the synapses remain in a dormant state of potentiation after brief tetanic conditioning), or whether the potentiation gradually decays as a function of time. These characteristics are thought to be of relevance to the overall function of the GABAergic system in the brain, but might also reveal important information on the nature and mechanisms of PTP. The results of this study have been presented as an abstract (Lambert and Korshøj, 2006).

2. Results

Experiments were performed on cultured hippocampal GABAergic neurons which evoked bicuculline-sensitive IPSCs on the postsynaptic neuron with which they were paired.

2.1. *Low-frequency depression and post-pause potentiation*

After exploratory stimulation of the presynaptic neuron to identify the GABAergic IPSC, stimulation was paused for a few minutes while the recording settled down. Thirty-six IPSCs were then evoked with low-frequency basal stimulation of 0.2 Hz. The first period of LFS showed two characteristic features (Fig. 1). (1) The first IPSC was substantially and significantly larger (by 24±7.8%; $P<0.0005$) than the subsequent IPSC. This would infer that low-frequency depression (LFD) (Akaneya et al., 2003) is manifest at a frequency of 0.2 Hz. In order to identify the initial amplitude decrement as LFD it would be necessary to make a detailed characterization with respect to time and frequency dependency and time course of recovery (Akaneya et al., 2003). This was not done since, for the purposes of comparison, we were primarily interested in using the same frequency as used in our previous studies (Jensen et al., 1999a,b). (2) Response amplitudes decreased by 15–20% during the first 3 min. This decline was time-dependent and irreversible, and thus had the characteristics of run-down (Akaneya et al., 2003, also see Section 3.1). Although the averaged results in Fig. 1 would seem to indicate that changes in IPSC amplitudes are phasic in nature, this oscillatory behavior was not seen for results from individual neurons and is considered to be an aberration of the averaging process.

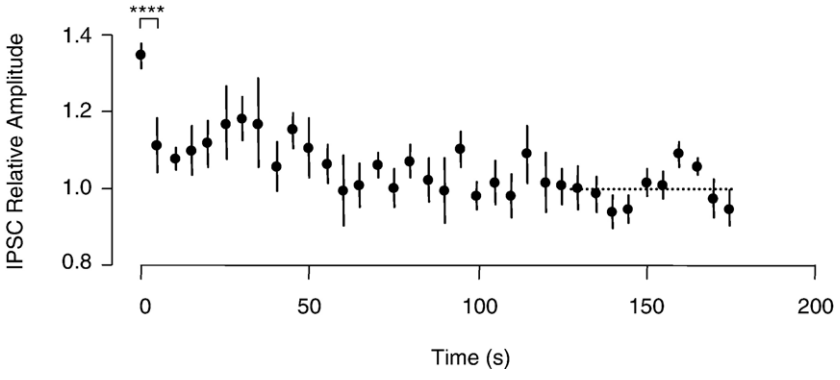

Fig. 1 – IPSCs evoked by low-frequency stimulation (LFS) of naïve GABAergic neurons show run-down. After the presynaptic neuron had been identified as GABAergic, it was rested for some minutes. On starting LFS at 0.2 Hz, the first IPSC was significantly larger than those following (**, $P<0.0005$, paired *t*-test for difference between the amplitudes of the first and second IPSCs). Subsequent IPSCs gradually declined by a further ~20% over the next 3 min (36 stimuli) and did not appear to reach a stable level. Data from 9 neurons normalized to the average of the last ten pulses (dotted line at 1.0). Error bars represent SEM.**